

APPLICATION OF VOLTAMMETRY TO DETERMINE VITAMIN C IN APPLE JUICES

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Abstract. The cyclic voltammetric method, which is based on the redox properties of ascorbic acid, was adopted to determine the concentration of vitamin C in apple juices. Recorded on voltammograms, the oxidation current of ascorbic acid was linearly dependent on the ascorbic acid concentration (a linear calibration curve was found in the range of up to 150 mg ascorbic acid/100 cm³ solution). The standard addition technique was used to determine the ascorbic acid concentration. To verify results, the titrimetric method was used. Differences between concentrations of vitamin C determined using the cyclic voltammetric and titrimetric methods are lower than 1 mg (in comparison to concentrations of ascorbic acid about 50 mg/100 cm³ of juice). The method – easy for automation – can be used directly without special preparation of studied apple juices samples before measurement of the vitamin C concentration.

Key words: ascorbic acid, cyclic voltammetry, apple juice, vitamin C, platinum electrode

INTRODUCTION

Ascorbic acid (AA) because of its antioxidant and therapeutic properties, is a valuable food component. Unfortunately, it is also a very labile substance which easily undergoes degradation caused by enzymes liberated from the raw material during technological processes, too high temperature or access of air. The losses of vitamin C during technological procedures forming part of the processing of juices can exceed even 50% [Horubała 1993]. That is why manufacturers of food products – bearing in mind the need to replenish vitamin C losses caused by the technological treatment and also to enhance the nutritive value of the product characterized by low content of this vitamin or to extend the shelf-life of food products – frequently add vitamin C to them.

It is worth emphasizing that, in some cases, excessive quantities of AA may result in the inhibition of natural processes occurring in food and, consequently, contribute to the deterioration of its taste (e.g. ascorbic acid added to apple pulp in the amount of

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250 mg/kg inhibits oxidation processes largely responsible for the proper aroma of apple juice [Czapski 1999]).

The requirement to assess the nutritive value of food products forces producers to constantly search for effective methods of determination of ascorbic acid not only during monitoring production processes but also to control the quality of finished products.

A wide range of methods of ascorbic acid determination can be found in literature on the subject and a considerable part of them is based on its reduction properties [Moszczyński and Pyć 1999, Pournaghi-Azar and Ojani 1997]. Titration methods [Ghani et al. 2002, Kabasakalis et al. 2000, Suntornsuk et al. 2002] are applied quite commonly, of which the most widespread is the Tillman's method recommended by the International Standardisation Organization (ISO) and the Association of Official Chemists (AOAC) [Danielczuk et al. 2004, Esteve et al. 1995]. However, this method does not allow determining the dehydroascorbic acid (DHAA), which is characterised by the same vitamin activity as the ascorbic acid [Moszczyński and Pyć 1999]. In addition, titration methods are not sufficiently selective as reduction substances present in food products interfere with the process of vitamin C determination and distort the obtained results [Arya et al. 2000, Moszczyński and Pyć 1999, Pournaghi-Azar and Ojani 1997].

Fluorometric methods provide more selective possibilities of vitamin C determination because they allow determination of the sum of AA and DHAA [Esteve et al. 1995, Yang et al. 1997]. Many of these methods are based on the DHAA reaction (obtained in the result of the earlier treatment of AA with oxidising agents) with *o*-phenylene diamine [Arya et al. 2000]. Both ISO and AOAC recommend the use of the fluorometric method employing the reaction of the dehydroascorbic acid with the *o*-phenylene diamine during vitamin C determination as a reference method [Arya et al. 2000, Danielczuk et al. 2004]. The disadvantage of the fluorometric methods is the need to control strictly the pH of the environment in which vitamin C is being determined because the fluorescence intensity of many substances depends on the pH of the solution [Arya et al. 2000]. Another shortcoming of these methods is the high cost of the required equipment.

