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DETERMINATION OF VITAMIN C CONTENT IN COMMERCIAL FRUIT JUICES BY VOLUMETRIC AND SPECTROPHOTOMETRIC METHODS

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Abstract

Ascorbic acid known as Vitamin C is the main constituents of fruit juice and it is an essential antioxidant. Presence of this organic acid plays an important role in human health and on the shelf life of the product. The purpose of this study was the determination of vitamin C content in some imported commercial fruit juices traded in Albania.

Samples were chosen randomly in Tirana's hypermarkets. Content of ascorbic acid was determined by redox titration with iodine solution and UV-spectrophotometric methods using potassium permanganate as a chromogenic reagent. Results were analyzed by two-sample t-test equal means.

Content of ascorbic acid in commercial fruit juices determined by volumetric methods varied from 5.28 mg/100mL to 39.63 mg/100mL. The level of ascorbic acid according to the spectrophotometric method varied from 8.7 mg/100mL to 49.33 mg/100mL. Based on two-sample t-test for equal means of vitamin C for the content in fruit juices revealed that there is no significant difference between two methods.

However, it was noticed that the level of vitamin C content was about three times higher than one declared on the labels of analyzed samples. Approximately 40 - 50% of the amount of ascorbic acid is lost after opening the package of commercial fruit juices and seven-day storage at the temperature of 4 Celsius degree.

***Key words**: Vitamin C, Commercial fruit juices, Volumetric method, UV-spectrophotometric method, Determination.*

1. Introduction

Fruit juices are consumed throughout the world due to the health benefits, the pleasant taste, their characteristic aroma and flavor. Natural fruit juices are good source of vitamins, minerals, soluble and insoluble fibers and major source of valuable bioactive compounds essential for human health [1, 2].

Due to their potential nutritional and biological fruit juices are foods with multiple implications for body balance [3]. Consumption of fruit juices as a part of a normal diet to prevent or treat cardiovascular diseases is becoming more fashionable [4].

Fruit juice is unfermented but fermentable liquid or juice intended for direct consumption, obtained from the edible portion of sound, appropriately mature and fresh fruit by mechanical extraction process and preserve, exclusive by chemical and physical means [5]. Fruit juice as well is a natural fluid that can be obtained by crushing or squeezing of fresh fruits or reconstituted concentrates [6, 7].

The most common ingredients in juices are fruits or reconstituted juice, water, preservatives, sugar, acid and color [8]. Fruit juices may be produced either by extracting the natural fruits with water resulting in water extractable juices. Those juices have all essential physical, chemical, organoleptic and nutritional properties of the fruits they are produced from [9].

In terms of chemical composition fruits consist of water and dry matter. The dry matter contains considerable amounts of vitamins (vitamin E and C), acids, sugars, polysaccharides, pectin, cellulose, polyphenols and minerals.

Minerals and vitamins could be added to enrich fruit juices [3].

According to Leahu et *al*., [3] consumption of fruit juices with pulp is recommended, being used in the treatment and prevention of cardiovascular diseases, liver disease, etc., natural juices are common sources of vitamins (mainly vitamin C) and minerals in daily dose.

Some manufacturers produce fruit juices from raw materials other than the fruit, or use concentrates in quantities below the legal requirements [10]. Preservatives such as sodium benzoate, sulphur dioxide, sorbic acid and ascorbic acid may be used to extend fruit juice shelf-life. These preservatives works effectively at pH usually below 4.3, therefore amount of acid added has to maintain the proper pH value. The commonly used acid is citric acid which should not exceed range 0.39 - 1.1 % in fruit juices [11].

The anti-oxidant components of fruit juice have beneficial long term health effects, such as decreasing the risk of cancer and heart disease [12]. Vitamin C does not provide energy to the body but it is essential and necessary nutrients for the growth of the body and also associated with the enzyme function in the body. It plays an important role not only in collagen biosynthesis, but also in iron absorption and immune response activation [13, 14, and 15].

The main sources of vitamin C are fruits and vegetables such as oranges, lemons, limes, citrus fruits, strawberries, melons, peppers, tomatoes, leafy vegetables, and potatoes spinach and etc. [16].

Even a healthy diet contains sufficient quantities of ascorbic acid; the body requires more of the vitamin after serious injury, major surgery, burns and when exposed to extremes temperature [14]. According to some previous studies minimum daily requirement of vitamin C for adults is 60 mg. The recommended daily doses are 25 to 30 mg/1000 kcal [17, 18, and 19]. In some cases, excessive quantities of ascorbic acid may result in the inhibition of natural processes occurring in food and can contribute to taste deterioration [20].

