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# CHANGES OF DEHYDROASCORBIC ACID CONTENT IN RELATION TO TOTAL CONTENT OF VITAMIN C IN SELECTED FRUITS AND VEGETABLES

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**Abstract.** Vitamin C performs many functions in the human body and is very important for its proper functioning. The main vitamin C sources in the human diet are fruits and vegetables. Changes of total content of vitamin C ($T_c$), L-ascorbic acid (AA) and dehydro-L-ascorbic acid (DHA) in selected fruits and vegetables during storage at 20°C until spoilage were determined. A decrease of $T_c$ and AA contents and an increase of DHA concentration were noted. Products of high acidity such as lemon and tomato were characterized by the highest retention of vitamin C. An increase of the DHA to $T_c$ ratio (%) was observed. On the first day of storage the DHA/$T_c$ ratio was higher than 10% in broccoli, cucumber and banana, while on the last day – it amounted over 40% in banana and cucumber, between 20 and 40% in parsley leaves and broccoli, and below 20% in tomato, cauliflower and lemon. In order to obtain correct results of vitamin C determination in fruits and vegetables it is necessary to take DHA content into consideration.

**Key words:** L-ascorbic acid, dehydro-L-ascorbic acid, differential method, TCEP, HPLC

## INTRODUCTION

Vitamin C contains L-ascorbic acid (AA) as well as dehydro-L-ascorbic acid (DHA). DHA exhibits properties of vitamin C because is easily reduced in an organism to AA [Nyyssonen et al. 2000]. The best known role of this acid in the human body is synthesis of collagen which constitutes 30–40% of all proteins [Catani et al. 2005]. Moreover, vitamin C regulates mechanisms of antioxidation which are related to the protection of cells and body fluid against oxidative stress [Chan 1993]. Multidirectional metabolic activity of this vitamin contributes to the enhancement of the immune system response against the selected bacterial or viral infections, and what is more, it prevents cancer formation [Block 1992] and improves the assimilation of iron [Hunt et al. 1990].

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Despite the availability of numerous methods there is a lack of internationally accepted method for determination of vitamin C. Table 1 presents the normalized methods, published in various sources, which are officially recommended for this purpose. Considerable part of them enables determination of only AA. For instance, the only norm of vitamin C analysis, which is currently valid in Poland, (PN-A-04019:1998), does not enable determination of total content of vitamin C in a sample. Furthermore, a serious drawback of this method is that reducers present in a sample reduce 2,6-dichlorophenolindophenol and overestimate results of the analysis [Arya et al. 1998]. Use of the methods from the Table 1 is caused by difficulty in a direct determining of DHA [Nováková et al. 2008] and general belief that content of this acid, especially in the fresh products, is much lower than in the case of AA. Not taking DHA into consideration during the determination of vitamin C leads to the negligible errors [Wills et al. 1984, Rapisarda and Pannuzzo 2008]. In analysis of this acid the most commonly used step is reduction to AA. To determine the total content of vitamin C it is necessary to add a reducer to a sample and subsequently measure content of AA as a sum of two its concentrations: initial and after derivatization. Tris(2-carboxyethyl)phosphine (TCEP) exhibiting high reactivity and stability in acidic solutions is currently considered to be the best reducer [Lykkesfeldt 2000, Wechtersbach and Cigić 2007, Sato et al. 2010]. In order to determine the content of DHA it is necessary to analyze additionally the content of AA before derivatization and subtract the obtained concentration from total content of vitamin C (differential method) [Lykkesfeldt 2000].