Chromatographic methods [Arya et al. 2000], especially the high performance liquid chromatography (HPLC) [Czerwiecki and Wilczyńska 1999, Esteve et al. 1995, Iwase and Ono 1998, Iwase 2000, Lykkesfeldt et al. 1995], provide an exceptionally sensitive and accurate tool that can be used for the determination of vitamin C. They allow a simultaneous determination of AA and DHAA [Esteve et al. 1995] but they require complicated and frequently expensive equipment, well qualified personnel and are usually both labour- and time-consuming.

Voltammetry which belongs to the group of polarographic methods [Cygański 2004, Hulanicki 2004] is widely applied in the area of agriculture and food production [Esteve et al. 1995, Łukaszewski et al. 2003, Łukaszewski et al. 1996, Łukaszewski and Zembrzuski 1992, Opydo and Zembrzuski 1993, Pournaghi-Azar and Ojani 1997]. Recently numerous investigations have been carried out aiming at the development of voltammetric methods to determine ascorbic acid [Florou et al. 2000, Pournaghi-Azar and Ojani 1995, Raoof et al. 2004, Vijaykumar et al. 2001, Yu and Chen 1997]. Because of low costs of the required equipment as well as the simplicity of the employed procedures required to determine vitamin C, voltammetry appears to offer an attractive alternative to the methods mentioned earlier, in particular during the routine quality control of some food products [Esteve et al. 1995]. The aim of this paper is to discuss

the possibility of application of the cyclic voltammetry to determine ascorbic acid in apple juices.

MATERIALS AND METHODS

The experimental material included: (a) "Fortuna" apple juice of the AGROS-FORTUNA Company, (b) "Garden" apple juice of the AGROS NOVA Company and (c) "Caprio" apple nectar of the MASPEX WADOWICE Company available in shops. Two methods were employed to determine the vitamin C concentration in the examined juices: the voltammetric method and, as a reference, the titration method using the 2,6-dichlorophenolindophenol (Tillmans reagent) as a self indicator [Arya et al. 2000, Lykkesfeldt et al. 1995].

A. The voltammetric method – consists in the registration of changes in the current (I) flowing through the system of electrodes in relation to the potential (E) changing in time, applied to a working electrode. The voltammetric curves $I = f(E)$, drawn on the basis of the registered data, constitute the basis for the performed qualitative and quantitative analyses [Szczepaniak 2002]. Voltammetric measurements in the discussed experiments were carried out in the typical three-electrode cell powered by a generator with a cyclic, triangular voltage changes (Fig. 1). The working electrode of 0.25 cm^2 area was made of platinum. A platinum wire of 23 cm^2 area acted as an auxiliary electrode. The potential of the working electrode, measured in relation to the reference electrode, Ag/AgCl/KCl, changed in the range from $-0.3 \div +0.7 \text{ V}$, while the time of the full change cycle lasted 140 s. Before each series of measurements, the working electrode was subjected to mechanical treatment with the aim to remove all the substances adsorbed on its surface and then washed with distilled water. In addition, the working electrode was cleaned before each measurement by applying to it pulse at -1.5 V for 3 s. Standard ascorbic acid solutions of 0.11 M were prepared on the basis of the supporting electrolyte solution, whose role was played by 0.34 M KCl. Precise measurements of the current were taken using a computer system of data acquisition and voltammogram visualisation based on the I-7018 and I-7520R modules of a Taiwanese company ICP-CON. Before the experiment, the most favourable range and speed of the cyclic voltage changes of the electronic generator were determined and the best concentration, from the point of view of the sensitivity of the measuring system, of the supporting electrolyte was selected.

The value of the current resulting from the presence of the supporting electrolyte and, in the case of juices, also other substances, the so-called 'background current' (I_t), was estimated on the basis of experimentally plotted voltammograms ($I = f(E)$). During the first stage, the linear relationship, $I_t = f(E)$, which constituted an extension of the segment of the voltammetric curves on which electrode reactions did not take place was determined (Fig. 2). Employing the obtained equation, the value of the background current (I_t) was calculated at the potential which corresponded to the electrochemical oxidation of the ascorbic acid on electrode. Next, the obtained values of the current (I_p) associated with the oxidation peak of ascorbic acid were decreased by the value of the determined background current (I_{tp}) (Fig. 2).

