DETERMINATION OF ASCORBIC ACID IN FRUIT JUICES

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Abstract: *The present study has in attention the determination of ascorbic acid in beverages. This presents important interests as the amount of C vitamin content in certain food products, especially in beverages, has to be known by the consumers. Moreover, the food industry should not add more than 20% than the amount of vitamin C that is declared on the label. We propose an electrochemical method for the determination of ascorbic acid using the standard addition method. It was found that in most of the cases, the declared concentration of C vitamin is much lower than the ones found through electrochemical detection.*

Keywords: ascorbic acid, glassy carbon, electrochemistry

INTRODUCTION

Antioxidants in food are the most debated issue. Although the body has its own antioxidant defence system that usually reacts in case of exposure to radiation and chemical pollutants, a nutrition rich in antioxidants helps to potentiate the assimilation and metabolism of antioxidants. A diet rich in antioxidants does not increase the body concentration of these substances. Their levels remain within physiological limits due to homeostasis regulatory systems, which act for most endogenous compounds (Borut, P. et al. 2013).

As the knowledge of the various aspects of free radicals has become more extensive, the importance of antioxidants in diet has grown, becoming a subject not only in the biochemistry field, but also in nutrition, pharmacology and medicine (Ramalingum, N. et al. 2014).

Of particular importance are vitamins with antioxidant action (A, E, C and bioflavonoids) that have the most powerful effect of preventing membrane lipid peroxidation and accumulation of compounds that affect the integrity of the vascular wall. Ascorbic acid participates in the metabolism of collagen, intervening in unaltered maintenance of the vascular walls structure. Additionally, it prevents the transformations of mucopolysaccharides from the heart, avoiding alteration of selective permeability and ordered spatial disruption of the fundamental substance (Flora, S. J., 2009). Between ascorbate and cholesterol is a significant negative correlation, probably due to the vitamin's participation in the formation of 7-dehydro-cholesterol and/or other catabolism products (Pinto, J. T. et al. 2014.

The controversial roles of vitamin C could be partly explained by both the influence of concentration (by accepting diametrically opposite effects in many processes) and the interaction with other antioxidants. Thus, the interactions of vitamins C and E can be compared. In fact, although they act in different environments, lipophilic and hydrophilic membranes, their reciprocal regeneration is possible especially at interfaces of cell membranes and low density lipoproteins (LDL) particles. Unlike vitamin E that acts just at the site of peroxidation of polyunsaturated fatty acids interrupting the chain of oxidation, ascorbic acid is located in heterogeneous environments, varied and as regeneration possibilities, benefiting from the intake of several antioxidants, such as the reduced form of nicotinamide adenine dinucleotide (NADH) by the catalyzed reaction of semidehydroascorbate reductase from the mitochondria (Krajčovičová-Kudláčková, M et al. 2004).

In this sense, vitamin C is more likely to be regenerated, so oxidation stops to the dehydroascorbic acid stage that can be easily regenerated by reacting with an H donor (Traber, M. G. et al. 2011).

Although it has been discovered since the 17th century, the exact role of this vitamin in human biology and health is still a mystery in terms of benefits (Naidu, K. A. 2003).

The determination of C vitamin from pharmaceutical or food samples can be performed through different analytical methods, such as spectrophotometry (Güçlü, K. et al. 2005, Tabata, M. et al., 1997), chromatography (Dennison, D.B. et al., 1981, Lykkesfeldt, J., 2000) flow injection (Grudpan, K. et al. 1999) chemiluminescence (Alwarthan, A.A. 1993), mass spectrometry (Conley, J.M., et al. 2008) and electrochemical methods (Baghizadeh, A. et al. 2015, Bijad, M., et al. 2013, Karimi-Maleh, H., et al., 2014, Sun, W., et al., 2007, and Wang, X., et al., 2013).

The electrochemical methods have several advantages in comparison to the other analytical methods, which reside in their high sensitivity, lower cost, good accuracy, high dynamic range and simplicity.

In this paper we present a rapid and simple method for the determination of ascorbic acid from different juices using the standard addition method and electrochemical detection.

MATERIALS AND METHODS

All electrochemical experiments were performed using a Voltalab 40 (PGZ 301) potentiostat (Radiometer Analytical, France) controlled by a Voltamaster 4 electrochemical software (version 7.08).

The working, reference and counter electrode electrodes were: glassy carbon electrode (working area 0.07 cm^2), Ag|AgCl electrode filled with 3 M KCl (BAS, Bioanalytical Systems, West Lafayette, IN, USA), and a coiled platinum electrode (23 cm). The used electrolyte was 0.02 M sodium acetate buffer, pH 4.0. Other solutions used in the experiments were the fruit juices chosen to be tested.

The glassy carbon electrodes were polished successively on alumina slurries with diameter of 5; 1; 0.3 and 0.05 μm (Buehler Ltd, USA) and then carefully rinsed with distilled water.

