UDC 615.072:577.164.2:535.33:614.31:613:292

DOI: 10.15587/2519-4852.2024.313637

THE CONTENT AND STABILITY OF ASCORBIC ACID IN COMMERCIAL FOOD SUPPLEMENTS

Ain Raal, Andres Meos, Agne Vutt, Herman Kirsimäe, Tetiana Ilina, Alla Kovalyova, Oleh Koshovyi

Ascorbic acid is a well-known compound found in many vegetables and fruits. In medical practice, it is used in the composition of many drugs and food supplements. Taking into account the different quality of production of food supplements on the one hand and the lability of ascorbic acid on the other, it is important to analyze the real content of ascorbic acid in food supplements containing it.

The aim. The aim of the study was to compare the content of ascorbic acid in food supplements from different manufacturers to establish the stability of ascorbic acid over time and the relationship between the content of ascorbic acid and the shelf life of food supplements, as well as to investigate the relationship between the stability of ascorbic acid and the nature of its origin.

Materials and methods. 13 preparations of food supplements containing ascobrinic acid, which were sold in Estonian pharmacies or online environments at the time of the work, were analysed. Quantitative analysis of ascorbic acid was performed using the HPLC method.

Research results. It was found that in 11 out of 13 food supplements, the ascorbic acid content ranged from 78.5 % to 115.2 % of the nominal. For two samples, the ascorbic acid content was very low compared to that provided by the manufacturer (54.6–56.3 %). The content of ascorbic acid in three preparations does not meet the standards set by the European Commission (from -20 % to +50 %). After 16 months of storage, a statistically significant change in the content of ascorbic acid occurred only in four samples, in which its content decreased (p<0.05) by about 7–21 mg. The ascorbic acid content in the other samples did not change for 16 months after storage of the opened packages. Statistical analysis of the data showed that the relative amount of ascorbic acid present in the preparation relative to the nominal was related to the origin of the substance, whereas the content of ascorbic acid of natural origin in preparations relative to the nominal was significantly lower than in preparations with a synthetic active substance.

Conclusions. The actual content of ascorbic acid in most solid preparations roughly corresponds to that stated on the label, usually slightly below it. In solid supplements containing ascorbic acid, its amount practically does not change after 16 months of shelf life. In preparations containing ascorbic acid of natural origin, it is found relatively less than in preparations containing synthetic ascorbic acid

Keywords: ascorbic acid, food supplements, shelf life, Estonia

How to cite:

Raal, A., Meos, A., Vutt, A., Kirsimäe, H., Ilina, T., Kovalyova, A., Koshovyi, O. (2024). The content and stability of ascorbic acid in commercial food supplements. ScienceRise: Pharmaceutical Science, 5 (51), 28–37. http://doi.org/10.15587/2519-4852.2024.313637

© The Author(s) 2024

This is an open access article under the Creative Commons CC BY license

1. Introduction

The chemical name of ascorbic acid (vitamin C) is (5R)-5-[(1S)-1,2-dihydroxyethyl]-3,4-dihydroxyfuran-2(5H)-one, molar mass 176,1. Ascorbic acid is a fairly strong acid with reducing properties, the bioactivity of which is lost under the action of oxygen and light, as well as when heated. Crystalline ascorbic acid is preserved for many years, but when stored as a solution, stabilizers are added to it, since ascorbic acid is already oxidized to dehydroascorbic acid under the action of weak oxidants [1–3].

Ascorbic acid has a number of important biofunctions in the human body [4, 5]. Vitamin C has antioxidant properties and plays an important role in the human immune system [6]. The human body is not able to synthesize ascorbic acid since the enzyme G-gulunolactosoxidase is absent. Therefore, people need to get ascorbic acid with food or dietary supplements [6–8]. The indispensable minimum daily intake of vitamin C should be 55–100 mg [1, 9]. In the European Union, in 2008, the

recommended daily dose of vitamin C was set at 80 mg (wwwl). Since vitamin C is found quite widely in food, there is no deficiency in the presence of a normal diet. Deficiency can easily occur in smokers, alcoholics and when taking antibiotics. 10 mg of vitamin C per day is enough to prevent the development of scurvy in adults.

Ascorbic acid is found in many fresh fruits and vegetables: oranges, lemons, grapes, watermelons, papayas, strawberries, mangoes, pineapples, cherries, raspberries, tomatoes, broccoli, cauliflowers, ordinary cabbages, green and red peppers [7, 10]. Plants are the main source of vitamin C for humans [11–13].

Acerola (*Malpighia glabra* L., *M. punicifolia* L., *M. emarginata* D.C.) or Barbados cherry or West Indian cherry belongs to the family *Malpighiaceae* Juss. Acerola is native to the south of North America, Central America and the north of South America, and is also common to South America, tropical regions of Asia and Africa. Acerola grows into a shrub or a small tree 3–5 meters

high. The fruit changes from green to red or yellow as it matures. Fresh fruit of acerola contains 1.5-3.5 % vitamin C [14–16].

Camu-camu (*Myrciaria dubia* (Kunth) McVaugh) belongs to the myrtle family (*Myrtaceae* Juss.) and is native to the Amazon territories but is also found in Peru, Venezuela and Columbia. Camu-camu is a shrub or tree that grows from four to eight meters in height [17, 18]. Camu-camu is rich in vitamin C. Vitamin C is found in green fruits in 1910 mg/100 g of pulp, 2061 mg/100 g of pulp is found in ripened, purple fruits [19–21].