The test of the suitability of the cyclic voltammetry method for the determination of vitamin C in apple juices and, simultaneously, the test of the above-described experimen-

tal stand was conducted on aqueous solutions of ascorbic acid prepared on the basis of 0.34 M solution of the supporting electrolyte (100 cm³) supplemented with 0.5 g citric acid to which successive quantities of the standard ascorbic acid solution were added (Fig. 3). From voltammograms obtained for different values of vitamin C concentration,

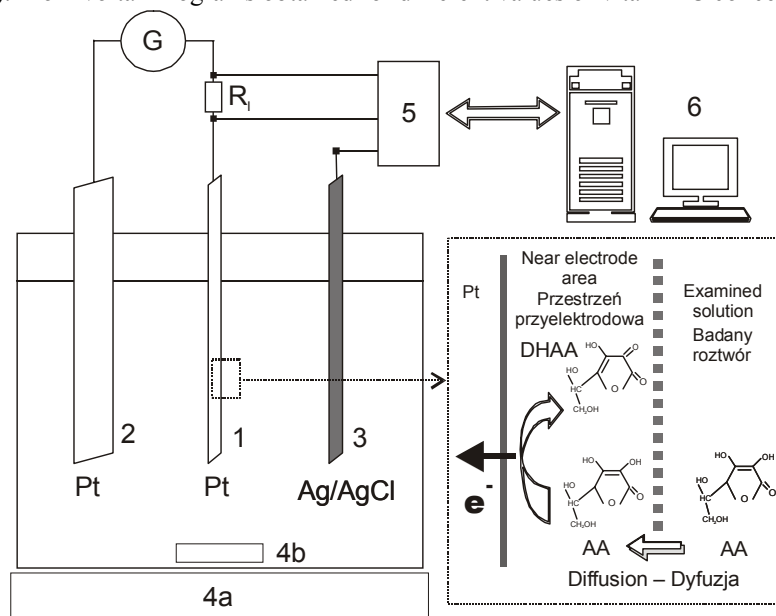


Fig. 1. Schematic diagram of the research stand: 1, 2, 3 – working, auxiliary and reference electrodes inside the cell (100 cm³); 4a and 4b – magnetic stirrer; 5 – data acquisition system; 6 – computer equipped with the program Vi_Volt for recording, visualization and archiving voltammograms; G – electronic generator; R₁ – measuring resistor; inside the rectangle on the right side: an idea of the vitamin C oxidation on the working electrode

Rys. 1. Schemat stanowiska badawczego: 1, 2, 3 – elektroda pracująca, pomocnicza i odniesienia w komorze pomiarowej (100 cm³); 4a i 4b – mieszkadło magnetyczne; 5 – system akwizycji danych; 6 – komputer z programem Vi_Volt do rejestracji, wizualizacji i archiwizacji voltamperogramów; G – generator elektroniczny; R₁ – rezystor pomiarowy; w prostokącie po prawej stronie: model pojęciowy elektrodowej oksydacji kwasu askorbinowego na elektrodzie pracującej

values of the current of oxidation peaks of ascorbic acid (I_p) were read and, after taking into account values of the current background, the character of the plot of the ($I_p - I_{tp}$) versus the ascorbic acid concentration was examined (Fig. 4).

The standard addition technique [Szczepaniak 2002, Cygański 2004] was employed to determine the concentration of vitamin C (C_S) in the examined apple juices. During the first stage of the experiment, a voltammetric curve was plotted for 100 cm³ juice supplemented with 2.5 g of the supporting electrolyte – KCl. Next, successive quantities of the standard ascorbic acid solution were added to the examined sample and voltammetric diagrams were made (Fig. 5). On the basis of the obtained curves, exactly the same way as it was done in the case of the aqueous solution of ascorbic acid, the background current was estimated and, once it was taken into account, the plot of the