Table 1. Officially recommended methods for determination of vitamin C
Tabela 1. Oficjalnie zalecane metody oznaczania witaminy C

| Source<br>Źródło | Use<br>Stosowanie | Method<br>Metoda |
|---|---|---|
| Polish Committee for Standardization, 1998 | PN-A-04019:1998 Food products. Determination of vitamin C content | 2,6-dichloroindophenol titrimetric and spectrophotometric method |
| AOAC Official Methods of Analysis, 18th ed., 2005 | L-ascorbic acid in vitamin preparations and juices, 5.1.14, 967.21 | 2,6-Dichloroindophenol titrimetric method |
| | L-ascorbic acid in vitamin preparations, 45.1.15, 967.22 | fluorometric determination with o-phenylenediamine |
| | L-ascorbic acid in food 45.1.16, 984.26 | fluorometric determination with o-phenylenediamine |
| | L-ascorbic acid in infant milk 50.1.09, 985.33 | 2,6-Dichloroindophenol titrimetric method |
| American Association of Cereal Chemists, Approved Methods, vol. 2, 2000. | L-ascorbic acid in cereal products AACC86-10 | spectrophotometric method |
| U.S. Pharmacopeia National Formulary, 2006, USP 29/NF 24 Nutritional Supplements Official Monographs | L-ascorbic acid in tablets and vitamin solutions | 2,6-Dichloroindophenol titrimetric method or fluorometric determination with o-phenylenediamine |
| | L-ascorbic acid in solutions for injection, tablets | iodine or 2,6-dichloroindophenol titrimetric method |
| British Pharmacopeia, 2007 | L-ascorbic acid in solutions for injection, tablets | iodine or cerium sulphate titrimetric method |
| Food Chemical Codex, 5th ed., 2004 | L-ascorbic acid | iodine titrimetric method |

The content of vitamin C varies among species and cultivars [Davey et al. 2000]. Its concentration is affected by many factors such as: genetic variability, maturity, climate, methods of cultivation, harvesting and storage [Lee and Kader 2000]. Processing methods and storage conditions of raw materials and products cause a gradual decrease of vitamin C concentration. Dynamic of the changes in vitamin C content in fruits and vegetables is a reason for determination of its both forms.

The aim of the study was analysis of changes in content of AA and DHA and total content of vitamin C in selected fruits and vegetables during storage.

## MATERIALS AND METHODS

Different types of fruits (banana, lemon) and vegetables (broccoli, cauliflower, cucumber, tomato, parsley leaves) were used in the study.

The material was stored in darkness at 20°C and constant humidity. Time intervals in which the determinations were made were selected individually to the product taking its life span into consideration. The first sample was analyzed on the day of purchase and the last one (from the same lot) just before the date since which the product has been unfit for consumption.

**Reagents.** The following reagents were used: L-ascorbic acid and tris(2-carboxyethyl)phosphine (Sigma-Aldrich, USA), metaphosphoric acid (Merck, Germany), orthophosphoric acids (POCH, Poland). All reagents were of analytical grade purity.

**Sample preparation for analysis.** Two analytical samples were prepared from each product by blending edible parts taken from at least three pieces. Each sample was analyzed two times.

The sample was weighted exact to 1 mg ($m_p$). Then 2% solution of metaphosphoric acid was added and the sample was weighted again ($m_{p+k}$). The dilution coefficient (F) was calculated and subsequently used to quantify the analyte in the sample.

$$F = \frac{m_{p+k}}{m_p} \tag{1}$$

The sample was homogenized for 1 min using Ultra Turrax T18 Basic, (Ika, Germany) homogenizer and then centrifuged for 5 min at 12 000×g. The supernatant was filtered through a 0.45 μm syringe filter. The obtained extract was divided into two parts; one part was used for determination of L-ascorbic acid ($AA_p$) and the other for determination of the total content of vitamin C ($T_c$) after reduction of dehydro-L--ascorbic acid. 0.2 mL of 100 mM solution of tris(2-carboxyethyl)phosphine was added to 2 mL of the extract and then the sample was continuously shaken for 30 min in the dark.