Rosehip (Rosa spp.) belongs to the rose-flowered (Rosaceae Juss.) family. Rosehips are predominantly deciduous, less often evergreen climbing or upright shrubs up to 3 meters high. Rosehips grow naturally in Estonia in meadows and alvar grasslands, sometimes also on the edge of forests. The fruit of rosehip is usually red, less often almost black. According to the European Pharmacopoeia, a rosehip dish (Rosae pseudo-fructus) must contain at least 0.3 % ascorbic acid. In fruits, vitamin C is on average 1–2 %, less often even up to 5 % [22]. There is very little vitamin C in the seeds [23, 24]. In previous work at the Institute of Pharmacy, it has been found that in fruits dried at room temperature of rosehip, ascorbic acid is contained in the ratio of 0.1-0.4 % to the air-dry material, with the temperature of drying not significantly affecting the ascorbic acid content [25].

The blackcurrant (*Ribes nigrum* L.) belongs to the family of currants (*Grossulariaceae* DC.). This is a deciduous shrub that grows to a height of 1–2 meters. The fruit is a black round berry. Black currant is widely cultivated as a berry shrub, in the wild it is found in log forests. Vitamin C is found in fresh berries up to 0.4 %, in leaves up to 0.25 % [23, 26, 27].

Ascorbic acid is also found in relatively large quantities in peppers (0.3 %), chilli peppers (0.5 %), lemon 27 mg, tangerine 22 mg, and orange 29 mg per 100 ml of freshly squeezed juice [25]. Medium-sized oranges contain 100 mg of ascorbic acid [23]. In the fresh leaves of the common primrose, the average content of ascorbic acid was found to be 1.1–2.4 % in relation to air-dry material, which exceeds the ascorbic acid content in dried rosehip fruits by about six times [28].

Food supplements define as "Foodstuffs the purpose of which is to supplement the normal diet and which are concentrated sources of nutrients or other substances with a nutritional or physiological effect, alone or in combination, marketed in dose form, namely forms such as capsules, pastilles, tablets, pills and other similar forms, sachets of powder, ampoules of liquids, drop dispensing bottles, and other similar forms of liquids and powders designed to be taken in measured small unit quantities" [29]. Defini-

tions of what is considered to be 'dietary supplements', or indeed specific types of supplements, have been reported to vary across American surveys [30–32].

The global dietary supplements market is expected to reach USD 340.62 billion by 2030, at a CAGR of 8.97 % from 2022 to 2030 [33].

Different countries have different requirements for the registration and quality of dietary supplements [34–36]. Therefore, questions periodically arise about their poor quality [37–39]. Adopting low-cost methods to design an effective fortification strategy to improve the stability of ascorbic acid during processing and storage is still the focus and challenge for researchers [1, 40].

Taking into account the possible poor production quality of food supplements on the one hand and the lability of ascorbic acid on the other hand, it is important to analyze the real content of ascorbic acid in food supplements containing it.

This work had the following objectives:

- 1) to compare the preparations of different manufacturers based on their ascorbic acid content;
- 2) to investigate the keeping quality of ascorbic acid and the relationship between the ascorbic acid content and the shelf life of the preparation;
- 3) to investigate the relationship between the keeping quality of ascorbic acid and its origin. To the best of our knowledge, such studies with dietary supplements have not been published before.

2. Planning (methodology) of research

The study protocol describing the different stages of the present research work is presented in the following flow chart (Fig. 1).

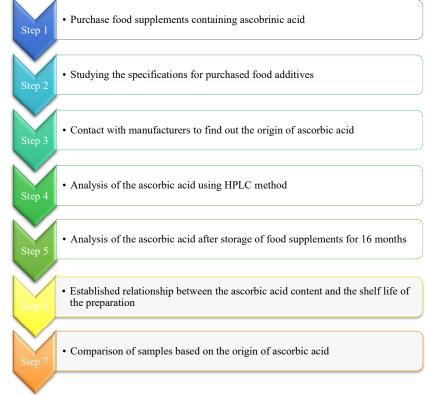


Fig. 1. Study protocol

3. Materials and methods

Food supplement studied.

13 preparations of food supplements containing ascorbic acid, sold in Estonian pharmacies or online environments at the time of the work, were analysed. All preparations were purchased between September 2012 and April 2013. Table 1 shows the names, pictures, shelf life, batch numbers and manufacturers of the studied preparations. Preparations are ranked by shelf life. Of the solid preparations, 4 represented ordinary tablets, 6 chewable tablets, 1 effervescent tablet, 1 suction tablet, and 1 preparation was in powder form. The packages of the preparations under study were opened immediately before the analysis of the content of ascorbic acid. The packages were stored in a dark closet at room temperature and examined again after 16 months.

Apparatus.

HPLC system PerkinElmer Series 200 bundled with autosampler and photo sequence detector. HPLC control program Total Chrom 6.3.2. Reagents used: demineralized water, standard substance L-ascorbic acid (L-ascorbic acid) SIGMA-ALDRICH ACS reagent ≥99 % 255564; acetonitrile (HPLC grade), potassium dihydrogen phosphate (KH₂PO₄). The reagents used in the work met at least the quality requirement of "analytical grade". Data was processed using the software program "Excel" (Microsoft, 2007). Statistical analysis of the data was carried out using the software program "STATISTICA (data analysis software system)" (StatSoft, Inc 2011, version 10).

The column is named Phenomenex Luna® NH2, with a length of 250 mm and a diameter of 4.6 mm. The average diameter of the particles in the column is 5 μm . The column contains aminopropylsilica gel (aminopropylsilyl silica gel) as a stationary phase. For the mobile phase, 0.05M phosphate buffer with acetonitrile in the ratio of 25:75 (v/v). Elution rate 1 ml per minute, elution time 22 minutes, temperature 45 °C. The volume of sample injected is 20 μl . The detector is a photo-sequence detector (DAD - Diod Array Detector), and measurements were made at a wavelength of 260 nm.

Determination of ascorbic acid content by HPLC method.

