Research Article

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# The effect of selected substances on the stability of standard solutions in voltammetric analysis of ascorbic acid in fruit juices

https://doi.org/10.1515/chem-2019-0078
received October 16, 2018; accepted February 20, 2019.

**Abstract:** The objective of the study was to identify suitable additives stabilizing standard solutions of ascorbic acid (AA) that would not cause interference in the analytical process with the use of voltammetry in the determination of the AA content in food products. In addition, the effect of various conditions of storage of selected fruit juices and drinks on the concentration of vitamin C was studied. The study demonstrated that AA degradation was inhibited the most effectively by tartaric acid and its optimum concentration was set to 200 mg $L^{1}$. Analysis of selected fruit juices stored in various temperature conditions confirmed that an elevation of temperature and extension of the time of storage caused a decrease in the content of vitamin C in the analyzed samples, while closing the packages caused a limitation of the changes in concentration of this vitamin. On the basis of literature data and of the results obtained in the present study it can be concluded that fruit juices should be stored at a temperature lower than room temperature to retain their nutritive value.

**Keywords:** ascorbic acid; differential pulse polarography; fruit juice; storage; vitamin C.

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## 1 Introduction

The role of ascorbic acid in food technology, apart from its vitamin properties, can be reduced to the protection of other nutrients which may undergo unfavourable transformations (mainly through oxidation) in the course of the technological process and storage, resulting in undesirable sensory changes in the product (colour, taste, flavour, clarity). As ascorbic acid can remove oxygen, it can be applied as an additive to air-tight sealed products containing air.

Determination of the content of vitamin C (ascorbic acid) in food products, fruit and vegetable preserves and raw materials for production is important not only from a nutritional point of view. Due to the sensitivity of that vitamin to the effect of physicochemical factors, its content in raw materials and food products is one of the primary indicators of the quality and correctness of technological processes applied, evaluation of the processing methods, and of biochemical changes taking place in products in storage. Ascorbic acid is one of a few organic compounds which display reducing properties in an acidic environment. This is determined by the presence of an enediol group in its molecule, which easily liberates electrons, transforming into an oxidised form. Chemical methods of quantitative assay of vitamin C are based on those properties of ascorbic acid and consist mainly of colour reactions, the phenomenon of light absorption, and fluorescence. A number of methods and analytical techniques of vitamin C assay have been developed for diverse matrices. Those include spectrometric titration [1, 2], voltammetry [3–7], potentiometry [8], fluorimetry [9, 10], flow injection analysis [11–14], spectrophotometry [15–18] and chromatography [19–23]. In addition, in recent years a number of new methods for the assay of vitamin C have been developed, based, for example, on chemically modified electrodes, biological sensors (biosensors) or on the techniques of mass spectrometry and atomic

absorption spectrometry [24–26]. A special note is due to the method of differential pulse polarography (DPP) which is characterised by a relatively low level of uncertainty of results in the assay of vitamin C. Among all methods for the assay of vitamin C in food products, the polarographic method creates the possibility of the simplest procedure in the analysis of that component. The polarographic techniques developed over the past decades were aimed at increasing the sensitivity of determinations, automation of measurement, and reduction in the use of pure mercury and other materials required in the procedure, and were also aimed at the elimination of all the drawbacks and shortcomings of the classic constant current polarography. In our previous study we confirmed the applicability of high-performance liquid chromatography with diode-array detection (HPLC-DAD) for determination of total content of vitamin C and ascorbic acid in various kinds of food [27], and demonstrated an absence of statistically significant differences in the AA content assayed by differential pulse polarography (DPP) and HPLC-DAD [28]. Differential pulse polarography is an equivalent method in relation to HPLC-DAD in terms of analytical parameters, and allows simpler and faster assay of ascorbic acid. A negative aspect of polarographic determination of vitamin C, that may have a detrimental effect on the accuracy of the measurement of its content, is the relatively low stability of that compound in the standard solution to the concentration of which – in every measurement – the curves of concentration of the assayed component in the sample are compared. As soon as after several hours, changes are visible in the concentration of vitamin C in standard solutions, prepared without any stabilising additives. That instability of the concentration of vitamin C solutions makes it necessary to prepare fresh standard solutions of vitamin C 3 – 4 times during a work-day, and in addition it enforces the necessity of flushing the entire sample injector system with those standards, and performing an additional series of test analyses. All of those operations cause unnecessary complications in the analytical procedure and extend its duration, and their omission causes a decrease of correctness of the results obtained.

Therefore, the objective of the study was to find suitable additives stabilising standard solutions of vitamin C, that would prevent to an optimum extent its rapid degradation, and at the same would not cause any disturbance in the analytical process by creating their own polarographic wave or causing any deformation of the polarographic curve of vitamin C, within the range of potentials used. In addition, we used in the study the method of differential pulse polarography (DPP) for the estimation of the effect of various storage conditions of fruit juices on the concentration of vitamin C.