**HPLC analysis.** A reversed phase HPLC technique was used for determination of total content of vitamin C, AA and DHA. Analyses were performed with the use of Varian HPLC system equipped with a diode-array detector (DAD, model 335), an isocratic pomp (model 210), a dosing valve 7725i (Rheodyne, USA) and a column thermo-

stat. Galaxie Chromatography Data System, version 1.9.302 (Varian, USA) was used for process control and data acquisition. Separations were made using a column Gemini (150 × 4.6 mm, 3 μm, C18) connected with a pre-column Gemini (C18 4 × 3 mm, Phenomenex, USA). The mobile phase was a solution of orthophosphoric acid at pH 2.8 pumped at a flow of 0.6 $mL \cdot min^{-1}$. The injection volume was 20 μL. Chromatograms (fig. 1) were recorded at 244 nm and column temperature of 30°C. AA identification was performed on the basis of retention time and UV absorption spectrum of the standard sample. The concentration of AA was calculated from equation of the calibration curve plotted for the standard solutions. The calibration graph was linear in the range from 2 to 80 $\mu g \cdot mL^{-1}$ and obeyed the equation $y = 1.71x - 0.15$, where $y$ and $x$ are the peak area (mAU·min) and AA concentration ($\mu g \cdot mL^{-1}$), respectively; and the linear correlation coefficient r = 0.999. The limit of detection was calculated on the basis of signal-to-noise ratio. The detection limit obtained was 0.21 $\mu g \cdot mL^{-1}$. The limit of quantification corresponds to the minimum quantity with which it is possible to quantify without uncertainty and the result obtained was 0.69 $\mu g \cdot mL^{-1}$. Accuracy of the method used was stated by conducting analysis of the certified reference material (BCR 431, IRMM, Belgium) sample and comparison of the obtained average (500 ± 10.2 $mg \cdot 100\ g^{-1}$) with the certified value (483 ± 9.8 $mg \cdot 100\ g^{-1}$).

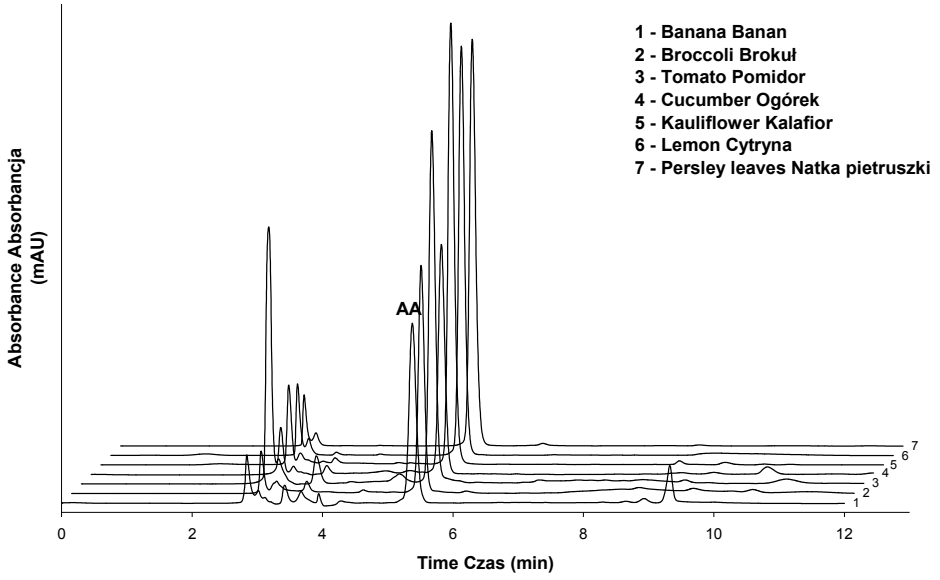


Fig. 1. The exemplary chromatograms of vitamin C extracts from the analyzed products
Rys. 1. Przykładowe chromatogramy ekstraktu witaminy C z analizowanych produktów

In the first stage the content of AA was analyzed in the sample (formula 2) and then DHA was reduced quantitatively using TCEP and total content of vitamin C was determined (formula 3). The concentration of DHA ($DHA_p$) was calculated by subtracting the initial concentration of AA from total concentration of vitamin C (differential

method). Calculation of analytes content were performed according to Gökmen et al. (2000) methodology with modification concerning the choice of reducer and manner of its addition to the extract of vitamin C.

$$AA_p = \frac{C_{AA} \cdot F}{10} \tag{2}$$

where: $C_{AA}$ – AA content in extract ($\mu g \cdot mL^{-1}$)

$$T_C = \frac{C_{TA} \cdot 1{,}1 \cdot F}{10} \tag{3}$$

where: $C_{TA}$ – AA content after reduction ($\mu g \cdot mL^{-1}$)

The obtained results were studied by analysis of variance and the significance of differences was determined using Tukey's test at 0.05 probability level.