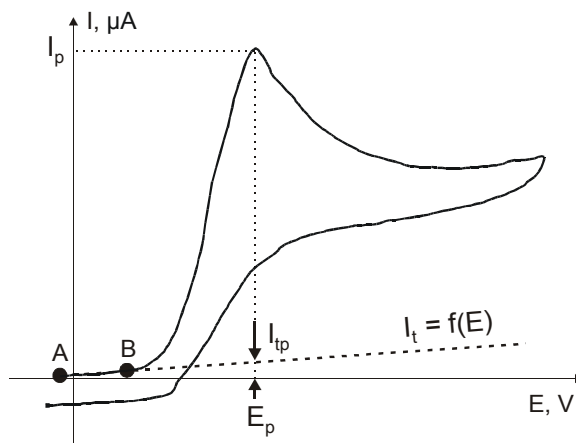


Fig. 2. Background current (I_{tp}) determination
Rys. 2. Wyznaczenie prądu tła (I_{tp})

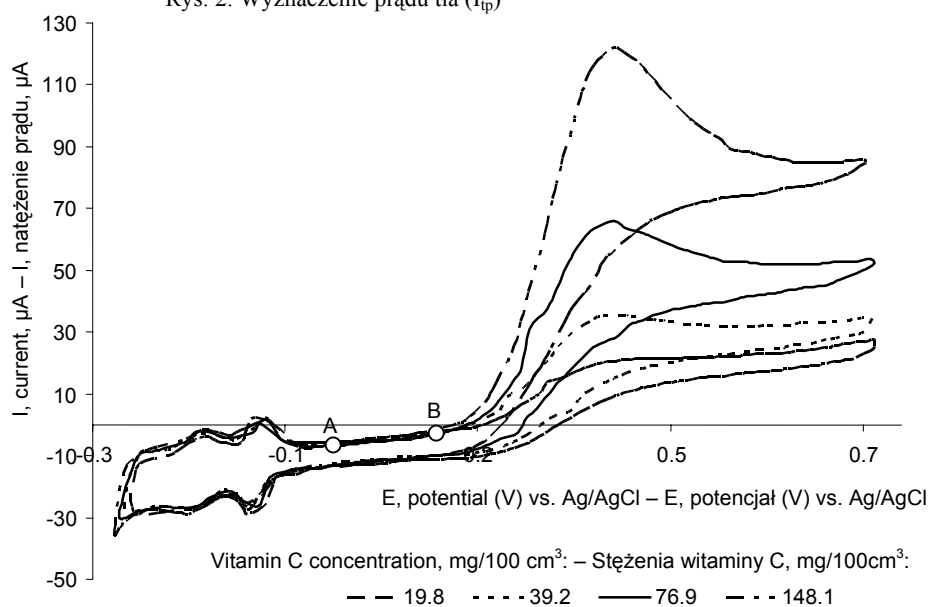
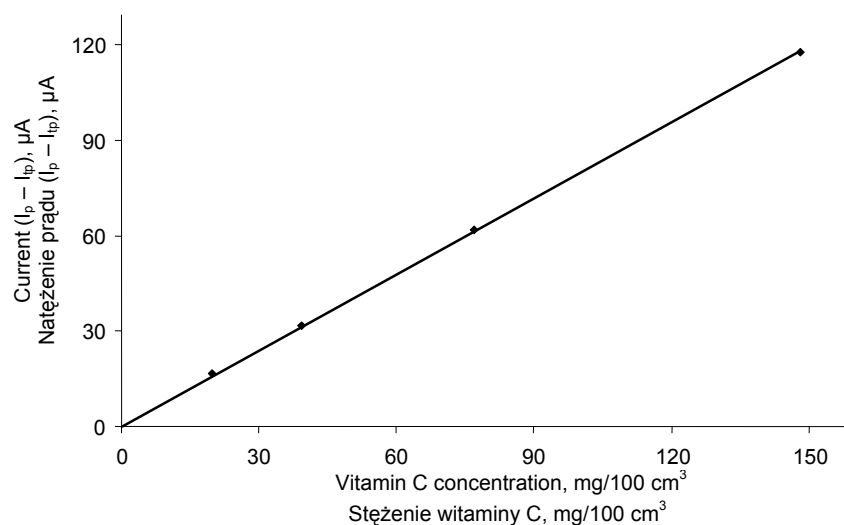