# 2 Materials and Methods

## 2.1 Experiment 1. Optimisation of conditions of vitamin C assay with the polarographic method

The test material consisted of the following solutions:
– standard ascorbic acid solution (100 mg $L^{-1}$) – control,
– standard ascorbic acid solutions (100 mg $L^{-1}$) with an addition (100 mg $L^{-1}$) of substances potentially inhibiting its degradation: buthylated hydroxyanisole (BHA), tartaric acid (TA), sodium citrate (SC), sodium sulphite (SS), caffeic acid (CA), disodium versenate (EDTA), formic acid (FA), dithiothreitol (DTT), tris-(2-carboxyethyl)phosphine (TCEP).

Ascorbic acid determinations were performed by the method of differential pulse polarography, using a voltammetric trace analyser Metrohm 746 VA (Switzerland) with a 747 VA stand. All analyses were performed in triplicate. The potential of the working electrode was measured in relation to an Ag/AgCl/KCl reference electrode. Qualitative determination of ascorbic acid was performed based on the comparison of the potential of its peak (obtained from sample analysis) with the potential of peaks obtained after adding the standard solution three times to the sample. A quantitative assay was performed by the method of triple standard addition by means of an automatic dosing unit Dosino 700 (Metrohm). The standard addition was selected during each determination in such a way that at the final stage a 3-4-fold higher mass of ascorbic acid was obtained in the measurement vessel. Ascorbic acid content in the sample ($AA_p$) was determined on the basis of formula (2):

$$AA_p = C_{AA} \cdot D \tag{2}$$

where: where: $C_{AA}$ – concentration of ascorbic acid in the extract (mg·$L^{-1}$), D - the dilution coefficient.

10 ml of acetate buffer with pH 4.6 and 0.5 ml of an extract of the analysed sample were added directly to the polarographic vessel. Prior to polarographic measurement, the solution was flushed with argon for 5 minutes to remove oxygen. The following conditions were applied during the polarographic analysis: initial potential -50 mV, final potential 200 mV, voltage step

time 0.6 s, voltage step 6 mV, pulse duration 40 ms, pulse amplitude 50 mV, the rate of potential change 10mV/s, drop size 0.38 $mm^2$.

### 2.2 Experiment 2. Estimation of the effect of various conditions of storage of fruit juices on the concentration of vitamin C

The test material consisted of the following fruit juices: orange juice, multi-fruit juice, and a blackcurrant drink in carton packs (Tetra Pack), from a single production batch. In addition, the study included the use of fruit juices extracted directly from fruits of orange, grapefruit and lemon. The juices and fruits were purchased in supermarkets in Lublin.

Samples of the commercial juices were taken directly from opened carton packs, stored in thermostat-controlled chambers at temperatures of 10°C, 20°C and 30°C. Juices pressed from fruits were placed in dark glass bottles, open and closed, respectively, and stored in thermostat-controlled conditions at temperatures of 10°C, 20°C and 30°C. Samples of the commercial juices and those pressed from fruits were taken for analysis from their packaging kept in the thermostat-controlled chamber after 48, 96 and 168 hours. Quantitative determinations of vitamin C were performed with the polarographic technique in conformance with the conditions described in experiment 1 using an additive, selected on the basis of the results of experiment 2, that inhibits degradation of the vitamin C standard (tartaric acid with concentration of 200 mg $L^{-1}$).

Ethical approval: The conducted research is not related to either human or animal use.

## 3 Results and Discussion

### 3.1 Experiment 1. Optimisation of conditions of vitamin C assay with the polarographic method

Dithiothreitol and tris(2-carboxyethyl)phosphine are known dehydroascorbic acid reducing agents, that minimise changes in the concentration of ascorbic acid in solutions. So far, no papers describing their use in polarographic analysis of vitamin C have been published, so it was necessary to verify whether they do not display their own polarographic peaks within the range of potentials used in the analysis of AA, or cause a deformation of its peak. Polarograms of solutions

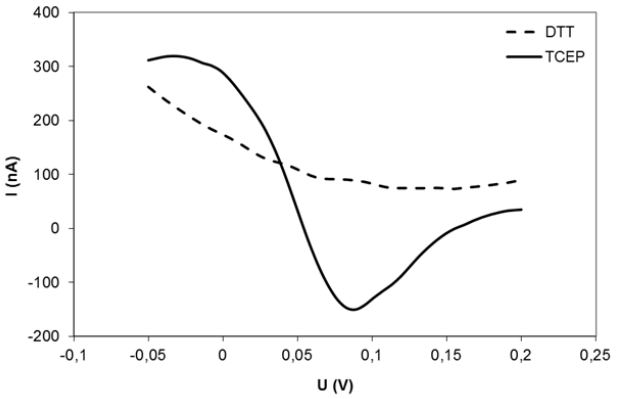


**Figure 1:** Polarograms of dithiothreitol (DTT) and tris(2-carboxyethyl) phosphine (TCEP) solutions.

of dithiothreitol and tris(2-carboxyethyl)phosphine solutions, in the range of potentials from -0.05V to 0.2V, are presented in Figure 1. An elevation of the base line is observed in the polarogram of the dithiothreitol solution and a distinct deformation in the polarogram of tris(2-carboxyethyl)phosphine in the range of potentials of the peak of ascorbic acid. This precludes the use of those substances for the stabilisation of standard solution during polarographic analysis.