## RESULTS AND DISCUSSION

Table 2 presents changes in content of AA, DHA and total content of vitamin C in selected fruits and vegetables stored at 20°C until the moment of not being suitable for consumption. On the first day of the study the highest amount of vitamin C was determined for parsley leaves (222.48 mg·100 $g^{-1}$), and the lowest – for cucumber (13.28 mg·100 $g^{-1}$). During storage a decrease in total content of vitamin C and AA was observed in the case of all products subjected to analysis. Maximum decrease in total content of vitamin C, equal 38.1% in comparison to the initial value, was obtained for cucumber and minimal – for tomato (15.6%). Lemon (82.9%) and tomato (84.4%) were characterized by the highest retention of vitamin C among all products. It is related to their high acidity which stabilizes both AA and DHA. Similar relationship between stability of vitamin C and acidity of citrus fruits was reported by Nagy [1980]. Cauliflower and broccoli belonging to *Cruciferae* family contain high total content of sulfur and glutathione. Albrecht et al. [1990] reported relationship between high retention of vitamin C during storage and total amount of sulfur in broccoli. Moreover, glutathione may reduce DHA to AA which has stabilizing influence on changes of vitamin C content. In the present studies, broccoli showed 75.4% and cauliflower 69.7% of the initial content of vitamin C after 6 days of storage.

Many reports on vitamin C content in fruits and vegetables have been published until now but they have not considered dehydro-L-ascorbic acid [Zhang 2003, Zhang and Hamauzu 2004, Frenich and Torres 2005, Koh and Wimalasiri 2009, Valente and Albuquerque 2011]. All products analyzed on the day of purchase contained DHA in concentration between 1.28 (tomato) and 12.36 (parsley leaves) mg $(100\ g)^{-1}$. An increase of its content was observed during storage but there was no uniform tendency for each of the products. The highest increase of DHA concentration (2.4 times) was noted for lemon and the lowest (0.38 times) – for broccoli.

Table 2. Changes in content (mg·100 $g^{-1}$) of L-ascorbic acid, dehydro-L-ascorbic acid and total vitamin C, and ratio (%) of dehydro-L-ascorbic acid to total vitamin C content in fruits and vegetables during storage at 20°C

Tabela 2. Zmiany zawartości (mg·100 $g^{-1}$) kwasu L-askorbinowego, dehydro-L-askorbinowego, całkowitej zawartości witaminy C i stosunku (%) zawartości kwasu dehydro-L-askorbinowego do całkowitej zawartości witaminy C w owocach i warzywach podczas przechowywania w 20°C