For the determination of ascorbic acid in samples prepared from preparations, the HPLC method given in the monograph of ascorbic acid in the European Pharmacopoeia (11.0) was used [22]. The measurement at a wavelength of 210 nm described in the European Pharmacopoeia is specific for determining impurities. In the present research, the determination of the ascorbic acid content was carried out at a wavelength of 260 nm, which corresponds to the absorption maximum of ascorbic acid. Previously, we have successfully applied such a modified method to determine the content of ascorbic acid in conifers as well as herbaceous plants, plant foods, and orange juices [25, 28, 41].

A tablet was taken from each package of solid preparation and weighed with analytical accuracy. The powders were carefully opened and the entire contents of the package were also weighed with analytical accuracy. After weighing, the tablet or powder was placed in a mortar, ground and stirred until a homogeneous powder mixture was obtained. The required amount of powder was then taken from the powder mixture to prepare a solution with an approximate ascorbic acid concentration of 0.5 mg/ml. A tablet was taken from each package of a solid preparation and weighed with analytical accuracy. The powders were carefully opened and the entire contents of the package were also weighed with analytical accuracy. After weighing, the tablet or powder was placed in a mortar, ground and stirred until a homogeneous powder mixture was obtained. The required amount of powder was then taken from the powder mixture to prepare a solution with an approximate ascorbic acid concentration of 0.5 mg/ml [28].

The required amount of powder was transferred to a measuring flask and the exact balance on the analytical balance was fixed. At the same time, the required amount of acetonitrile 5% aqueous solution was prepared. For this purpose, 100% acetonitrile was used, which was diluted with distilled water to the desired concentration. The relatively polar organic solvent acetonitrile is often used by the HPLC method in the analysis of vitamin C vitamins, both as a solvent and as an eluent. In a 0.2% solution of acetonitrile (v/v), the decomposition of ascorbic acid is slowed down four times. Starting from a 2.0% solution of acetonitrile, the stability of ascorbic acid reaches its maximum, and with further increase in concentration, there is no change [42].

The preparation powder in the volumetric flask was dissolved in a 5 % solution of acetonitrile, which was initially added to 1/3 of the volume of the volumetric flask. After initial mixing, the cap-coated flasks were placed on the ultrasonic bath for two five-minute cycles, stirring in between, to ensure the extraction and dissolution of the full formulation of ascorbic acid from the material. The measuring flasks were then supplemented to the mark and stirred ten times by rotating the pistons once more. The test solutions were filtered through a nylon 0.45 µm pore membrane filter and the filtrate was collected in glass vials of autosampler (2 ml). The solutions of some herbal preparations were too cloudy after preparation, so they had to be centrifuged in plastic vials at a speed of 13 000 rpm for 5 minutes and then filtered through a membrane filter.

The reference standard was prepared by weighing the standard substance L-ascorbic acid at an analytical balance of ~50 mg. It was transferred to a 100 ml volumetric flask and dissolved in a 5 % acetonitrile solution. The standard solution was mixed, made up to the mark and stirred again by inverting the flask. The reference standard was then moved to autosampler vials. Solutions of the reference standard were always prepared with samples. For chromatography using a solution of acetonitrile in a buffer solution of 0,05 M potassium dihydrogen phosphate in a ratio of 75:25, was used as an eluent. A 75 % solution of acetonitrile was used to pre-wash the column. Between tests, 2-propanol was used as a storage solution for the aminocolumn.

Table 1 Names, photos, shelf life, batch numbers and manufacturers of the studied preparations

Product name	os, shelf life, batch numbe Photo	Best before	Serial number	Producer
Vivasan Acerola-Or- angen-Tabletten "Acerola"	ACEROLA Mereda Prochigner	02/2014	0791930101	SWISS CAPS AG, Switzerland
Acerola Plus 100	ACEROLA Plus 100	12/2015	NM 087	OY Valioravinto AB, Finland
Alva Acerola	alva Accrols	02/2015	12105	Alva naturkosmetik gmbh, Germany
Acerola Sweet	Transferred Comments of the Co	02/2014	2046	Hankintatukku OY, Finland
Acerola C-vita Blackcurrant	Acerola C-vita Blackcurant	12/2014	1578902	Vitabalans oy, Finland
Litozin®	Sold Squares - Terrord Squares COO The Cook of the C	04/2014	0942	Axellus A/S, Denmark
Bio-C-Zinc®	Colored a control of position of the colored and the colored a	10/2013	1103136	Pharma Nord ApS, Denmark
Nutrilite™ vitamiin C	Vitamin C	05/2014	2145FG8A	Access Business Group LLC, USA
BioCare® Vit-C-Plex	BioCare* Vit-C-Plex Transmit control Transmit	02/2014	27876	BioCare® Limited, UK

Continuation of Table 1

Camu Camu	Minu Canu	07/2013	L 02 TCC 100713	Raab Vitalfood GmbH, Germany
Neorauta	EORANUTATION OF THE PROPERTY O	03/2015	2103	Hankintatukku OY, Fin- land
Gnld Nutritionals All C	ALL C	11/2013	L112699	Thompson & Capper Ltd, UK
Livol Extra C Cold	ENTRA C COLD Page 1 and	08/2013	415744	Axellus A/S, Denmark

The software program "Excel" (Microsoft, 2007) was used for data processing. Dixon's Q-test was used to find the drop-out sizes among the samples. Dixon's Q-test is, to this day, the main tool for finding deviant results in the case of a small selection (<10). The results are ranked in ascending order to find the deviant results, and, depending on the need, the value of O corresponding to the first or then the last result is found. If the value of Q with a 95 % confidence interval is greater than the critical value of Dixon Q given in the tables, then the exclusion of this result is statistically justified [43]. The probability of error in this case is less than 5 % [44]. In other words, with a probability of 95 %, it is not a case of variability of a random result but a determined or systematic error [43]. Using this method, one result from samples 3, 9 and 10 was excluded from the calculations (Table 2).