The working electrode prepared in this way was placed in an electrochemical cell (Ag|AgCl reference electrode (3 M KCl), and platinum wire as an auxiliary electrode). The electrodes were connected to the potentiostat.

The operating temperature of the entire system was maintained constant at 20 °C.

Reagents were prepared with distilled water. The standard solution of 1% ascorbic acid was prepared using solid ascorbic acid and 0.02 M sodium acetate buffer, pH 4.0 to limit ascorbic acid losses to less than 5%.

The fresh fruit juices were prepared at the time of use and used without further dilution.

The purpose of this experiment is to determine the concentration of ascorbic acid in commercial and home-made juices using analytical voltammetry and the standard addition method.

To the cell were initially added 10 mL of the analyte, then two successive additions of standard solution of 1 ml each were made, and each time the resulting current was measured.

RESULTS AND DISCUSSIONS

The standard addition method is used to determine the amount of ascorbic acid in juices. The basic analytical relationship is:

$$i_{aa} = k \cdot c_{aa} \quad (1),$$

where: i_{aa} is the oxidation current.

c_{aa} is the concentration of ascorbic acid.

k is a constant which depends on the type of electrodes, the number of electrons and on the dissolution capacity of ascorbic acid.

Since the value of k is not known for the juice samples, the standard addition method is used. This involves the addition of a known amount of the standard with the concentration, c_s, then the current i_{an} is measured. The value of the current i_{an} can be related to the amount of standard addition:

$$i_{an} = k \cdot c_{an} = k \frac{c_{aa} \cdot V_0 + c_s \cdot \Delta V}{V_0 + \Delta V} \quad (2)$$

Where:

n is 2 or 3

V_0 = the initial volume of the solution to be analyzed (10 mL).

ΔV = the volume of the added standard solution of C vitamin.

From the relationships (1) and (2) the concentration of ascorbic acid can be determined).

$$c_{aa} = \frac{i_{aa} \cdot c_s \cdot \Delta V}{i_{an}(V_0 + \Delta V) - i_{aa} \cdot V_0} \qquad (3)$$

The results obtained during the experiments are presented in the following tables according to the registered voltammograms (data not shown).

Table 1. The measured and calculated parameters for the first standard addition

	i_{aa}, μA	i_{a2}, μA	c_{aa}, mg/mL	
			determined	**label declared**
Commercial Orange Juice	7.72	14.00	100.52	15
Commercial tomatoes juice	6.11	31.04	21.79	15
Homemade tomato juice	2.41	38.39	6.05	19
Commercial grapefruit juice	4.55	13.21	45.59	35
Commercial lemon juice	3.43	17.53	21.64	9
Fresh Orange juice	11.85	23.56	84.25	53
Fresh Grapefruit juice	5.76	19.51	36.69	34
Fresh Lemon juice	7.26	29.91	28.31	45

As it can be observed, the determined values of C vitamin present in the analyzed juices are higher than the label declared values indicated by the producer (the case of commercially juices). This can be explained taking into account that on the label is stated just the amount of C vitamin intentionally added to the juice, without taking into account the amount of C vitamin naturally contained by the fruit (Rodríguez-Comesana et al. 2002).

Table 2. The measured and calculated parameters for the second standard addition

	i_{aa}, μA	i_{a3}, μA	c_{aa}, mg/mL	
			determined	**label declared**
Commercial Orange Juice	7.72	26.9	62.87	15
Commercial tomatoes juice	6.11	48.06	23.70	15
Homemade tomato juice	2.41	51.47	8.12	19
Commercial grapefruit juice	4.55	18.02	53.30	35
Commercial lemon juice	3.43	28.14	22.61	9
Fresh Orange juice	11.85	27.53	111.87	53
Fresh Grapefruit juice	5.76	29.93	38.20	34
Fresh Lemon juice	7.26	35.12	41.62	45

Another explaination can be attributed to the fact that the amount indicated on the label must be present in the product on the last day of its validity. The amount of ascorbic acid introduced into the product is higher and is calculated taking into account the degradation rate with temperature (Johnson, O. R, et al, 2013).

It is believed that the ascorbic acid losses of juice packaged in Tetra Pack are about 50% each month at room temperature.

CONCLUSIONS

The differences found between the values on commercially purchased juice packs and the measured ones can be attributed either to the loss of ascorbic acid due to the storage conditions or to the fact that the producer does not take into account the initial concentration of vitamin C that exists initially un the fruits juice. Another possible explanation might be attributed to the complexity of the juice matrix that can be change during the spiking of the sample with C vitamin prepared in 0.02 M

acetate buffer pH 4.0. Further experiments will be necessary to validate the electrochemical method purposed in this study and to check the influence of the buffers on the matrix of the studied juices.

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