The polarographic curves obtained for the remaining analysed substances indicate that each of the proposed compounds can be used as a stabilising additive, potentially inhibiting the degradation of vitamin C. None of these substances gave its own polarographic wave in the range of used potentials and did not cause distortion of the polarographic curve of vitamin C.

Studies have shown that all substances selected in the experiment stabilized the concentration of ascorbic acid after 24 h storage in a comparable extent. Analysis of the effect of the substances (100 mg $L^{-1}$) potentially inhibiting degradation of vitamin C in standard solution with concentration of 100 mg $L^{-1}$ stored at room temperature indicates that after 48 h from the preparation of the solution, the optimal inhibition of vitamin C degradation was obtained in the case of buthylated hydroxyanisole (vitamin C concentration decrease by 2.93%) and tartaric acid (vitamin C concentration decrease by 2.43%). The addition of the other substances resulted in a decrease of vitamin C concentration in standard solution within the range of 4,59% for disodium ethylenediaminetetraacetate to 38.90% for formic acid, at vitamin C concentration decrease by 50.01% in standard solution without any content of any additives (Figure 2).

It was observed that sodium sulphite and tartaric acid were the most effective inhibitors after prolonging

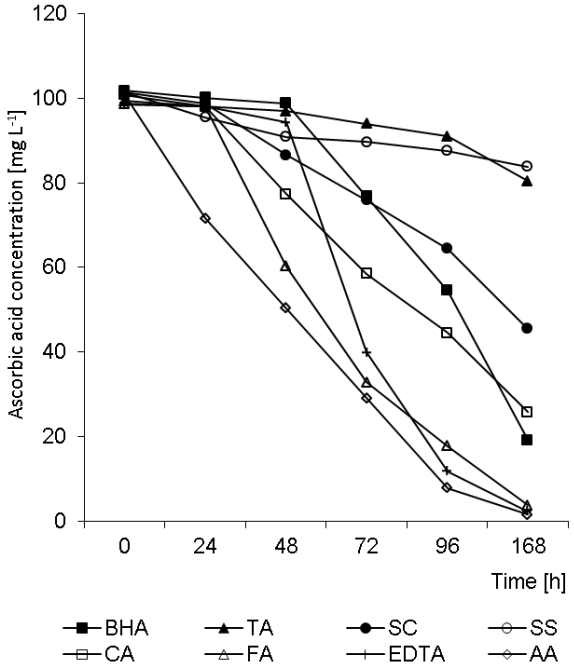


**Figure 2:** Changes in the concentration of ascorbic acid in a standard solution (100 mg $L^{-1}$) with the addition of selected substances: buthylated hydroxyanisole (BHA), tartaric acid (TA), sodium citrate (SC), sodium sulphite (SS), caffeic acid (CA), disodium versenate (EDTA), formic acid (FA).

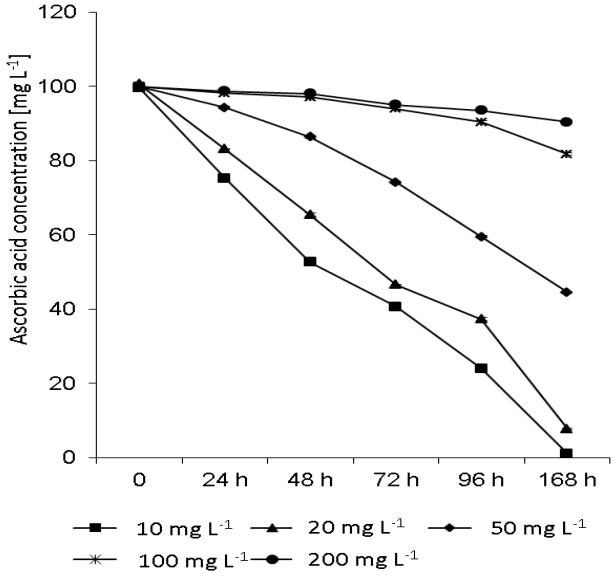


**Figure 3:** Changes of vitamin C concentration in standard solution (100 mg $L^{-1}$) with an addition of tartaric acid (TA) (within the range of concentrations of 10-200 mg $L^{-1}$) stored at room temperature.