Assessment of the origin of ascorbic acid in preparations.

The purpose of the research was to analyze vitamin preparations of natural origin, but one of the most interesting questions was to find out the true origin of the ascorbic acid contained in the preparations. When purchasing preparations, proceed from the text indicated on the label. In the course of the work, it turned out that under certain conditions, a distinction can be made whether the preparation contains natural or synthetic ascorbic acid. The label usually states that ascorbic acid comes from herbal extracts, powders or flours. As this information may not have been reliable, as synthetic ascorbic acid may have been added to natural ascorbic acid, the manufacturers or importers of the preparations

were contacted for further information. The first letter asked for information about vitamin C vitamers, their origins, and their quantity. By the second letter, data on the ascorbic acid content specified in their preparation were sent individually to each manufacturer and if the first letter was not answered, the original question was re-submitted. The e-mails of manufacturers or importers were searched on the Internet and, if possible, the letter was sent to several addresses. 14 preparations were produced by 12 manufacturers, and 6 of them corresponded; the remaining preparations were grouped according to what was stated on the label (Table 2).

Thanks to feedback from manufacturers, it was possible to group 6 preparations with great certainty by origin. In the event that most of the ascorbic acid contained in the preparation was obtained synthetically, they were grouped under synthetic ones. 8 preparations had to be grouped according to the information on the label only. In total, 3 preparations were found to contain ascorbic acid of unknown origin, 8 preparations of natural origin and 3 ascorbic acid of synthetic origin (Table 2). Eight preparations of natural origin contained ascorbic acid from various sources. The ascorbic acid in the Camu Camu preparation came from the camu-camu plant, Apropos® C bambini and NEORAUTA rosehip. The remaining preparations contained acerola cherry extracts, powders or concentrates as sources of ascorbic acid.

Statistical analysis.

Analysis of variance is used when comparing the mean of more than two populations. The test of multiple comparisons of averages following variance analysis provides an answer to the question of which averages actually differ. The decision is made by comparing the averages of the samples in pairs [45].

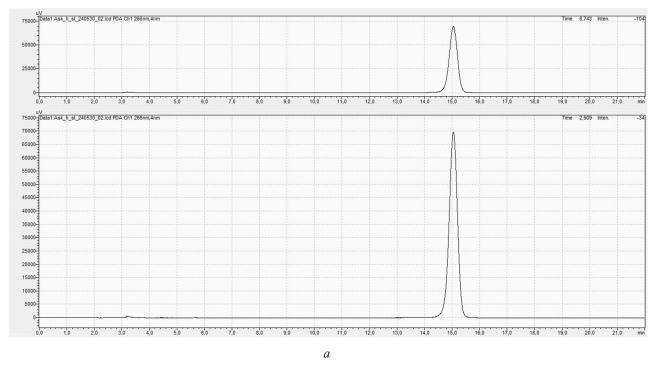
Single-factor dispersion analysis was used to compare groups of vitamin preparations of natural, synthetic, and unknown origins. The factor was the origin of ascorbic acid, and its effect on the average ascorbic acid content in different groups was investigated. As a hypothesis, it was tested whether the average content of ascorbic acid groups of different origins with respect to the nominal differs from each other or not. To carry out

the variance analysis, the statistical program "Statistica" was used.

4. Results

Quantitative content of ascorbic acid in food supplements studied.

The results of the HPLC analysis show that the average ascorbic acid content of 8 preparations out of 13 is between 90 % and 110 % of the nominal (Table 2 and Fig. 2). The least ascorbic acid contained in sample 13 was 56.3 % of the nominal on the package.



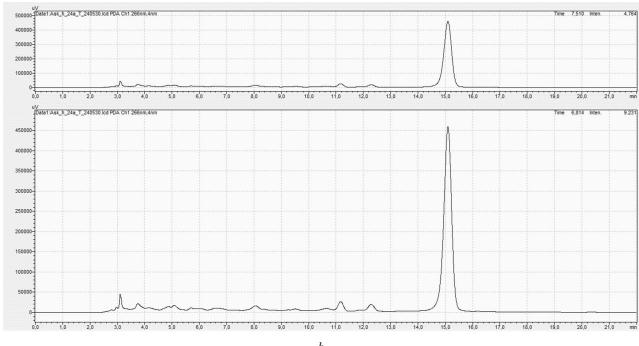


Fig. 2. HPLC chromatograms of ascorbic acid: a – the standard sample of ascorbic acid; b – the typical chromatogram of a studied sample

Table 2
The average content of ascorbic acid after opening the packages of food supplements and 16 months later with the origin of ascorbic acid used by producers

Commis	Average content of ascorbic acid (% of nominal on the package)		Ouisin of assorbis said/saymas of information	
Sample	After opening the packages*	16 months later**	Origin of ascorbic acid/source of information	
1	115.2±11.3	130.7±3.1	No information	
2	105.4±5.7	109.0±4.1	Semisyntetic/Producer	
3	104.3±9.9	90.2±6.0	Natural/Producer	
4	99.5±10.8	104.0±6.4	Semisyntetic/Producer	
5	97.8±15.3	122.7±8.5	Semisyntetic/Producer	
6	95.9±4.1	103.9±1.0	Natural/Producer	
7	91.6±5.7	101.4±3.3	Natural/ Packaging labelling	
8	91.5±3.3	88.0±6.3	No information	
9	88.4±4.6	89.1±2.2	Natural/ Packaging labelling	
10	81.2±10.5	75.6±2.3	Natural/ Packaging labelling	
11	78.5±9.3	75.4±21.5	No information	
12	54.6±4.1	58.3±1.0	Natural/Producer	
13	56.3±6.8	57.0±3.8	Natural/ Packaging labelling	

Note: * – average of 8–11 samples have been analyzed; ** – average of 6–13 samples have been analyzed. Sample numbers do not correspond to the sequence above in Table 1.