Fig. 3. Cyclic voltammograms of ascorbic acid in supporting electrolyte solution – 0.34 M KCl with additional amount of citric acid (0.5 g/100 cm³ solution of KCl); potential scan rate: 16 mVs⁻¹; the distance between points A and B used for the calculation of the background current

Rys. 3. Voltamperogramy cykliczne dla witaminy C w roztworze elektrolitu wspomagającego – 0,34 M KCl z dodatkiem kwasu cytrynowego (0,5 g/100 cm³ roztworu KCl); szybkość zmian napięcia: 16 mVs⁻¹; odcinek pomiędzy punktami A i B służący do wyznaczenia prądu tła



Rys. 4. Relationships between the oxidation peaks current and ascorbic acid concentration in 0.34 M KCl solution

Rys. 4. Zależności natężenia prądu pików odpowiadających elektrodowej oksydacji kwasu askorbinowego od stężenia witaminy C w roztworze 0,34 M KCl

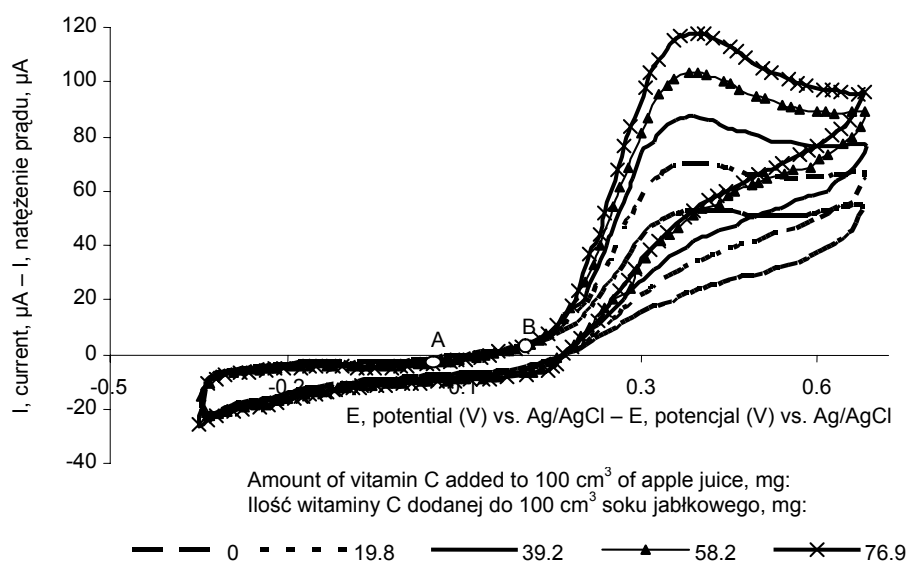


Fig. 5. Cyclic voltammograms of apple juice with addition of supporting electrolyte – 0.34 M KCl without and with the addition of ascorbic acid; potential scan rate: 16 mVs⁻¹; the segment between points A and B used for the calculation of the background current

Rys. 5. Voltamperogramy cykliczne dla soku jabłkowego z dodatkiem elektrolitu wspomagającego – 0,34 M KCl bez i z dodatkową zawartością witaminy C; szybkość zmian napięcia: 16 mVs⁻¹; odcinek pomiędzy punktami A i B służący do wyznaczenia prądu tła

$(I_p - I_{tp})$ versus the ascorbic acid concentration in the juice was drawn (Fig. 6). The content of vitamin C in the examined juice was determined graphically by extrapolating the obtained straight line to the value of current equal zero as well as analytically, with the assistance of the formula presented below, which was elaborated on the basis of the least squares method:

$$C_S = \frac{\sum_{i=1}^n (I_{pi} - I_{tpi}) \times \sum_{i=1}^n C_i^2 - \sum_{i=1}^n C_i \times \sum_{i=1}^n C_i \times (I_{pi} - I_{tpi})}{n \sum_{i=1}^n C_i \times (I_{pi} - I_{tpi}) - \sum_{i=1}^n C_i \times \sum_{i=1}^n (I_{pi} - I_{tpi})} \quad (1)$$

where:

- i – consecutive number of the measurement,
- n – amount of measurements,
- C_s – concentration of ascorbic acid in the examined sample,
- C – concentration of ascorbic acid added in the form of the standard solution in the examined sample. The remaining symbols are explained in the text.