the waystorage time. In the case of the addition of sodium sulphite and tartaric acid it was demonstrated that the decrease of vitamin C concentration in the standard solution was lower in the case of tartaric acid after 48h, 72h and 96h of "ageing" the standard of the vitamin. Therefore, tartaric acid was selected as the substance that provided the optimal stabilisation of the vitamin C. Then the effects of adding tartaric acid in various concentrations (10, 20, 50, 100, 200 mg $L^{-1}$) on inhibiting the degradation of vitamin C in the standard solution were compared. As follows from the data in Figure 3, standard solutions of vitamin C with an addition of TA at concentrations of 10, 20 and 50 mg $L^{-1}$ did not produce the desired effect. In the case of standard solutions stabilised with tartaric acid in concentrations of 100 and 200 mg $L^{-1}$, a decrease of the content of vitamin C was also observed, but in comparison with the above concentrations it was small, and after 168 h of "ageing" of the standard solution the content of vitamin C amounted to: for TA with concentration of 100 mg $L^{-1}$– 81.82 mg $L^{-1}$ (decrease of vitamin C concentration by about 18.0%), for TA with concentration of 200 mg $L^{-1}$– 90.45 mg $L^{-1}$ (decrease of vitamin C concentration by about 9.6%). Therefore, on the basis of the above results it was concluded that tartaric acid with a concentration of 200 mg $L^{-1}$ is a good agent for inhibiting the degradation of vitamin C in standard solution, maintaining a high concentration of that vitamin during polarographic measurements.

## 3.2 Experiment 2. Estimation of the effect of various conditions of storage of fruit juices on the concentration of vitamin C

Concentration of vitamin C in samples of orange and multi-fruit juices, as well as blackcurrant drink taken directly after opening of the carton packs was at the level of 301.62 mg $L^{-1}$, 188.08 mg $L^{-1}$and 183.13 mg $L^{-1}$, respectively. Subsequent storage of the juices in the opened packages in an air atmosphere led to a decrease in vitamin C concentration that was related to both the temperature and the time of storage. At a storage temperature of 10°C the content of vitamin C in the orange juice decreased by about 10% (Figure 4), in the multi-fruit juice by about 12% (Figure 5), and in the blackcurrant drink by about 20%, relative to the initial value (Figure 6). At the storage temperature of 20°C and 30°C, the decrease in vitamin C content was as follows: for the orange juice by about 13% and 13%, for the multi-fruit juice by about 21% and 35%, and for the blackcurrant drink by about 36% and 39%. After storage at 20°C for 168 h a decrease of vitamin C content by about 32% was noted in the case of the

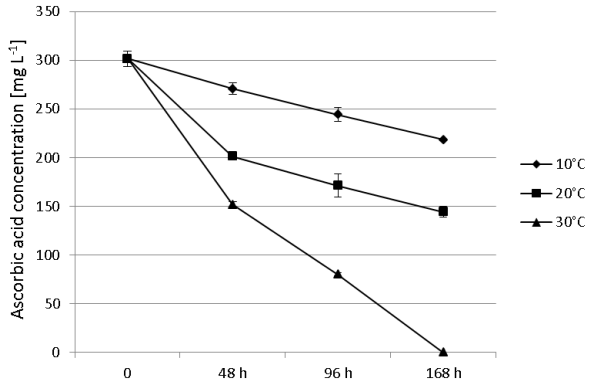


**Figure 4:** Changes in the concentration of ascorbic acid during storage of orange juice.

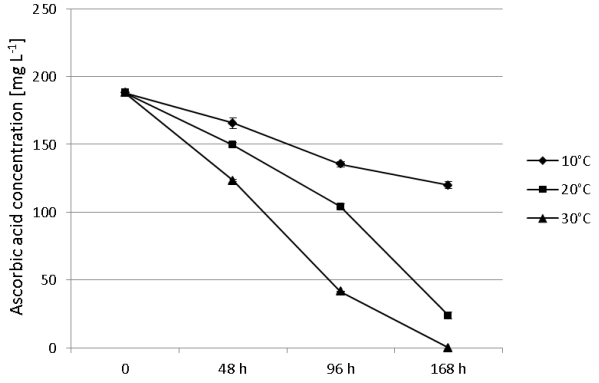


**Figure 5:** Changes in the concentration of ascorbic acid during storage of multi-fruit juice.

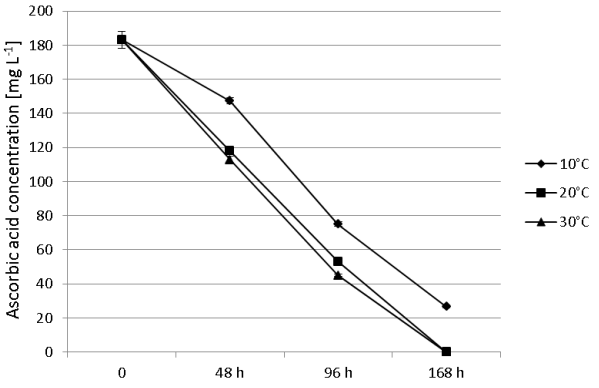


**Figure 6:** Changes in the concentration of ascorbic acid during storage of blackcurrant drink.

orange juice, by about 87% in the multi-fruit juice, while in the case of the blackcurrant drink the concentration of vitamin C decreased to zero. Storage of the juices for 168 h at 30°C resulted in the total degradation of ascorbic acid.