Significantly lower ascorbic acid levels than other preparations were also present in sample 12, 54.6 % of the nominal, respectively. Sample 12 also differed from the other in appearance – the tablets were very brittle and unevenly shaped. At the same time, the ascorbic acid content in them was uniform – a standard deviation of 4.4. In the remaining preparations, the biggest difference in quantity between the actual ascorbic acid content and what was stated on the label was in sample 11 – an average of 78.5 % of the nominal. Relatively, the largest amount of ascorbic acid was contained in sample 1, 115.2 % of the nominal. Of the amount indicated on the label, sample 2 and sample 3 also contained more.

The sample 5 contains synthetic ascorbic acid and an additional dog rosehip (*Rosa canina*) powder. The results showed that the content was 97.8 % of the nominal content of synthetic ascorbic acid. Thus, the dog rosehip powder did not add to the amount of ascorbic acid, or the amount of synthetic ascorbic acid in the preparation was less than indicated on the label.

Stability of ascorbic acid in food supplements studied. Data on the shelf life of ascorbic acid after storage of preparations for 16 months are given in Table 2. In 2012, tolerances for nutrition information were introduced by the European Commission [46]. In vitamin preparations used as food supplements, the vitamin content may vary from -20 % to +50 % compared to what is indicated on the packaging. In this context, it can be noted that the content of ascorbic acid in three preparations does not meet the standards set by the European Commission: samples 13 (57.0 % nominal), 12 (58.3 %) and 10 (75.6 %). Sample 11 (75.4 %) also contained ascorbic acid less than 80 % of what was indicated on the package, but a large standard deviation does not allow a final estimate to be made here. The average ascorbic acid content of the remaining preparations was within the permissible variability limits.

Relationship between the ascorbic acid content and the shelf life of the preparation.

Using the Excel software program, an attempt was made to find a correlation, i.e. a relationship between the av-

erage ascorbic acid content of the preparation (% of the nominal) and the age of the preparation. To find the age, the date of the last analysis was taken and the number of days remaining from that date to the end of the shelf life was found.

Pearson's correlation coefficient when comparing all preparations was 0.14. Thus, the absolute value of the correlation coefficient is less than 0.3, and this is a weak relationship.

In other words, the value of one trait (age) does not influence the behaviour of another trait (ascorbic acid content (% of nominal)) – the traits are independent.

Comparison of preparations based on the origin of ascorbic acid.

The result of the variance analysis, with a probability of less than 0.01, showed that the factor of origin affected the average ascorbic acid content in the preparations, and the average ascorbic acid levels between the groups differed from each other. In ascorbic acid preparations of unknown origin, the average content of ascorbic acid was 97.6 % of the nominal with a 95 % confidence interval of 90.6 %–104.5 % (N=35); in preparations of natural origin, the average content is 74.0 %, with a 95 % confidence interval of 68.8 % to 79.2 % (N=63); in preparations of synthetic origin, the mean content is 100.5 %, with a 95 % confidence interval of 92.7 %–108.3 % (N=28).

A multiple comparison tests of Bonferroni with a probability of less than 0.001 confirmed that the average levels of ascorbic acid preparations of natural and synthetic and natural and unknown origin relative to the nominal were different from each other.

5. Discussion

In the sample 13 preparation, ascorbic acid came from rosehip flour. The preparation additionally contained blood meal and iron amino acid chelate. Ascorbic acid helps both inorganic and amino acid-bound iron to be better absorbed from the gastrointestinal tract [47]. The low ascorbic acid content of sample 13 may be due to the reaction or binding of ascorbic acid to blood meal or iron amino acid chelate. Also, rosehip flour can be low in ascorbic acid or

ascorbic acid decomposed in the preparation since heavy metals accelerate the decomposition of ascorbic acid.

The results carried out by Ševeljova on the same topic and method showed that in all 8 solid preparations studied, the ascorbic acid content ranged from 90.1–104.2 % of the nominal [48]. In the present research, in 11 out of 13 preparations, the ascorbic acid content ranged from 78.5 % to 115.2 % of the nominal. For 2 preparations, samples 12 and 13, the ascorbic acid content was very low compared to that provided by the manufacturer.

If we compare the content of ascorbic acid after the opening of the packages and 16 months later, the T tests show that a statistically significant change occurred only in the samples 3, 8, and 10, in which the substance content had decreased (p<0.05) by approximately 7–21 mg. This may be because this preparation had already exceeded its shelf life by five months by the time of the analysis. It was not possible to detect differences in the remaining preparations, so the content of ascorbic acid in them had not changed for 16 months after storing the open packages.

Ascorbic acid can be stored in crystalline form for many years [3]. Ševeljova, in addition to the content of ascorbic acid in solid and liquid preparations, also studied the effect of preservation. His results show that in most solid preparations the amount of ascorbic acid remained the same or turned out to be even higher. The latter may have been due to an experimental error, as well as the variability of the tablets [48]. This is also confirmed by the result found in the present work that there is practically no relationship between the ascorbic acid content (% of the nominal) and the shelf life. By preserving vitamin C solid preparations under the prescribed conditions, there is no high probability of fearing a significant decrease in the content of ascorbic acid.

Statistical analysis of the data showed that the relative amount of ascorbic acid present in the preparation relative to the nominal was related to the origin of the substance, whereas the content of ascorbic acid of natural origin in preparations relative to the nominal was significantly lower than in preparations with a synthetic active substance.