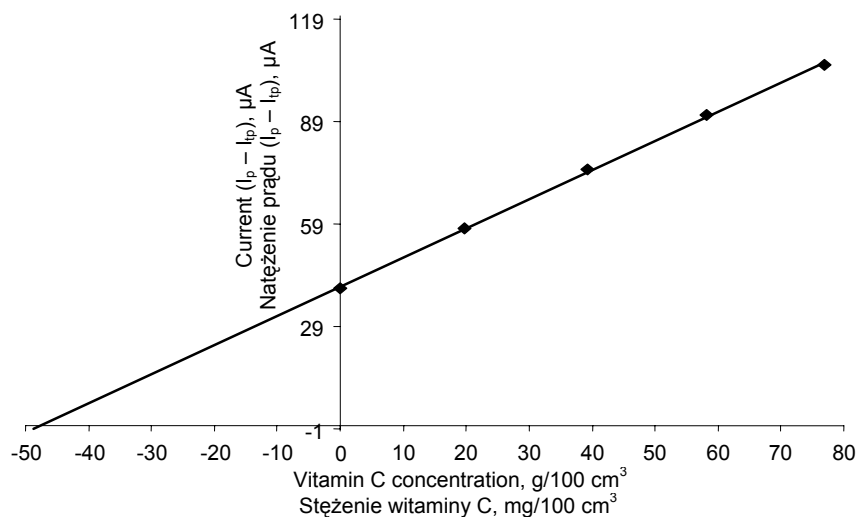


Fig. 6. Relationships between the oxidation peaks current and ascorbic acid concentration in apple juice with the addition of supporting electrolyte – 0.34 M KCl

Rys. 6. Zależności napięcia prądu pików odpowiadających elektrodowej oksydacji kwasu askorbinowego od stężenia witaminy C w soku jabłkowym z dodatkiem elektrolitu wspomagającego – 0,34 M KCl

B. Titration method. The determination was carried out in accordance with the Polish Standard PN-A-04019. Oxalic acid was applied as the regulator of acidity and 2,6-dichlorophenolindophenol (Tillmans reagent) as a self indicator.

All the applied reagents were of the analytical grade and distilled water was used to make all solutions. All assays were taken in six repetitions.

RESULTS AND DISCUSSION

The test results of both the experimental stand as well as the suitability of the cyclic voltammetry technique for the determination of vitamin C are presented in the form of voltammograms in Figure 3. Both their shape and the value of the E_p potential (associated with the electrochemical oxidation of AA to DHAA on the platinum electrode) at which peak current values (I_p) were recorded, was about 0.37[V] and they were similar to those known from the recent publications on this subject [Ernst and Knoll 2001, Mu and Kann 2002].

The straight line equation representing the background current in the aqueous solution of ascorbic acid supplemented with the supporting electrolyte and citric acid was developed on the basis of point coordinates (of AB segment on Fig. 3) situated on voltammograms within the range of potentials in which the electrode reactions did not occur: $I_t = 27.93 \cdot E - 6.72 \mu\text{A}$. The value of the background current for the E_p , $I_{tp} = I_t(E_p)$ potential was 3.6 μA . This value was taken into account in the course of determining the correlation between the current of the electrochemical oxidation of ascorbic acid and the concentration of vitamin C (Fig. 4). It was found that the dependence [$I_p - I_{tp}$] on vitamin C concentration for the examined concentrations had a linear character (correlation coefficient $R^2 = 0.99$) and, therefore, the voltammetric method could have been employed to determine vitamin C.