| | Time (days) Czas (dni) | $AA_p$ M ± S.D. (mg/100 g) | $T_c$ M ± S.D. (mg/100 g) | $DHA_p$ M ± S.D. (mg/100 g) | Ratio Udział $DHA_p : T_c$ (%) |
|---|---|---|---|---|---|
| Persley leaves Natka pietruszki | 0 | 210.12 ± 7.17 a | 222.48 ± 8.33 a | 12.36 ± 2.66 a | 5.6 |
| | 1 | 183.72 ± 7.20 b | 203.30 ± 11.06 ad | 19.58 ± 5.71 a | 9.6 |
| | 2 | 156.64 ± 7.21 c | 186.37 ± 6.90 bd | 29.73 ± 3.99 b | 16.0 |
| | 3 | 111.44 ± 7.76 d | 144.89 ± 11.36 c | 33.45 ± 3.61 c | 23.1 |
| Tomato Pomidor | 0 | 17.64 ± 1.36 a | 18.92 ± 1.45 a | 1.28 ± 0.26 a | 6.8 |
| | 2 | 18.22 ± 0.84 a | 19.70 ± 1.07 a | 1.48 ± 0.45 a | 7.5 |
| | 4 | 15.73 ± 1.25 a | 18.02 ± 0.77 ac | 2.29 ± 0.93 ac | 12.7 |
| | 6 | 12.99 ± 0.96 b | 15.96 ± 0.69 bc | 2.97 ± 0.63 bc | 18.6 |
| Kauliflower Kalafior | 0 | 86.57 ± 3.47 a | 94.98 ± 4.76 a | 8.41 ± 1.46 a | 8.9 |
| | 2 | 67.58 ± 3.48 b | 74.55 ± 4.94 be | 6.97 ± 1.58 a | 9.3 |
| | 4 | 58.77 ± 2.78 ce | 68.30 ± 3.69 ce | 9.53 ± 1.47 ac | 13.9 |
| | 6 | 53.83 ± 2.00 de | 66.22 ± 3.11 de | 12.39 ± 1.88 bc | 18.7 |
| Broccoli Brokuł | 0 | 68.65 ± 3.61 a | 80.80 ± 4.43 a | 12.15 ± 1.45 a | 15.0 |
| | 2 | 59.15 ± 4.32 b | 72.99 ± 5.89 ad | 13.84 ± 1.73 ac | 19.0 |
| | 4 | 51.32 ± 2.23 c | 67.28 ± 4.33 bde | 15.96 ± 2.27 ad | 23.7 |
| | 6 | 44.21 ± 2.72 d | 60.95 ± 4.44 ce | 16.74 ± 1.95 bcd | 27.5 |
| Cucumber Ogórek | 0 | 11.64 ± 0.49 a | 13.28 ± 0.61 a | 1.64 ± 0.52 a | 12.4 |
| | 4 | 9.24 ± 0.70 bf | 10.93 ± 1.24 be | 1.69 ± 0.59 a | 15.4 |
| | 8 | 9.47 ± 0.80 cf | 11.82 ± 1.20 ae | 2.35 ± 0.41 a | 19.9 |
| | 12 | 7.56 ± 0.50 d | 10.97 ± 0.47 ce | 3.41 ± 0.65 bd | 31.1 |
| | 16 | 4.73 ± 0.28 e | 8.22 ± 0.65 d | 3.49 ± 0.55 cd | 42.5 |
| Banana Banan | 0 | 13.18 ± 0.60 a | 15.42 ± 0.82 a | 2.24 ± 0.54 a | 14.5 |
| | 4 | 10.49 ± 0.65 b | 13.46 ± 0.72 bg | 2.97 ± 0.56 af | 22.0 |
| | 8 | 8.19 ± 0.59 cg | 11.77 ± 0.86 cgh | 3.58 ± 0.42 bfg | 30.4 |
| | 12 | 7.59 ± 0.42 dgh | 11.80 ± 0.78 dgi | 4.21 ± 0.75 cfg | 35.7 |
| | 16 | 8.08 ± 0.37 eg | 12.39 ± 0.74 egi | 4.31 ± 0.58 dg | 34.8 |
| | 20 | 5.58 ± 0.47 fh | 10.23 ± 0.83 fhi | 4.65 ± 0.59 eg | 45.5 |
| Lemon Cytryna | 0 | 71.17 ± 5.13 a | 73.75 ± 5.62 a | 2.58 ± 0.58 a | 3.5 |
| | 1 | 66.54 ± 6.47 ae | 70.16 ± 6.01 ad | 3.62 ± 0.60 ab | 5.2 |
| | 2 | 64.71 ± 2.88 af | 68.10 ± 2.80 ad | 3.39 ± 0.82 ab | 5.0 |
| | 3 | 60.28 ± 3.41 befg | 65.44 ± 3.69 ad | 5.16 ± 0.76 be | 7.9 |
| | 4 | 56.75 ± 3.57 cefg | 63.32 ± 4.30 bd | 6.57 ± 0.88 ce | 10.4 |
| | 5 | 52.39 ± 3.58 dg | 61.16 ± 5.66 cd | 8.77 ± 2.19 d | 14.3 |

Various letters indicate statistically significant differences ($p < 0.05$)
Różne litery wskazują na istotne statystycznie różnice na poziomie istotności < 0,05
M ± S.D – mean ± standard deviation
M ± S.D – średnia ± odchylenie standardowe