According to the Bonferron test, the average content of preparations containing synthetic ascorbic acid relative to the nominal did not differ from the average content of ascorbic acid preparations of unknown origin. Ascorbic acid preparations of unknown origin are, therefore, also likely to contain synthetic ascorbic acid.

Practical relevance. The stability of ascorbic acid in solid forms of dietary supplements during 16 months of storage after opening the packages has been confirmed.

Research limitations. 13 food supplements were analyzed. The origin of ascorbic acid was known for sure only for 6 of them. For the rest, the origin of ascorbic acid was determined only by the information on the label.

Prospects for further research. The obtained results create the basis for the study of the stability of ascorbic acid in liquid forms of dietary supplements.

6. Conclusions

The actual content of ascorbic acid in most solid preparations roughly corresponds to that stated on the label, usually slightly below it.

In solid supplements containing ascorbic acid, its amount practically does not change after 16 months of shelf life.

In preparations containing ascorbic acid of natural origin, it is found relatively less than in preparations containing synthetic ascorbic acid.

Preparations containing ascorbic acid of unknown origin are likely to use synthetic ascorbic acid.

Conflicts of interest

The authors declare that they have no conflict of interest concerning this research, whether financial, personal, authorship or otherwise, that could affect the research and its results presented in this paper.

Funding

This work was supported by the European Union in the MSCA4Ukraine project "Design and development of 3D-printed medicines for bioactive materials of Ukrainian and Estonian medicinal plants origin" [ID number 1232466].

Data availability

The datasets used and/or analyzed during the current study are available from the author and/or corresponding author upon reasonable request.

Use of artificial intelligence

The authors confirm they did not use artificial intelligence technologies when creating the current work.

Acknowledgement

The authors sincerely thank the Armed Forces of Ukraine for defending Ukrainian statehood and independence, as well as the partners who stand with Ukraine.

References

- 1. Yin, X., Chen, K., Cheng, H., Chen, X., Feng, S., Song, Y., Liang, L. (2022). Chemical Stability of Ascorbic Acid Integrated into Commercial Products: A Review on Bioactivity and Delivery Technology. Antioxidants, 11 (1), 153. https://doi.org/10.3390/antiox11010153
- 2. Pilarski, B., Wyrzykowski, D., Młodzianowski, J. (2023). A New Approach for Studying the Stability and Degradation Products of Ascorbic acid in Solutions. Journal of Solution Chemistry, 52 (6), 639–657. https://doi.org/10.1007/s10953-023-01260-9
- 3. Shelke, O., Susarla, K. P. C., Bankar, M. (2024). Understand the Stabilization Engineering of Ascorbic Acid, Mapping the Scheme for Stabilization, and Advancement. AAPS PharmSciTech, 25 (6). https://doi.org/10.1208/s12249-024-02882-y
 - 4. Krečak, I., Babić, G., Skelin, M. (2022). Scurvy. Acta dermatovenerologica Croatica: ADC, 30 (1), 59-60.
- 5. Bhoot, H. R., Zamwar, U. M., Chakole, S., Anjankar, A. (2023). Dietary Sources, Bioavailability, and Functions of Ascorbic Acid (Vitamin C) and Its Role in the Common Cold, Tissue Healing, and Iron Metabolism. Cureus, 15 (11), e49308. https://doi.org/10.7759/cureus.49308

