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ORIGINAL ARTICLE

THE CONTENT OF ASCORBIC AND DEHYDROASCORBIC ACIDS AND VITAMIN C IN NON-PRESERVED JUICES, DEPENDING ON THEIR TYPE AND STORAGE TIME

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ABSTRACT
Background. Vitamin C is one of the most important water-soluble vitamins. It is responsible for many important functions in the body, including: it has a positive effect on maintaining immunity, protects the body against free radicals, and also participates in the synthesis of hormones. Juices can be a good source of this vitamin. Most of the juices available on the market are processed products. Untreated juices, which do not contain added preservatives, sugar and are not pasteurized, constitute a smaller group on the market. Therefore, this group of juices can be a valuable product in human nutrition.
Objective. The aim of the study was to analyze the content of ascorbic acid (AA), dehydroascorbic acid (DHAA) and vitamin C (TAA) in non-preserved juices, depending on their type and storage time.
Material and methods. The analysis of TAA, AA and DHAA content in juices was carried out in ten types of non-preserved juices from two companies (A and B), purchased in a chain of retail outlets. The analyzed juices in company A were: sauerkraut and carrot, grapefruit, orange, apple and mandarin, while in company B: orange, apple, apple and quince, grapefruit and mandarin. In test 1, the first ten juices were analyzed, in test 2 - another ten juices after one month, in test 3 - juices from test 2 were used, and three days after opening the package and storing the juices in standard refrigeration conditions, the stability test of AA was analyzed. The AA and TAA contents were determined using the high performance liquid chromatography (HPLC) method. The DHAA content was calculated by subtracting the AA content from the TAA content.
Results. The highest TAA content was found in citrus juices, i.e. grapefruit, orange and mandarin, and the lowest in sauerkraut and carrot juices and apple juice. Moreover, ascorbic acid in apple juice was characterized by the lowest durability.
Conclusions. In the production of non-preserved apple juice, consideration should be given to the natural protection of ascorbic acid by the addition of citrus or other fruit juice, vegetable juice or by using a mild technology in the production process.

Key words: *vitamin C, ascorbic acid, dehydroascorbic acid, non-preserved juices*

STRESZCZENIE
Wprowadzenie. Witamina C jest jedną z najważniejszych witamin rozpuszczalnych w wodzie. Odpowiedzialna jest za wiele ważnych funkcji w organizmie m.in.: wpływa korzystnie na utrzymanie odporności, chroni organizm przed działaniem wolnych rodników, a także ma udział w syntezie hormonów. Soki mogą stanowić dobre źródło tej witaminy. Większość dostępnych na rynku soków, to produkty przetworzone. Mniejszą grupę na rynku stanowią soki niepoddane utrwaleniu, które nie zawierają dodatku konserwantów, cukru i nie są poddawane procesowi pasteryzacji. W związku z tym ta grupa soków może być wartościowym produktem w żywieniu człowieka.
Cel badań. Celem pracy była analiza zawartości kwasu askorbinowego (AA), dehydroaskorbinowego (DHAA) oraz witaminy C (TAA) w sokach niepoddanych utrwaleniu, w zależności od ich rodzaju oraz czasu przechowywania.
Materiał i metody. Analizę zawartości TAA oraz AA i DHAA przeprowadzono w dziecięciu rodzajach soków niepoddanych utrwaleniu produkcji dwóch firm (A i B), zakupionych w sieci placówek handlowych. Analizowanymi sokami firmy A były: sok z kiszonej kapusty i marchwi, grejpfruta, pomarańczy, jabłka i mandarynki, natomiast, produkcji firmy B: sok z pomarańczy, jabłek, jabłek i pigwy, grejpfruta i mandarynki. W teście 1 analizie poddano pierwsze dziesięć soków, w teście 2 - po upływie miesiąca - kolejne dziesięć soków, w teście 3 - wykorzystano soki z testu 2 i po upływie trzech dni od otwarcia opakowania i przechowywania soków w standardowych warunkach chłodniczych, poddano

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analizie sprawdzenia trwałości AA. Zawartość AA i TAA w sokach oznaczano metodą wysokosprawnej chromatografii cieczowej (HPLC). Zawartość DHAA obliczano odejmując oznaczoną zawartość AA od zawartości TAA.
Wyniki. Największą zawartością witaminy C charakteryzowały się soki cytrusowe, tj. z grejpfruta, pomarańczy i mandarynki, a najmniejszą soki z kapusty kiszonej i marchwi oraz sok jabłkowy. Ponadto kwas askorbinowy w soku jabłkowym cechował się najmniejszą trwałością.
Wnioski. W produkcji soku jabłkowego niepoddanego utrwaleniu należałoby rozważyć naturalną ochronę kwasu askorbinowego poprzez dodatek soku z owoców cytrusowych lub innych owoców, warzyw lub poprzez zastosowanie łagodnej technologii w procesie produkcji.