The changes in vitamin C concentration in fresh fruit juices, stored in open and closed containers at 10° C, 20° C and 30° C, were compared. The content of vitamin C in juice pressed from fresh fruits was 513.63 mg $L^{-1}$ in the case of the orange juice, in the case of grapefruit juice – 341.78 mg $L^{-1}$, and in the case of the lemon juice – 398.44 mg $L^{-1}$. Differences in the concentration of vitamin C were also observed after 48 hours of storage of the juices in glass vessels, open and closed, respectively. In the case of the orange juice stored at 30°C in an open glass vessel, vitamin C concentration decreased by about 64%, while in the same juice but stored in a closed vessel the concentration of vitamin C decreased by about 44% (Figure 7). The highest decrease in vitamin C concentration after 48 h of storage was observed in the case of the lemon juice, by about 72% and about 55%, for the open and closed vessels, respectively (Figure 8). The study showed that lemon juice was characterised by the greatest decrease of vitamin C concentration among all of the experimental systems – after 168 h of storage at 10°C the content of vitamin C was lower by about 91% in the open packing, and about 82% in the closed one. The concentration of vitamin C in grapefruit juice after 168 h of storage at 10°C and 30°C decreased, respectively, by about 72% (an open vessel) and about 63% (a closed vessel), and by about 92% (an open vessel) and by about 81% (a closed vessel), which indicates that the grapefruit juice was the most stable in terms of the content of vitamin C (Figure 9).

In the presented study, juices pressed directly from fruits contained higher amount of vitamin C compared to the commercial products, which generally supports the results obtained earlier by Lebiedzińska et al. [29]. Ajibola et al. demonstrated that fruit juices stored at room temperature were characterised by a greater loss of vitamin C in comparison to juices stored in refrigerated conditions. The degradation of vitamin C is related to a number of chemical transformations, a majority of which proceed with the participation of enzymes (present in natural non-pasteurised products) that become active with temperature increase. In addition, unprotected food matrices can also be media for pathogenic microorganisms (Bacillus subtilis, Candida sp.), that cause a decrease in the content of nutrients in food products [30]. Studies conducted by Remini et al. [31], Juarez-Enriquez et al. [32] and by Sapei and Hwa [33] also confirm a greater decrease of the AA content in food products stored at room temperature (19-28°C) as compared to refrigerated

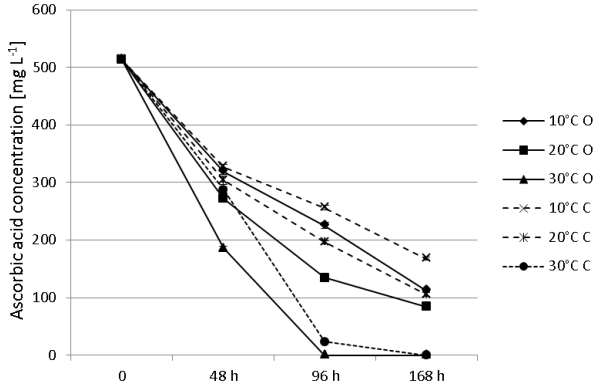


**Figure 7:** Changes in ascorbic acid concentration during storage of freshly squeezed orange juice in open (O) and closed (C) containers.

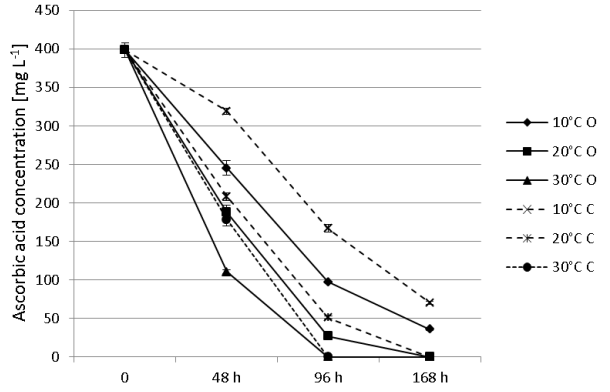


**Figure 8:** Changes in the concentration of ascorbic acid during storage of freshly squeezed lemon juice in open (O) and closed (C) containers.