Vitamin C or L-ascorbic acid (AA) is a water soluble essential nutrient which is not synthesized by the human organism; it is easily degraded by enzymes and possibly could be decomposed during the storage. The chemical formula of ascorbic acid is $C_6H_8O_6$ with a white or slightly yellow crystal or powder [21, 22, and 20].

Vitamin C is instability because of its strong reducing agent and it is degraded by oxidizing agents such as atmospheric oxygen, heating, light, etc. Also, vitamin C can be easily lost by boiling due to its water-solubility [15, 23]. Ascorbic acid has limited stability and may be lost from foods during storage, thermal processes, preparation and cooking [24].

Ascorbic acid (AA) is used commonly in the beverage industry, especially in fruit juices. The addition of vitamin C in fruit juices is done for some technical reasons such as: to restore the vitamin C lost value during processing and to contribute to the product's appearance and shelf life. AA may be added in some different stages of juices production process to prevent enzymatic browning and flavor deterioration [26, 27].

For all reasons mentioned above the vitamin C content in fruit juices represents an indicator of quality, which has to be carefully monitored, regarding its variation during manufacturing and storage conditions [15]. Due to its importance and facility of degradation, it is of great importance to quantify correctly the levels of vitamin C in fruit juices [27].

The development of rapid, simple, and inexpensive analytical methods is the areas of growing interest, especially since quick decisions are needed in environmental and industrial fields [28, 28]. Many analytical methods can be used for the quantitative determination of vitamin C content in fruit juices, such as: titrimetry, spectrophotometry, biological methods, electrochemical methods (voltametry, fluorometry, and potentiometry) and chromatographic method [30, 31, and 28].

Vitamin C is usually analyzed by classical techniques such as volumetric methods - titration with an oxidant solution such as dichlorophenol indophenol (DCPIP) [32], potassium iodates [33], or bromate [34]. Not all analytical methods are suitable for the determination of both forms (ascorbic acid and oxidized form, dehydroascorbic acid) of ascorbic acid because two forms of the vitamin C, ascorbic acid and its oxidized form dehydroascorbic acid possess the different chemical, optical and electrochemical properties [30].

Spectrophotometry is one of the most frequently used simple methods, because vitamin C is able to absorb UV ray [35]. Direct ultraviolet (UV) spectrophotometry can provide a fast, simple and reliable method for the determination of L-ascorbic acid. Absorption of UV light by the sample matrix is a major problem due to the interference appeared by absorption of other rays [36, 37].

This paper aims to determine the content of ascorbic acid in some commercial fruit juices by both titrimetric and spectrophotometric methods. Obtained results were compared in order to define if there was a significant difference between two methods and to evaluate the simplest, accurate and reliable analytical method for determination of vitamin C content in commercial fruit juices.

2. Materials and Methods

2.1 Sample collections

Recently the trend of fruit juice production and consumption has been increased significantly in Albania as well as other countries. Different imported brands and types of packed fruit juices are traded in markets.

Subject of this study were most preferred and consumed brands of commercial fruit juices. 3 to 5 different types of 5 selected brands were purchased from Tirana's hypermarkets during April to June (2018), which were chosen randomly. Fruit juices samples were stored in 4 ^{0}C prior analyzing.

The analyses were carried out within 24 hours of sample collection in the Laboratories of Chemistry Department and Food Center, Faculty of Biotechnology and Food, Agricultural University of Tirana.

2.2 Reagents and equipment used for determination of vitamin C content in fruit juices

All reagents were chemically pure and of analytical grade (Merck and Fisher), volumetric glassware (flask, burettes, pipettes, cylinders, etc.) and other equipment were calibrated.

Determination of vitamin C content through Spectrophotometric method was performed by using an UV-vis spectrophotometer type SPECORD 40, Analytic Jena AG Germany.

2.3 Evaluation of total acid ascorbic content in fruit juices by titration method

A standard solution of potassium iodate (KIO_3) was used as an oxidizing agent for volumetric determination of vitamin C. Continuous standardization of potassium iodate solution was carried out by using a standard solution of ascorbic acid.

10 mL samples of each selected fruit juices samples was pipetted into a conical flask where was added 5 mL potassium iodide (KI 0.2 M), 2.5 mL hydrochloric acid (HCl 1 M) and a few drops of starch solution. This solution was titrated against standard solution of KIO_3 (0.01 M) until the dark blue color was appeared.