Słowa kluczowe: *witamina C, kwas askorbinowy, kwas dehydroaskorbinowy, soki niepoddane utrwaleniu*

INTRODUCTION

Fruit and some vegetable juices are a significant sources of vitamin C in human diet. Vitamin C is the collective term for L-ascorbic acid (product of its one-electron oxidation, L-ascorbyl radical) and L-dehydroascorbic acid [4, 27]. Vitamin C is water soluble and is responsible for many important functions in the body, including promotes detoxification of the body, is beneficial for maintaining immunity, protects the body against free radicals, stimulates collagen production, increases the bioavailability of non-heme iron, is involved in the synthesis of hormones [9, 20]. Therefore, vitamin C is important for maintaining the health of the human body, preventing colds, cancer, cardiovascular diseases, hypertension, and supporting the healing of wounds [1, 3, 18, 21, 23], but also an association has been shown between vitamin C intake and the prevention or treatment of Covid-19 infections [5, 22]. There are studies that have also shown the effect of consuming foods rich in vitamin C, i.e. vegetables and fruits, including juices, on the mental health of children and adolescents [11, 12].

The main source of vitamin C are fresh vegetables, fruits and juices [17]. However vitamin C is readily oxidized and degraded at different rates depending on storage conditions. In the presence of oxygen ascorbic acid (AA) is oxidized to dehydroascorbic acid (DHAA), which has vitamin C activity [4]. Most of the commercially available juices are processed, and synthetic vitamin C is added during the process. There is little information about the content of vitamin C in fresh fruit juices, which do not contain any preservatives or sugar. They are produced even without pasteurization, which keeps the flavor and aroma of fresh fruits and vegetables.

Common fruit juices were associated with a significant reduction in risk and markers of cancer, improvement cardiovascular parameters, including reduced blood pressure and endothelial function, attenuated cognitive impairment of aging and neurodegeneration in humans. Beneficial effects on bone health, recurrent UTI in high risk women and pulmonary function were reported. All juices had significant anti-oxidant activity *in vivo* and *in vitro* in addition to other mechanisms related to disease risk such as cell signaling, inflammation, cell proliferation, and apoptosis. The possible health-related and disease prevention benefits of consuming 100% juice as part of a balanced diet should be appreciated [13]. These benefits are due, among others, the presence of vitamin C, which is listed as the essential nutrient of fresh juices.

The aim of this study was to determine the content of L-ascorbic acid and the sum of ascorbic and dehydroascorbic acids in the ten types of fruit and vegetable juices, not preserved, purchased at retail, depending on its type and storage time.

MATERIAL AND METHODS

The juices from the two of the most important Polish producers were selected to the study. Fruit juices were obtained by directly mechanical extraction processes from the fragmented fruits and/or vegetables. They were naturally cloudy, unpasteurized, without dyes and preservatives. The finished product was packed in glass bottles in order to guarantee the maintenance of the original aroma and flavor of fresh fruits and vegetables. The technological process was conducted in accordance with the Hazard Analysis and Critical Control Points (HACCP) system and the standards of Good Agricultural Practice, and International Featured Standards.

Juices purchased twice at an interval of one month to check the repeatability of the vitamin C content in different batches of juices (test 1 and 2). To check the stability of ascorbic acid determination was repeated on a second batch of samples stored in the refrigerator after three days of opening the package (test 3). Manufacturers recommended storing the juices in the refrigerator, up to 4 days (except sauerkraut juice -7 days).

To study were selected following juices: sauerkraut and carrot mix, grapefruit, orange, apple, mandarin (Producer A); orange, apple, apple-quince, grapefruit, mandarin (Producer B).