- 6. Carr, A., Maggini, S. (2017). Vitamin C and Immune Function. Nutrients, 9 (11), 1211. https://doi.org/10.3390/nu9111211
- 7. Nowak, D. (2021). Vitamin C in Human Health and Disease. Nutrients, 13 (5), 1595. https://doi.org/10.3390/nu13051595
- 8. Wang, J. L. (2019). Vitamin C in Human Health and Disease. Springer Dordrecht, 184. https://doi.org/10.1007/978-94-024-1713-5
- 9. Doseděl, M., Jirkovský, E., Macáková, K., Krčmová, L., Javorská, L., Pourová, J. et al. (2021). Vitamin C Sources, Physiological Role, Kinetics, Deficiency, Use, Toxicity, and Determination. Nutrients, 13 (2), 615. https://doi.org/10.3390/nu13020615
- 10. Abdullah, M., Jamil, R. T., Attia, F. N. (2023). Vitamin C (Ascorbic Acid) Treasure Island: StatPearls Publishing. Available at: https://www.ncbi.nlm.nih.gov/books/NBK499877/
- 11. Granger, M., Eck, P. (2018). Dietary Vitamin C in Human Health. Advances in food and nutrition research, 281–310. https://doi.org/10.1016/bs.afnr.2017.11.006
- 12. Paciolla, C., Fortunato, S., Dipierro, N., Paradiso, A., De Leonardis, S., Mastropasqua, L., de Pinto, M. C. (2019). Vitamin C in Plants: From Functions to Biofortification. Antioxidants, 8 (11), 519. https://doi.org/10.3390/antiox8110519
- 13. Belwal, T., Devkota, H. P., Hassan, H. A., Ahluwalia, S., Ramadan, M. F., Mocan, A., Atanasov, A. G. (2018). Phytopharmacology of Acerola (Malpighia spp.) and its potential as functional food. Trends in Food Science & Technology, 74, 99–106. https://doi.org/10.1016/j.tifs.2018.01.014
- 14. Poletto, P., Álvarez-Rivera, G., López, G.-D., Borges, O. M. A., Mendiola, J. A., Ibáñez, E., Cifuentes, A. (2021). Recovery of ascorbic acid, phenolic compounds and carotenoids from acerola by-products: An opportunity for their valorization. LWT, 146, 111654. https://doi.org/10.1016/j.lwt.2021.111654
- 15. Carneiro Ferreira, I., Pereira da Silva, V., Vilvert, J. C., França Souza, F., Freitas, S. T., dos Santos Lima, M. (2021). Brazilian varieties of acerola (Malpighia emarginataDC.) produced under tropical semi-arid conditions: Bioactive phenolic compounds, sugars, organic acids, and antioxidant capacity. Journal of Food Biochemistry, 45 (8). https://doi.org/10.1111/jfbc.13829
- 16. Olędzki, R., Harasym, J. (2024). Acerola (Malpighia emarginata) Anti-Inflammatory Activity A Review. International Journal of Molecular Sciences, 25 (4), 2089. https://doi.org/10.3390/ijms25042089
- 17. Šmíd, J., Kalousová, M., Mandák, B., Houška, J., Chládová, A., Pinedo, M., Lojka, B. (2017). Morphological and genetic diversity of camu-camu [Myrciaria dubia (Kunth) McVaugh] in the Peruvian Amazon. PLOS ONE, 12(6), e0179886. https://doi.org/10.1371/journal.pone.0179886
- 18. CAMU CAMU (Myrciaria dubia) (2006). FAO. Available at: https://www.ipcinfo.org/fileadmin/user_upload/inpho/InfoSheet pdfs/CAMU CAMU.pdf
- 19. Conceição, N., Albuquerque, B. R., Pereira, C., Corrêa, R. C. G., Lopes, C. B., Calhelha, R. C. et al. (2019). By-Products of Camu-Camu [Myrciaria dubia (Kunth) McVaugh] as Promising Sources of Bioactive High Added-Value Food Ingredients: Functionalization of Yogurts. Molecules, 25 (1), 70. https://doi.org/10.3390/molecules25010070
- 20. García-Chacón, J. M., Marín-Loaiza, J. C., Osorio, C. (2023). Camu Camu (Myrciaria dubia (Kunth) McVaugh): An Amazonian Fruit with Biofunctional Properties—A Review. ACS Omega, 8 (6), 5169–5183. https://doi.org/10.1021/acsomega.2c07245
- 21. Cunha-Santos, E. C. E., Viganó, J., Neves, D. A., Martínez, J., Godoy, H. T. (2019). Vitamin C in camu-camu [Myrciaria dubia (H.B.K.) McVaugh]: evaluation of extraction and analytical methods. Food Research International, 115, 160–166. https://doi.org/10.1016/j.foodres.2018.08.031
 - 22. European Pharmacopoeia (2022). Strasbourg: Council of Europe.
- 23. Medveckienė, B., Kulaitienė, J., Jarienė, E., Vaitkevičienė, N., Hallman, E. (2020). Carotenoids, Polyphenols, and Ascorbic Acid in Organic Rosehips (Rosa spp.) Cultivated in Lithuania. Applied Sciences, 10 (15), 5337. https://doi.org/10.3390/app10155337
- 24. Butkevičiūtė, A., Urbštaitė, R., Liaudanskas, M., Obelevičius, K., Janulis, V. (2022). Phenolic Content and Antioxidant Activity in Fruit of the Genus Rosa L. Antioxidants, 11 (5), 912. https://doi.org/10.3390/antiox11050912
 - 25. Kask, M., Meos, A., Raal, A. (2013). Askorbiinhappe sisaldusest apelsinimahlas. Eesti Rohuteadlane, 2, 13–15.
- 26. Karaklajic-Stajic, Z., Tomic, J., Pesakovic, M., Paunovic, S. M., Stampar, F., Mikulic-Petkovsek, M. et al. (2023). Black Queens of Fruits: Chemical Composition of Blackberry (Rubus subg. rubus Watson) and Black Currant (Ribes nigrum L.) Cultivars Selected in Serbia. Foods, 12 (14), 2775. https://doi.org/10.3390/foods12142775
- 27. Cortez, R. E., Gonzalez de Mejia, E. (2019). Blackcurrants (Ribes nigrum): A Review on Chemistry, Processing, and Health Benefits. Journal of Food Science, 84 (9), 2387–2401. https://doi.org/10.1111/1750-3841.14781
- 28. Meos, A., Zaharova, I., Kask, M., Raal, A. (2017). Content of Ascorbic Acid in Common Cowslip (Primula veris L.) Compared to Common Food Plants and Orange Juices. Acta Biologica Cracoviensia s. Botanica, 59 (1), 113–120. https://doi.org/10.1515/abcsb-2016-0020
- 29. European Commission. Directive 2002/46/EC of the European Parliament and of the Council of 10 June 2002 on the approximation of the laws of the Member States relating to food supplements (2002). Off J Eur Communities, L183/51. http://data.europa.eu/eli/dir/2002/46/oj
- 30. In brief: What are dietary supplements? (2024). Cologne: Institute for Quality and Efficiency in Health Care (IQWiG). InformedHealth.org. Available at: https://www.ncbi.nlm.nih.gov/books/NBK279425/
- 31. Regulation of Dietary Supplements: Background and Issues for Congress (2021). CRS Report. Available at: https://sgp.fas.org/crs/misc/R43062.pdf
- 32. FDA 101: Dietary Supplements (2022). FDA. Available at: https://www.fda.gov/consumers/consumer-updates/fda-101-dietary-supplements
- 33. Dietary Supplements Market Size by Ingredient Type (Vitamins, Minerals, Enzymes, Probiotics, Fibers & Specialty Carbohydrates, Botanicals, Proteins & Amino Acids, Omega Fatty Acids, and Others), Application (Energy & Weight Management, Bone & Joint Health, Immunity, Diabetes, Lungs, Skin/Hair, Mental Health, Menopause, Prenatal Health, Anti-Aging, Insomnia, Sexual Health, Anti-Cancer, Cardiac

Health, Gastrointestinal Health, General Health, and Others), Form Type, Distribution Channel, Regions, Global Industry Analysis, Share, Growth, Trends, and Forecast 2022 to 2030 (2024). Available at: https://www.thebrainyinsights.com/report/dietary-supplements-market-12888