Analysis of vitamin C content by titration was conducted with three replications. Calculation of vitamin C content in fruit juice samples was carried out by using the relationship of oxidation – reduction reaction of potassium iodate with acid ascorbic (1 mol IO_3^- is equal to 3 mol of $C_6H_8O_6$) and based on equation according to other similar studies [38 - 41].

$$IO_3^- + 3\,C_6H_8O_6 \rightarrow 3\,C_6H_6O_6 + 3\,H_2O + I^- \qquad (1)$$

The amount of vitamin C in fruit juices was calculated by using following relationship:

$$\frac{M_1\,V_1}{n_1} = \frac{M_2\,V_2}{n_2}$$

Where: M = molar concentration, V = volume , and n = moles number.

2.4 Evaluation of total acid ascorbic content in fruit juices by spectrophotometric method

The determination of vitamin C in commercial fruit juices was also conducted by spectrophotometric method using potassium permanganate as a chromogenic reagent. The reduction of absorbance was measured when a potassium permanganate solution reacted with the solution of ascorbic acid in acid medium, as shown in equation (2):

$$2KMnO_4 + 5C_6H_8O_6 + 3H_2SO_4 = 2MnSO_4 + 5C_6H_6O_6 + K_2SO_4 + 8H_2O \qquad (2)$$

where vitamin C consumes $KMnO_4$ (violet color) causing a decrease in the absorbance at 525-530 nm. This method is based also on previous studies [22, 38].

2.5 Preparation of standard solutions and calibration curve of ascorbic acid

Standard solutions of ascorbic acid were prepared within the samples range (1 to 100 mg/L). Ascorbic acid (0.01 g) was dissolved in small amount of 0.5 % oxalic acid solution and completed with distilled water to obtain a concentration of 100 mg/L.

The solution of chromogenic agent with 100 mg/L concentration was prepared by dissolving previously 0.01 g of $KMnO_4$ in 5 molar solution of sulfuric acid in a 100 mL volumetric flask and completed to the mark with distilled water. 1 mL of chromogenic agent was added in a series of 10 mL standard solution with different concentrations of ascorbic acid and after 5 min was performed the absorbance measurement of each solution at 530 nm against blank [22, 38].

Two calibration curves with different concentration range of ascorbic acid (0.1 - 4 mg/L and 10 - 100 mg/L respectively) were prepared (Figures 1 and 2).

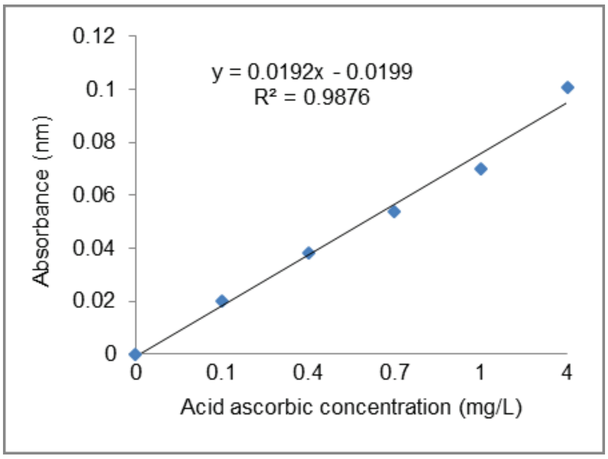

Figure 1. Calibration curve for determination of vitamin C content (concentration of AA range 0.1 to 4 mg/L)

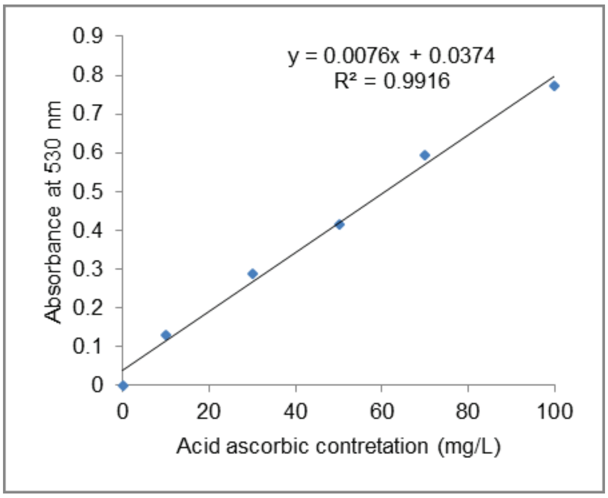

Figure 2. Calibration curve for determination of vitamin C content (concentration of AA range 10 to 100 mg/L)

2.6 Statistical analysis

Measurements of vitamin C in commercial fruit juices were carried out with 3 replications and presented as min, max, mean, standard deviation (std), confidence interval (C.I), and coefficient of variation (C.V). Statistical analysis of data was performed by using the SPSS ver. 22 software and graphs were drawn by using software GraphPad Prism version 8.02 (2019).