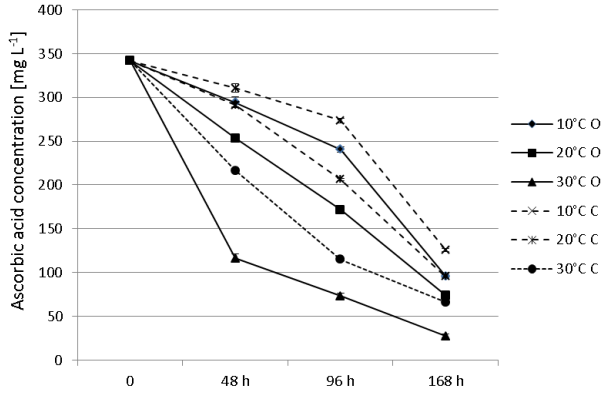


**Figure 9:** Changes in ascorbic acid concentration during storage of freshly squeezed grapefruit juice in open (O) and closed (C) containers.

storage conditions (4-8°C). Also, in the study concerning the effect of the storage conditions of Brussels sprouts vitamin C degradation was observed that intensified with the increase of the storage temperature [34]. Stešková et al. report that the temperature, the form of the vitamin and the matrix are factors affecting the stability of vitamin C in food [35]. Lower storage temperature leads to the retention of a higher content of vitamin C in the product. Earlier reports indicate that the climate, temperature, exposure to light, and storage have an effect on the content of vitamin C in fruits, and thus also in juices extracted from them [36]. The content of vitamin C is also affected by the location of the cultivation (climatic conditions) and by the degree of fruit ripeness. Fruits from tropical regions have a lower content of vitamin C than fruits produced in countries with a cooler climate [37]. Research shows that juice heating causes a decrease in its content of vitamin C [38], which may be related to the activation of processes of degradation of that substance under the effect of temperature. One of the methods of limiting the changes concerning the concentration of vitamin C in fruit juices is their storage in closed packages. Mazurek and Jamroz [39] observed a decrease of vitamin C concentration in commercial orange, grapefruit and apple juices and a blackcurrant drink stored in open packages in refrigerated conditions and at room temperature. A greater decrease of vitamin C content in the analysed samples was observed in the case of samples stored at room temperature, and in addition those authors observed also a significantly greater decrease in vitamin C content in the blackcurrant drink, compared to the remaining analysed samples, which can be attributed to a higher content of water in the ready product relative to fruit juice, and the related lower concentration of active substances naturally present in blackcurrant drink. Kalisz and Kurowska [40] observed a decrease in the content of vitamin C in strawberry semi-concentrates stored in refrigerated conditions in closed glass containers. The cited authors report that vitamin C, in the presence of products of degradation of anthocyanins or though non-enzymatic oxidation, may intensify the degradation of those pigments, which was observed in the experiment conducted by Kalisz and Kurowska in the form of a reduction in the concentration of anthocyanin pigments. The higher concentration of ascorbic acid than that of anthocyanins is related to intensification of degradation of polyphenolic pigments [40–43]. Analysis of literature data and of the results obtained in the presented study indicates that, to retain their nutritive value, fruit juices should be stored at a temperature lower than room temperature [44–47].

# 4 Conclusion

The study demonstrated that the optimum substance inhibiting the degradation of ascorbic acid in standard solution is tartaric acid, and therefore it can be used as a stabilising agent for standard solutions of vitamin C in analytical measurements performed with the use of the voltammetric technique. Analysis of real samples confirmed the utility value of the use of stabilising agents for standard solutions of vitamin C in routine analyses in the area of estimation of vitamin C content in fruit juices. In addition, the analysis of selected samples of fruit juices stored in various temperature conditions confirmed that an elevation of the storage temperature and extension of the time of storage caused a decrease in the content of vitamin C in the analysed samples, while closing the packages caused a limitation of the changes in vitamin C content in the stored juices.