During the next phase of investigations, the content of vitamin C in apple juices with the assistance of the voltammetric technique was determined. Examples of voltammograms (for the "Fortuna" apple juice) are presented in Figure 5. The background current equation for standard voltammographic curves, determined in the same way as in the case of aqueous solutions of vitamin C, had the following form: $I_t = 41.71 \cdot E - 3.16 \mu\text{A}$, while the value of the background current (I_{tp}) equalled 12.35 μA . The content of vitamin C was determined using the standard addition technique (on the basis of the plot for current peaks [$I_p - I_{tp}$] as a function of vitamin C concentration – Fig. 6).

In order to validate measurement results obtained with the assistance of the cyclic voltammetry, they were compared with results obtained using the reference method. The selected reference method, similarly to other researchers [Danielczuk et al. 2004], was the Tillmans method, which is recommended by the ISO and AOAC as a routine analytical method. Vitamin C contents determined using the two methods are presented in Table 1. The precision of the measurement (carried out in the same measurement conditions for the same juice sample), i.e. the degree of dispersion of the set of results of vitamin C content in the examined juices, was expressed by values of standard deviation (SD) [Gawęcki and Wagner 1984, Lewis 1984] which, for three measurement series (for A, B and C juices), for the voltammetric method were slightly higher than the corresponding values in the reference method (Table 1). The calculated values (SD) correspond to the values of measurement repeatability, frequently applied in practice, expressed by the maximal difference between the results of a series of measurements. In the case of the voltammetric method, the measurement repeatability expressed in this way amounted to 2.44; 1.15 and 2.66 mg/100 cm³ of juice and 0.96; 0.70 and 0.68 mg/100 cm³ of juice for the reference method. The accuracy of the titration method was higher than that of the voltammetric method, which corroborates the opinion of other researchers [Danielczuk et al. 2004] about the possibility of its application as a reference

method for the voltammetric method. We believe that the accuracy of the voltammetric method can be improved by the automation of the measurement process.

Table 1. Comparison of vitamin C content in apple juice: "Fortuna" – juice A, "Garden" – juice B and "Caprio" – juice C obtained by voltammetric method and 2,6-dichlorophenolindophenol titration method, treated as the reference method

Tabela 1. Porównanie zawartości witaminy C w sokach jabłkowych: „Fortuna” – sok A, „Garden” – sok B oraz „Caprio” – sok C otrzymanych metodą voltamperometryczną i metodą Tillmansa, traktowaną jako odniesienie

Sample number Numer próby	Juice A – Sok A		Juice B – Sok B		Juice C – Sok C	
	method V metoda V	reference method metoda odniesienia T	method V metoda V	reference method metoda odniesienia T	method V metoda V	reference method metoda odniesienia T
	mg/100 cm ³ of juice mg/100 cm ³ soku	mg/100 cm ³ of juice mg/100 cm ³ soku	mg/100 cm ³ of juice mg/100 cm ³ soku	mg/100 cm ³ of juice mg/100 cm ³ soku	mg/100 cm ³ of juice mg/100 cm ³ soku	mg/100 cm ³ of juice mg/100 cm ³ soku
1	46.45	46.92	48.67	48.19	45.01	45.88
2	48.76	46.59	49.74	48.19	47.67	45.55
3	47.72	46.59	49.22	48.19	45.96	46.23
4	47.56	47.24	49.71	48.55	46.85	45.55
5	46.84	46.92	48.59	48.19	45.44	45.88
6	46.32	46.28	49.11	47.85	45.70	45.82
Min.	46.32	46.28	48.59	47.85	45.01	45.55
Max	48.76	47.24	49.74	48.55	47.67	46.23
X	47.28	46.76	49.17	48.19	46.11	45.82
SD	0.843	0.308	0.449	0.202	0.897	0.231

Statistics of differences between the voltammetric (V) and reference (T) methods for three different juices (A, B and C)

Mean of differences $X_V - X_T$: 0.59 mg/100 cm³ juice.

Max value of differences $X_V - X_T$: 0.98 mg/100 cm³ juice.

Min value of differences $X_V - X_T$: 0.29 mg/100 cm³ juice.

Standard deviation of differences $X_V - X_T$: 0.29 mg/100 cm³ juice.

V – voltammetric method.

T – 2,6-Dichlorophenolindophenol titration method.