The comparison of two methods used for determination of ascorbic acid content in fruit juices samples was done by paired sample t-test for equal means of vitamin C content. A p value < 0.05 was considered statistically significant [42].

3. Results and Discussion

The main goal of our research was the determination of vitamin C content on commercial fruit juices available in Tirana market.

Different types of commercial fruit juices (apple, orange, cherry and blueberry) of 5 most preferred and frequently consumed brands were selected. A total of 18 commercial fruit juices were analyzed in our study. All the collected samples were labeled with specific code ranging from L1 to L18.

The pH was recorded on pH meter Hanna, model HI 2210. There was observed no differences in pH values of fruit juices samples as shown in Figure 3.

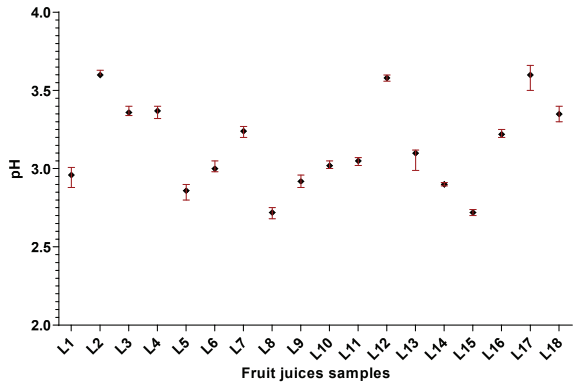

Figure 3. pH values of fruit juices samples

Table 1 presents statistical results of pH values of fruit juices samples with range 2.68 to 3.66.

Table 1. Statistical results of pH data

	Statistical parameters					
	min	max	$\bar{x}$	std	C.I	C.V
pH	2.68	3.66	3.14	0.28	0.08	8.92

The results of ascorbic acid content in fruit juices samples obtained from two employed methods are shown in Figure 4. A standard solution of 10 mg/100 mL of AA was evaluated by using both analytical methods. Based on results almost all analyzed samples presented a higher level of vitamin C content determined by spectrophotometric method compared to volumetric one. Samples L1 to L4 of the same brand, practically showed no differences on amounts of vitamin C.

Figure 4. Vitamin C content in commercial fruit juices determined by volumetric and spectrophotometric methods

The highest differences on vitamin C content were observed on samples L9 and L16, which belongs to blueberry types of fruit juices. According to Zanini *et al*., [22], the color and reducing sugar of industrial fruit juices influence on absorbance values.

Potassium permanganate as chromogenic reagent reacts with other reducing substances present in the juice and not only with the vitamin C [22]. Based on obtained results, as well as other studies, a higher level of vitamin C content determined by spectrophotometric method than titrimetric method was reported.

Statistical results of data obtained by both analytical methods employed for determination of vitamin C content are shown in Table 2. The vitamin C content of fruit juices samples was found in range of 5.28 mg/100 mL to 39.63 mg/100 mL and 15.67 mg/100 mL to 49.33 mg/100 mL with confidence interval 2.53 to 2.35 for volumetric and spechtrophotometric methods, respectively.

Evaluation of analytical methods used for determination of vitamin C content in fruit juices samples was performed by paired samples t-test. Due to the reasons mentioned above, results of two cases (L9 and L16) from the set of data underlying the statistical analysis were excluded. Based on paired samples t-test was observed no statistically significant difference between two analytical methods (Table 3).

Following the obtained results, statistical tests and based on other studies, the spectrophotometric method could be more consistent and reliable if we take into account the impact of the color interference [22, 38].

The content of vitamin C in commercial fruit juices samples was measured a week after opened and stored at room temperature (20 - 25 ^{0}C). Determination of vitamin C was conducted according to iodometric method.

As shown in Figure 5, a significant reduction of vitamin C level in all analyzed samples there was observed.