**Conflict of interest:** Authors declare no conflict of interest.

# References

[1] Willie F.U., Ekop A.S., Titrimetric determination of vitamin C in natural and commercial fruit juice samples using a locally extracted dye, Biosci. Biotechnol. Res. Asia, 2007, 4, 429–436.
[2] ISO 6557-2:1984, Fruits, vegetables and derived products -- Determination of ascorbic acid content -- Part 2: Routine methods, 1984.
[3] Kozar S., Bujak A., Eder-Trifunovic J., Kniewald G., Determination of L-ascorbic acid in fresh and processed fruit and vegetables by differential pulse polarography, Fresenius. J. Anal. Chem., 1988, 760–763.
[4] Pournaghi-Azar M.H., Ojani R., A selective catalytic voltammetric determination of vitamin C in pharmaceutical preparations and complex matrices of fresh fruit juices, Talanta, 1997, 44, 297–303.
[5] Ijeri V.S., Jaiswal P. V., Srivastava A.K., Chemically modified electrodes based on macrocyclic compounds for determination of Vitamin C by electrocatalytic oxidation, Anal. Chim. Acta, 2001, 439, 291–297.
[6] Wawrzyniak J., Ryniecki A., Zembrzuski W., Application of voltammetry to determine vitamin C in apple juices, Acta Sci. Pol. Technol. Aliment., 2005, 4, 5–16.
[7] Esteve M.J., Farre R., Frigola A., Lopez J.C., Romera J.M., Ramirez M., *et al.*, Comparison of voltammetric and high performance liquid chromatographic methods for ascorbic acid determination in infant formulas, Food Chem., 1995, 52, 99–102.
[8] Pandey N.K., Spectrophotometric and Titrimetric Determinations of Ascorbic Acid, Anal. Chem., 1982, 54, 793–796.
[9] Ensafi A.A., Rezaei B., Flow Injection Analysis Determination of Ascorbic Acid with Spectrofluorimetric Detection, Anal. Lett., 1998, 31, 333–342.
[10] Wu X., Diao Y., Sun C., Yang J., Wang Y., Sun S., Fluorimetric determination of ascorbic acid with o-phenylenediamine, Talanta, 2003, 59, 95–99.
[11] Pereira A.V., Fatibello-Filho O., Flow injection spectrophotometric determination of L-ascorbic acid in pharmaceutical formulations with on-line solid-phase reactor containing copper (II) phosphate, In: Analytica Chimica Acta, 1998, 55–62.
[12] Tabata M., Morita H., Spectrophotometric determination of a nanomolar amount of ascorbic acid using its catalytic effect on copper(II) porphyrin formation, Talanta, 1997, 44, 151–157.
[13] Themelis D.G., Tzanavaras P.D., Kika F.S., On-line dilution flow injection manifold for the selective spectrophotometric determination of ascorbic acid based on the Fe(II)-2,2'-dipyridyl-2-pyridylhydrazone complex formation., Talanta, 2001, 55, 127–134.
[14] Molina-Diaz A., Ortega-Carmona I., Pascual-Reguera M.I., Indirect spectrophotometric determination of ascorbic acid with ferrozine by flow-injection analysis., Talanta, 1998, 47, 531–536.
[15] Jaselskis B., Nelapaty J., Spectrophotometric Determination of Micro Amounts of Ascorbic Acid in Citrus Fruits, Anal. Chem., 1972, 44, 379–381.
[16] Baker W.L., Lowe T., Sensitive ascorbic acid assay for the analysis of pharmaceutical products and fruit juices, Analyst, 1985, 110, 1189–1191.
[17] Ferreira S.L.C., Bandeira M.L.S.F., Lemos V. a., dos Santos H.C., Costa a. C.S., de Jesus D.S., Sensitive spectrophotometric determination of ascorbic acid in fruit juices and pharmaceutical formulations using 2-(5-bromo-2-pyridylazo)-5-diethylaminophenol (Br-PADAP), Fresenius. J. Anal. Chem., 1997, 357, 1174–1178.
[18] Arya S.., Mahajan M., Jain P., Spectrophotometric determination of vitamin C with iron(II)-4-(2-pyridylazo) resorcinol complex, Anal. Chim. Acta, 2001, 427, 245–251.
[19] Gennaro M.C., Bertolo P.L., L-Ascorbic Acid Determination in Fruits and Medical Formulations by Ion Interaction Reagent Reverse Phase HPLC Technique, J. Liq. Chromatogr., 1990, 13, 1419–1434.
[20] Romero Rodriguez M.A., Vazquez Oderiz M.L., Lopez Hernandez J., Simal Lozano J., Determination of vitamin C and organic acids in various fruits by HPLC., J. Chromatogr. Sci., 1992, 30, 433–437.
[21] Kall M.A., Andersen C., Improved method for simultaneous determination of ascorbic acid and dehydroascorbic acid, isoascorbic acid and dehydroisoascorbic acid in food and biological samples., J. Chromatogr. B. Biomed. Sci. Appl., 1999, 730, 101–111.
[22] de Quiros A.R.B., Lopez-Hernandez J., Simal-Lozano J., Determination of vitamin C in sea urchin: Comparison of two HPLC methods, Chromatographia, 2001, 53, S246–S249.
[23] Lykkesfeldt J., Determination of ascorbic acid and dehydroascorbic acid in biological samples by high-performance liquid chromatography using subtraction methods: reliable reduction with tris[2-carboxyethyl]phosphine hydrochloride., Anal. Biochem., 2000, 282, 89–93.