Wills et al. [1984] carried out similar studies but did not detect DHA in banana during the whole period of storage, while the present studies showed that its content increased from 2.24 mg·100 $g^{-1}$ on the first day to 4.65 mg·100 $g^{-1}$ on the last day of storage. Moreover, most of the results of DHA determination obtained in the present study were higher than those reported by the authors mentioned above. Detection of DHA at 214 nm by Wills et al. [1984] is the reason of these discrepancies. DHA in solution shows high absorption of ultraviolet light at 185 nm but absorbs poorly in the region between 210 and 227 nm which limits its analysis because of spectral interferences of sample matrix and mobile phase components [Ball 2006]. Moreover, Gökmen et al. [2000] revealed that the relative sensitivity of DHA determination amounts to 0.0114 at 254 nm and 0.0305 at 210 nm on the assumption that it equals 1 for AA. Differential method used in this study shows higher selectivity and sensitivity towards DHA than use of spectrophotometric detection at low range of ultraviolet [Novakova et al. 2008].

Lowering of vitamin C content is caused by AA oxidation to DHA which is hydrolyzed very easily to 2,3-diketogulonic acid (not having vitamin C activity), but the observed increase of DHA content was not proportional. AA concentration in parsley leaves on the last day of storage decreased by 98.68 mg·100 $g^{-1}$ and simultaneously DHA content increased by only 21.09 mg·100 $g^{-1}$. A similar relationship was observed in the case of the remaining products. It may be related to the lower stability of DHA in comparison to AA.

Considerable decrease of AA content with simultaneous increase of DHA content causes higher ratio of the latter in total concentration of vitamin C during storage (tab. 2). The highest ratio of DHA was observed in the case of banana after 20 days of storage (44.5%) and the lowest – for the products of high acidity: lemon (14.3%) and tomato (18.6%). The use of the method which allows to determine only AA cause lowering the results, especially for long-term stored products.

## CONCLUSIONS

1. During storage of selected fruits and vegetables:

– total content of vitamin C as well as L-ascorbic acid concentration decline and content of dehydro-L-ascorbic acid increases

– an increase of DHA content is not proportional to a decrease of AA content

– the DHA ratio in total content of vitamin C rises.

2. DHA content has to be taken into consideration when selecting a method for vitamin C determination.

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## ZMIANY ZAWARTOŚCI KWASU DEHYDRO-L-ASKORBINOWEGO W STOSUNKU DO CAŁKOWITEJ ZAWARTOŚCI WITAMINY C W WYBRANYCH OWOCACH I WARZYWACH

**Streszczenie.** Witamina C pełni w organizmie człowieka wiele funkcji i ma istotne znaczenie dla jego prawidłowego funkcjonowania, a jej głównym źródłem w diecie są owoce i warzywa. Określono zmiany całkowitej zawartości witaminy C, kwasu L-askorbinowego i kwasu dehydro-L-askorbinowego w wybranych owocach (banan, cytryna) i warzywach (brokuł, kalafior, ogórek, pomidor, natka pietruszki) podczas przechowywania w 20$^{o}$C aż do nieprzydatności do spożycia. Wykazano obniżanie całkowitej zawartość witaminy C i kwasu L-askorbinowego oraz podwyższanie zawartości kwasu dehydro-L--askorbinowego. Największą retencją witaminy C cechują się produkty o wysokiej kwasowości, takie jak cytryna i pomidor. Wykazano wzrost udziału kwasu dehydro-L--askorbinowego w całkowitej zawartości witaminy C. W pierwszym dniu przechowywania udział ten był większy od 10% tylko w brokule, ogórku i bananie, zaś w ostatnim był większy od 40% w bananie i ogórku, pomiędzy 20 a 40% w natce pietruszki i brokule, mniejszy od 20% w pomidorze, kalafiorze, i cytrynie. W celu otrzymania poprawnych wyników oznaczania zawartości witaminy C w owocach i warzywach zastosowane metody muszą uwzględniać zawartość kwasu dehydro-L-askorbinowego.

**Słowa kluczowe:** kwas L-askorbinowy, kwas dehydro-L-askorbinowy, metoda różnicowa, owoce i warzywa

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