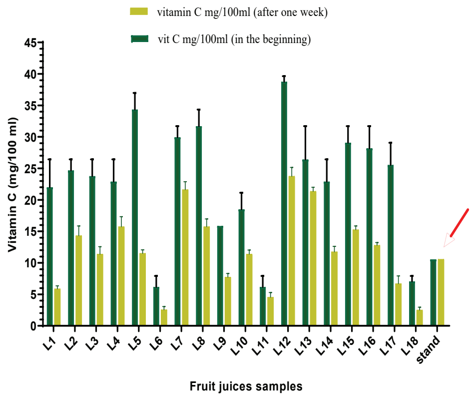

Figure 5. Vitamin C content of commercial fruit juice samples before and a week after

As noted in Table 4, the paired samples t-test indicates a statistically significant difference ($p < 0.05$) between means of vitamin C content measured before and a week after, which was decreased.

Table 2. Statistical results of vitamin C determined by two analytical methods

	Statistical parameters					
	min	max	$\bar{x}$	std	C.I.	C.V.
Volumetric method	5.28	39.63	22.99	9.49	2.53	41.25
Spectrophotometric method	15.67	49.33	27.87	8.80	2.35	31.57

Table 3. Paired Samples t-test of vitamin C determined by volumetric and spectrophotometric methods

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% confidence interval of the difference				
					Lower	Upper			
Pair 1	**Volumetric - Spectrophotometric**	-2.78738	6.93189	1.73297	**-6.48112**	**.90637**	-1.608	15	**.129**

Table 4. Paired Samples t-Test of vitamin C content determined before and a week after

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% confidence interval of the difference				
					Lower	Upper			
Pair 1	**Before - a week after**	10.9699	5.75857	1.35731	**8.10626**	**13.83359**	8.082	17	.000

Table 5 presents statistical results of vitamin C content evaluated before and a week after opening the package of commercial fruit juices samples. The concentration of vitamin C a week after was found in range of 1.94 mg/100 mL to 25.16 mg/100 mL. Storage of commercial fruit juices in opened conditions and at room temperature for 7 days results that total amount of vitamin C losses ranged from 14.2% to 76% (calculated average losses was 47.7%).

Consumption of commercial fruit juices in a short period of time (after it opened) ensures a higher content of vitamin C than the stored one. The storage temperature plays an important role in the decrease of total vitamin C amounts [16, 3, and 43].

It was observed that the concentrations of vitamin C in all analyzed commercial fruit juices samples were higher than those indicated on labels by producers, as presented in Table 6 the p value is smaller than 0.05. Declared level of vitamin C varied from 9 mg/100 mL to 12 mg/100 mL. The producer added a higher quantity of ascorbic acid due to the fact that time and conditions of storage influence on its losses [22, 44, and 45].

Table 5.Statistical results of vitamin C content measured before and a week after

	Statistical parameters						
	min	max	$\bar{x}$	std	C.I.	C.V.	Losses
Before	5.28	39.63	22.99	9.49	2.53	41.25	
A week after	1.94	25.16	12.02	6.27	1.67	52.14	47.7%

Table 6. Paired Samples t-Test of vitamin C concentration analyzed and declared on the label

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% confidence interval of the difference: Lower	95% confidence interval of the difference: Upper			
Pair 1	**Analyzed - Label**	**12.1046**	9.60049	2.26286	**7.33034**	**16.87877**	5.349	17	.000

4. Conclusions

- Ascorbic acid content in fruit juices plays an important role to human health and is considered as an indicator parameter of quality. Hence, a carefully quantification of the level of vitamin C in fruit juices is of great importance.

-This research was focused mainly on the determination of vitamin C content in commercial fruit juices by two analytical methods.

- The amount of vitamin C in commercial fruit juices measured by volumetric methods ranged from 5.28 mg/100 mL to 39.63 mg/100 mL and by spectrophotometric method ranged from 15.67 mg/100 mL to 49.33 mg/100 mL.

- From the paired samples t-test was observed no statistically significant difference between two analytical methods when we excluded from the set of statistical data the samples which compromise the color of chromogenic reagent used by spectrophotometric method.

- Based on other studies as well as on the results of the current study we suggest the spectrophotometric method as a reliable and simple method for rapid determination of vitamin C content in industrial fruit juices. This method could be applicable for determination of vitamin C concentration in fruit juices if we take into account and remove all interferences (juice color, reducing sugars and other reducing substances).

- The time and storage temperature significantly affects on the amount of ascorbic acid loses after opening the package of commercial fruit juices. Nearly 50 % of total amount of vitamin C in opened commercial fruit juices stored for seven-day at room temperature losses.

- Obtained results noticed that the concentration of vitamin C of analyzed commercial fruit juices samples was significantly higher (nearly three times more) than those indicated on the labels by producers.

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