[24] Gensler M., Rossmann A., Schmidt H.-L., Detection of Added L-Ascorbic Acid in Fruit Juices by Isotope Ratio Mass Spectrometry, J. Agric. Food Chem., 1995, 43, 2662–2666.
[25] del Carmen Yebra-Biurrun M., Cespón-Romero R.M., Bermejo-Barrera P., Indirect flow-injection determination of ascorbic acid by flame atomic absorption spectrometry, Microchim. Acta, 1997, 126, 53–58.
[26] Fatibello-Filho O., Vieira I. da C., L-ascorbic acid determination in pharmaceutical formulations using a biosensor based on carbon paste modified with crude extract of zucchini (Cucurbita pepo) , J. Brazilian Chem. Soc. , 2000, 11, 412–418.
[27] Mazurek A., Jamroz J., Precision of dehydroascorbic acid quantitation with the use of the subtraction method - Validation of HPLC-DAD method for determination of total vitamin C in food, Food Chem., 2015, 173, 543–550.
[28] Mazurek A., Włodarczyk-Stasiak M., Jamroz J., Validation of differential pulse polarographic method of ascorbic acid assay in food – comparison with the chromatographic reference method, Acta Aliment. An Int. J. Food Sci., 2018, 4.
[29] Lebiedzińska A., Czaja J., Petrykowska K., Szefer P., Evaluation of vitamin C content in fruit juices and nectars, Bromatol. i Chem. Toksykol., 2012, 45, 390–396.
[30] Ajibola V.O., Babatunde O. a, Suleiman S., The effect of storage method on the vitamin C content in some tropical fruit juices, Trends Appl. Sci. Res., 2009, 79–84.
[31] Remini H., Mertz C., Belbahi A., Achir N., Dornier M., Madani K., Degradation kinetic modelling of ascorbic acid and colour intensity in pasteurised blood orange juice during storage, Food Chem., 2015, 173, 665–673.
[32] Juarez-Enriquez E., Salmeron-Ochoa I., Gutierrez-Mendez N., Ramaswamy H.S., Ortega-Rivas E., Shelf life studies on apple juice pasteurised by ultrahigh hydrostatic pressure, LWT - Food Sci. Technol., 2015, 62, 915–919.
[33] Sapei L., Hwa L., Study on the Kinetics of Vitamin C Degradation in Fresh Strawberry Juices, Procedia Chem., 2014, 9, 62–68.
[34] Sikorska-Zimny K., Badełek E., Changes in vitamin c content during storage of two cultivars of brussels sprout Ajax F1 and Louis F1, Zesz. Nauk. Inst. Ogrod., 2014, 22, 121–127.
[35] Stešková A., Morochovičová M., Lešková E., Vitamin C degradation during storage of fortified foods, J. Food Nutr. Res., 2006, 45, 55–61.
[36] Gil M.I., Aguayo E., Kader A.A., Quality changes and nutrient retention in fresh-cut versus whole fruits during storage, J. Agric. Food Chem., 2006, 54, 4284–4296.
[37] Padayatty S.J., Katz A., Wang Y., Eck P., Kwon O., Lee J.-H., *et al.*, Vitamin C as an Antioxidant: Evaluation of Its Role in Disease Prevention, J. Am. Coll. Nutr., 2003, 22, 18–35.
[38] El-ishaq A., Obirinakem S., Effect of Temperature and Storage on Vitamin C Content in Fruits Juice, Int. J. Chem. Biomol. Sci., 2015, 1, 17–21.
[39] Mazurek A., Jamroz J., Vitamin C stability in fruit juices and black currant nectar during storage, Acta Agrophysica, 2010, 16, 93–100.
[40] Kalisz S., Kurowska M., Changes in the Content of Polyphenols and Ascorbic Acid in Strawberry Juices and Semiconcentrates During Their StorageNo Title, Żywność Nauk. - Technol. - Jakość, 2005, 2, 62–71.
[41] Garzon G.A., Wrolstad R.E., Comparison of the Stability of Pelargonidin-based Anthocyanins in Strawberry Juice and Concentrate, J. Food Sci., 2002, 67, 1288–1299.
[42] Kalisz B., Kalisz S., Oszmiański J., Influence Of Skullcap Flavones On Antioxidant Activity And Anthocyanins And Colour Stabilization Of Strawberry's Canned Fruits And Jams, ACTA Acta Sci. Pol., Technol. Aliment, 2004, 3, 73–83.
[43] Kalt W., Forney C.F., Martin A., Prior R.L., Antioxidant capacity, vitamin C, phenolics, and anthocyanins after fresh storage of small fruits., J. Agric. Food Chem., 1999, 47, 4638–44.
[44] Njoku P.C., Ayuk A.A., Okoye C.V., Temperature Effects on Vitamin C Content in Citrus Fruits, Pakistan J. Nutr., 2011, 10, 1168–1169.
[45] Santini C., Pellei M., Papini G., Morresi B., Galassi R., Ricci S., *et al.*, In vitro antitumour activity of water soluble Cu(I), Ag(I) and Au(I) complexes supported by hydrophilic alkyl phosphine ligands, J. Inorg. Biochem., 2011, 105, 232–240.
[46] Ibrahim M., Effect of Different Storage Condition on pH and Vitamin C Content in Some Selected Fruit Juices (Pineapple, Pawpaw and Watermelon), Int. J. Biochem. Res. Rev., 2016, 11, 1–5.
[47] Zhang J., Han H., Xia J., Gao M., Degradation Kinetics of Vitamin C in Orange and Orange Juice during Storage, Adv. J. Food Sci. Technol., 2016, 12, 555–561.