X – average value.

SD – standard deviation.

Statystyka różnic między metodą voltamperometryczną (V) a odniesieniem dla trzech różnych soków (A, B i C)

Wartość średnia różnic $X_V - X_T$: 0,59 mg/100 cm³ juice.

Wartość maksymalna różnic $X_V - X_T$: 0,98 mg/100 cm³ juice.

Wartość minimalna różnic $X_V - X_T$: 0,29 mg/100 cm³ juice.

Odchylenie standardowe różnic $X_V - X_T$: 0,29 mg/100 cm³ juice.

V – metoda voltamperometryczna.

T – 2,6-Dichlorophenolindophenol. Metoda Tillmansa.

X – wartość średnia.

SD – odchylenie standardowe.

The mean vitamin C content in the three examined juices determined using the voltammetric method was slightly higher (by 0.59 mg/100 cm³ juice) than the similar mean value determined by the Tillmans method (Table 1). A similar regularity was found by other researchers, who compared results of determination of the vitamin C content in milk-based infant formula obtained using the voltammetric method and with the assistance of liquid chromatography (HPLC) [Esteve et al. 1995].

The accuracy of the voltammetric method was expressed in percent as the ratio of the maximal differences value ($X_V - X_T$, Table 1) between the mean vitamin C contents in the examined samples of different juices determined by the voltammetric (V) and reference (T) methods to the value of the mean vitamin C content in the examined samples determined by the reference method [Taylor 1982]. It is evident from Table 1 that the accuracy defined in this way was $100 \cdot 0.98 / [(46.76 + 48.19 + 45.82) / 3] = 2.1\%$. This relatively high accuracy, as well as the low value of the standard deviation between the two methods (Table 1), indicates that the voltammetric method may be used in practice as an alternative method, especially for juice quality control.

CONCLUSIONS

1. The linear character of the plot of peak currents ($I_p - I_{p_0}$) versus the ascorbic acid concentration obtained for the ascorbic acid systems in the solution of the supporting electrolyte supplemented with the citric acid indicates the possibility of application of the cyclic voltammetry to assess vitamin C concentration.

2. There were small differences between mean values of the vitamin C content in the examined apple juices obtained using the voltammetric and Tillmans methods, the latter being the reference method. The mean value of differences amounted to 0.59 mg/100 cm³ juice (at the mean concentration for the reference method – 46.92 mg/100 cm³ juice) at 0.29 standard deviation. This points to the possibility of application of the voltammetric method as an alternative to other, very frequently expensive, methods, especially in practical applications to assess juice quality.

3. The voltammetric method of vitamin C determination does not require expensive equipment and can be used directly without special sample preparation of the examined apple juices prior to measurements. In addition, it is possible to automate the measuring process.

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ZASTOSOWANIE WOLTAMPEROMETRII DO OZNACZANIA ZAWARTOŚCI WITAMINY C W SOKACH JABŁKOWYCH

Streszczenie. Metodę woltamperometrii cyklicznej, opartą na oksydoredukcyjnych właściwościach kwasu askorbinowego, przystosowano do oznaczania witaminy C w sokach jabłkowych. Zarejestrowane na woltamperogramach natężenie prądu odpowiadające pikom oksydacyjnym było liniowo zależne od stężenia kwasu askorbinowego (badano stężenia do 150 mg witaminy C/100 cm³ roztworu). Zawartość witaminy C określano metodą wielokrotnie dodawanego wzorca. Wyniki zweryfikowano metodą miareczkową. Różnice pomiędzy zawartością witaminy C w badanych sokach wyznaczone metodą woltamperometryczną i metodą miareczkową wynosiły mniej niż 1 mg (w stężeniach kwasu askorbinowego ok. 50 mg /100 cm³ soku). Metoda – łatwa do zautomatyzowania – może być stosowana bezpośrednio, bez specjalnego przygotowania prób badanych soków jabłkowych przed pomiarami stężenia witaminy C.

Słowa kluczowe: kwas askorbinowy, woltamperometria cykliczna, sok jabłkowy, witamina C, elektroda platynowa

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