Determination of vitamin C

The following reagents were used: L-dehydroascorbic acid (Cat.nr. 261556); Sodium dihydrogen phosphate (NaH_2PO_4) (Cat.nr. S8282);

Dodecyltrimetyl ammonium chloride (Cat.nr. 44242); Disodium ethylenediamine-N,N,N',N',-tetraacetic acid (Na_2EDTA) (Cat.nr. E0399); Tris[2-carboxyethyl] phosphine hydrochloride (TCEP) (Cat.nr. C4706); Trizmabuffer (0,3M) (Cat.nr. T1449); meta-Phosphoric acid (MPA) (Cat.nr. 04103) were obtained from Sigma-Aldrich, USA. All reagents were of analytical grade purity. The mobile phase consisted of 2% of acetonitrile in the aqueous part prepared as follows: 2.5 mmol/L NaH_2PO_4, 2.5 mmol/L dodecyl-tri-methyl ammonium chloride and 1.25 mmol/L Na_2EDTA dissolved in water.

Ascorbic acid (AA) and vitamin C (total ascorbic acid - TAA) were determined. The method of extraction was adapted from *Karlsen* et al. [16]. A juice sample (0.5 mL) were mixed with 10% MPA (0.5 mL) and centrifuged at 3500 x g in 15 min. Than two volumes of 300 µL of the supernatant were used for the determination of AA and TAA.

The clear supernatant (300 µL) was diluted with the aqueous part of the mobile phase (300 µL), mix and centrifuged as previously. Than 100 µL of supernatant was injected onto the HPLC column to AA determination. Second volume of supernatant (300 µL) was mixed with the aqueous part of the mobile phase (150 µL) and the solution (150 µL) of the TCEP (2.3 mmol/L of TCEP in trizmabuffer (800 mmol/L; pH 9.0). The sample was kept 7 min in the dark at room temperature for the reduction of dehydroascorbic acid (DHAA). After centrifugation 100 µL of the supernatant was injected onto the HPLC column to TAA determination. The content of DHAA was calculated by subtracting the content of AA from TAA content.

Chromatographic analyzes were carried out using high-performance liquid chromatograph with a UV-VIS detector (Gilson Company, Middleton, WI, USA). For AA and TAA separation the isocratic method was achieved on a Discovery® C18 column (5 µm, 15 cm x 4,6 mm, SUPELCO; Supelco Analytical cat. no. 504955, Bellafonte, PA, USA) connected with a pre-column of the same company. The flow rate was 0.75 ml/min, and the wavelength was λ= 264 nm. Accuracy of the method was estimated by analysis of the standards of AA. The limit of quantification (LOQ) of the method was 44 µg (0.25 µmola)/L.

Statistical analysis

The mean and standard deviation of the three tests were calculated for the ascorbic acid, dehydroascorbic acid vitamin C content. Differences between the three testes (test 1 vs. test 2 and test 2 vs. test 3) were compared using the *Mann-Whitney* U test. The significance level of $p<0.05$ was used. The following software was used: Statistica 13.

RESULTS

The content of TAA in the analyzed samples of unpreserved juices was diversified. Regardless of the producer, the highest amounts of TAA were found in orange and grapefruit juice and mandarin juice, i.e. citrus juices in tests 1 and 2 in both producers A and B (Table 1 and Table 2). This is about half the amount of fresh fruit in oranges, the content of TAA is 49 mg/100 g of fresh weight of the product, in grapefruit 40 mg/100 g, and in mandarins 30 mg/100 g [17]. The lowest TAA was found in sauerkraut and carrot brine and apple juice in tests 1 and 2 in both juice producers (Table 1 and Table 2). These values were two times lower than declared by the manufacturer.

The content of AA from two different batches differed statistically significantly in grapefruit (20.78±0.01 vs. 15.78±0.58) and apple juice (2.77±0.25 vs. 10.98±0.06) in producer A (Table 1) and orange (15.93±0.39 vs. 37.66±0.31) in producer B (Table 2). The content of DHAA differed statistically significantly between the two batches of the tested juices (Table 1 and Table 2), except for the mandarin juice in producer B (Table 2). On the other hand, the content of TAA from two different batches differed statistically significantly in apple (5.33±0.95 vs. 11.68±0.30) and mandarin juice (17.05±0.37 vs. 13.38±0.02) in producer A (Table 1) and in orange juice (20.36±0.84 vs. 37.93±0.06) in producer B (Table 2).