- 34. Lentjes, M. A. H. (2018). The balance between food and dietary supplements in the general population. Proceedings of the Nutrition Society, 78 (1), 97–109. https://doi.org/10.1017/s0029665118002525
- 35. Saini, P., Ahmed, M., Iqbal, U., Yadav, N.; Rajakumari, R., Thomas, S. (Eds.) (2024). Challenges in Stability and Safety Evaluation of Nutraceutical and Nanonutraceutical Formulations. Handbook of Nutraceuticals. Cham: Springer. https://doi.org/10.1007/978-3-030-69677-1 16-1
- 36. Dey, P, Jain, N, Nagaich, U. (2018). Nutraceuticals: an overview of regulations. International Journal of Pharmaceutical and Life Sciences, 9, 5762–5766.
- 37. Zhang, F. F., Barr, S. I., McNulty, H., Li, D., Blumberg, J. B. (2020). Health effects of vitamin and mineral supplements. BMJ, m2511. https://doi.org/10.1136/bmj.m2511
- 38. Rao, K. S. (2018). Safety Assessment of Nutraceuticals. Biology, Engineering, Medicine and Science Reports, 3 (2), 70–72. https://doi.org/10.5530/bems.3.2.9
- 39. White, C. M. (2020). Dietary Supplements Pose Real Dangers to Patients. Annals of Pharmacotherapy, 54 (8), 815–819. https://doi.org/10.1177/1060028019900504
- 40. Giannakourou, M. C., Taoukis, P. S. (2021). Effect of Alternative Preservation Steps and Storage on Vitamin C Stability in Fruit and Vegetable Products: Critical Review and Kinetic Modelling Approaches. Foods, 10 (11), 2630. https://doi.org/10.3390/foods10112630
- 41. Raal, A., Nisuma, K., Meos, A. (2018). Pinus sylvestris L. and other conifers as natural sources of ascorbic acid. Journal of Pharmacy & Pharmacognosy Research, 6 (1), 89–95. https://doi.org/10.56499/jppres17.287 6.2.89
- 42. Golubitskii, G. B., Budko, E. V., Basova, E. M., Kostarnoi, A. V., Ivanov, V. M. (2007). Stability of ascorbic acid in aqueous and aqueous-organic solutions for quantitative determination. Journal of Analytical Chemistry, 62 (8), 742–747. https://doi.org/10.1134/s1061934807080096
- 43. Harvey, D. Critical Values for Dixon's Q-Test. Available at: https://chem.libretexts.org/Bookshelves/Analytical_Chemistry/Analytical Chemistry Volume I (Harvey)/10%3A Appendix/10.06%3A Critical Values for Dixon's Q-Test
- 44. Siddiqui, M. R., AlOthman, Z. A., Rahman, N. (2017). Analytical techniques in pharmaceutical analysis: A review. Arabian Journal of Chemistry, 10, S1409–S1421. https://doi.org/10.1016/j.arabjc.2013.04.016
- 45. Saisana, M.; Maggino, F. (Ed.) (2023). Analysis of Variance. Encyclopedia of Quality of Life and Well-Being Research. Cham: Springer, 183–187. https://doi.org/10.1007/978-3-031-17299-1 83
- 46. Guidance Document Tolerances: Simplified Summary Table (2012). The European Commission. Available at: https://food.ec.europa.eu/system/files/2016-10/labelling_nutrition-vitamins_minerals-guidance_tolerances_summary_table_012013_en.pdf
- 47. Bae, D.-H., Gholam Azad, M., Kalinowski, D. S., Lane, D. J. R., Jansson, P. J., Richardson, D. R. (2020). Ascorbate and Tumor Cell Iron Metabolism: The Evolving Story and Its Link to Pathology. Antioxidants & Redox Signaling, 33 (12), 816–838. https://doi.org/10.1089/ars.2019.7903
- 48. Ševeljova, O. (2012). Vitamiin C sisaldus ja selle muutus säilitamisel ravimpreparaatides. Tartu: Proviisoriõppe uurimistöö, Tartu Ülikool.

Received date 05.08.2024 Accepted date 15.10.2024 Published date 31.10.2024

Raal Ain*, PhD, Professor, Institute of Pharmacy, University of Tartu, Nooruse str., 1, Tartu, Estonia, 50411

Andres Meos, PhD, Associate Professor, Institute of Pharmacy, University of Tartu, Nooruse str., 1, Tartu, Estonia, 50411

Agne Vutt, Master Student, Institute of Pharmacy, University of Tartu, Nooruse str., 1, Tartu, Estonia, 50411

Herman Kirsimäe, Master Student, Institute of Pharmacy, University of Tartu Nooruse str., 1, Tartu, Estonia, 50411

Ilina Tetiana, Doctor of Pharmaceutical Sciences, Professor, Department of Pharmaceutical Management, Drug Technology and Pharmacognosy, Ivano-Frankivsk National Medical University, Halytska str., 2, Ivano-Frankivsk, Ukraine, 76018

Alla Kovaleva, Doctor of Pharmaceutical Sciences, Professor, Department of Pharmacognosy, National University of Pharmacy, Hryhoriia Skovorody str., 53, Kharkiv, Ukraine, 61002

Oleh Koshovyi, Doctor of Pharmaceutical Sciences, Professor, Department of Pharmacognosy, National University of Pharmacy, Hryhoriia Skovorody str., 53, Kharkiv, Ukraine, 61002; Doctor of Pharmaceutical Science, Institute of Pharmacy, University of Tartu, Nooruse str., 1, Tartu, Estonia, 50411

*Corresponding author: Raal Ain, e-mail: ain.raal@ut.ee