After three days of refrigerated storage (test 3), the content of AA and TAA in apple juices was below the limit of quantification in both producers (Table 3). Moreover, the content of AA (37.66±0.31 vs. 28.43±0.63) and TAA (37.93±0.06 vs. 33.91±1.01) in orange juice producer B and AA content (20.03±0.12 vs. 15.70±0.18) in mandarin juice in producer B decreased significantly (Table 3). However, after three days of refrigerated storage, the content of DHAA in grapefruit juice and mandarin juice increased in producer B and orange juice in both producers (Table 3).

The highest amounts of TAA were found in citrus juices: grapefruit, mandarin and orange (Table 3), which seem to be the most stable during storage, as the decrease in TAA was slight (up to 10%).

DISCUSSION

The study showed that the highest content of TAA was found in citrus juices, i.e. grapefruit, orange and mandarin, and the lowest in sauerkraut and carrot juices and apple juice. Moreover, AA in apple juice showed the lowest stability.

Vitamin C-rich juices, like citrus juices, are also good sources of polyphenols. Vitamin C and polyphenols act synergistically and define the

Table 1. The mean content of AA, DHAA and TAA in samples of A producer's juices taken in tests 1 and 2

Juice#	Sauerkraut and carrot mix		Grapefruit		Orange		Apple		Mandarin	
Test	1	2	1	2	1	2	1	2	1	2
AA* [mg/100 ml]	5.11±0.78****	6.13±0.31	20.78±0.01a	15.78±0.58b	20.28±0.01	22.48±0.76	2.77±0.25a	10.98±0.06b	15.93±0.36	12.84±0.53
DHAA** [mg/100 ml]	2.00±0.56a	0.51±0.35b	0.83±0.16a	2.35±0.15b	1.60±0.04a	0.26±0.06b	2.56±0.70a	0.69±0.24b	1.11±0.01a	0.53±0.50b
TAA*** [mg/100 ml]	7.11±0.21	6.65±0.03	21.53±0.17	18.13±0.42	21.88±0.04	22.75±0.01	5.33±0.95a	11.68±0.30b	17.05±0.37a	13.38±0.02b
Declared TAA content [mg/100 ml]	15.0		38.0		43.1		15.0		27.1	
Difference between declared and determined value [%]	44-47		47-56		50-52		35-77		49-62	

[#] Juices analyzed immediately after opening the package
*Ascorbic acid; **Dehydroascorbic acid; ***sum of AA and DHAA (vitamin C); ****mean ± standard deviation
a, b-statistically significant (*Mann-Whitney* U test); $p<0.05$

Table 2. The mean content of AA, DHA and TAA in the samples of B producer's juices taken in tests 1 and 2

Juice#	Orange		Apple		Apple-quince		Grapefruit		Mandarin	
Test	1	2	1	2	1	2	1	2	1	2
AA* [mg/100 ml]	15.93± ±0.39****a	37.66±0.31b	10.36±0.03	10.83±0.02	10.24±0.15	12.73±1.24	19.88±0.32	21.84±1.27	20.9±0.17	20.03±0.12
DHAA** [mg/100 ml]	4.43±0.44a	0.27±0.24b	2.93±1.28a	0.09±0.02b	1.60±0.28a	0.01±0.00b	2.33±0.01a	0.59±0.18b	0.01±0.00	0.01±0.00
TAA*** [mg/100 ml]	20.36±0.84a	37.93±0.06b	13.30±1.32	10.93±0.05	11.85±0.44	12.74±1.25	22.22±0.34	22.43±1.86	20.92±0.12	20.04±0.00
Declared TAA content [mg/100 ml]	43.1		15.0		-		38.0		27.1	
Difference between declared and determined content [%]	47-88		72-88		-		58-59		73-77	

[#] Juices analysed immediately after opening the package
*Ascorbic acid; **Dehydroascorbic acid; ***sum of AA and DHAA (vitamin C); ****mean ± standard deviation
a, b-statistically significant (*Mann-Whitney* U test); $p<0.05$

Table 3. Comparison of the average content of AA, DHAA and TAA in the juices of both producers (A and B) before and after three days of refrigerated storage

Juice[#]	Test	AA* content [mg/100 ml]	DHAA** content [mg/100 ml]	TAA*** content [mg/100 ml]	Declared TAA value [mg/100 ml]
Sauerkraut and carrot fix Producer A	2	6.13±0.31	0.51±0.35	6.65±0.03	15.0
	3	4.62±0.35	0.82±0.10	5.44±0.25	
Grapefruit Producer A	2	15.48±0.58	2.35±0.16	18.13±0.42	38.0
	3	14.07±0.01	2.76±0.05	16.84±0.05	
Grapefruit Producer B	2	21.84±1.27	0.59±0.58[a]	22.22±1.86	
	3	22.41±1.86	2.06±0.11[b]	24.47±1.98	
Orange Producer A	2	22.48±0.25	0.26±0.26[a]	22.75±0.01	43.1
	3	20.61±0.11	2.71±0.14[b]	23.33±0.26	
Orange Producer B	2	37.66±0.31[a]	0.27±0.24[a]	37.93±0.06[a]	
	3	28.43±0.63[b]	5.48±0.38[b]	33.91±1.01[b]	
Apple Producer A	2	10.98±0.06[a]	0.69±0.24[a]	11.68±0.30[a]	15.0
	3	<LOQ[##]	<LOQ	<LOQ	
Apple Producer B	2	10.83±0.02[a]	0.09±0.02[a]	10.93±0.05[a]	
	3	<LOQ	<LOQ	<LOQ	
Apple-quince Producer B	2	12.73±1.24[a]	0.01±0.01[a]	12.74±1.25[a]	-
	3	<LOQ	<LOQ	<LOQ	
Mandarin Producer A	2	12.84±0.53	0.53±0.50	13.38±0.02	27.1
	3	12.28±0.36	0.98±0.37	13.26±0.01	
Mandarin Producer B	2	20.03±0.12[a]	0.01±0.01[a]	20.04±0.12	
	3	15.70±0.18[b]	1.79±0.49[b]	17.49±0.30	

[#] Juices analyzed immediately after opening the package (Test 2) and after three days of refrigerated storage (Test 3)
[##] LOQ - limit of quantification - 44 µg (0.25 µmola)/L
* AA - Ascorbic acid; ** DHAA - Dehydroascorbic acid ; *** TAA - sum of AA and DHAA (vitamin C)
a, b - statistically significant (*Mann-Whitney* U test); $p<0.05$

antioxidant properties of juices. Many studies have shown that bioactive compounds, for example, polyphenols, and so on can play an important role in reducing oxidative stress and protect against various diseases [19]. In a study by *Soriano-Maldonado* et al. [24] showed that a joint consumption of apple juice natural antioxidants such as vitamin C and polyphenols might provide mild favorable effects on cardiometabolic markers, as compared to apple polyphenols alone.

The amounts declared by producers do not differ significantly from the expected vitamin C content in fresh fruit. However, as shown in this study, the TAA content in unfixed juices was lower. AA degradation occurs in both the aerobic and anaerobic pathways [14] and depends on many factors such as oxygen, light, heat, temperature, and storage time [7, 8, 26]. As demonstrated by *Johnston* and *Bowling* [15], ready-to-eat orange juices had a higher concentration of oxidized vitamin C compared to orange juices from frozen concentrates, but the rate of vitamin C decrease after opening was similar for all juices, about 2% per day. Taking into account the factors influencing the vitamin C content in the finished product: on the one hand, the fruit varieties, their ripeness and storage method, and on the other hand, the access of oxygen, light during production processes and the method of storage, transport and display on store shelves, there should be a batch of juices for the determination of vitamin C content.

The research also shows that the lowest content of TAA was in the juice of sauerkraut and carrots and apple. Moreover, AA in apple juice showed the lowest stability. It seems that in the production of fruit juices, the possibility of naturally protecting AA in apple juice by the addition of citrus juice or other fruits, vegetables [2, 10] or by using a gentle technology in the production process should be considered [6, 25].

CONCLUSIONS

Based on the research, it can be concluded that from the nutritional point of view, unpasteurized juices, mainly from citrus fruits, can be a valuable source of TAA. It seems that in the production of fruit juices, the possibility of naturally protecting AA in apple juice by the addition of citrus juice or other fruits, vegetables. The conducted studies should be treated as a pilot, and to draw clear conclusions, systematic long-term studies and a larger number of samples for analysis are necessary.

Conflict of interests

The authors declare no conflict of interests.

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