DOI: https://doi.org/10.55302/JAFES18721045bm

DETERMINATION OF VITAMINS AS ADDITIVES FOR FORTIFICATION OF REFRESHING SOFT DRINKS

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Abstract
Soft drinks are sweetened, based on water, usually contain a certain amount of fruit juice, fruit pulp or other natural ingredients and they have a balanced acidity. Their nutritional and energy value is derived mainly from the content of sugars, but besides them there are also mineral elements, vitamins, enzymes and amino acids in minor amounts. Soft drinks are classified as fruit juices and refreshing beverages. Refreshing beverages can be clear or with pulp. The main ingredient is water, then sugar or artificial sweetener, fruit juice or fruit base or plant based extracts or based on cereals, with addition of carbon dioxide and allowed additives in the prescribed amounts. In recent times a trend is to fortify the refreshing beverages with certain vitamins or minerals. Ascorbic acid is commonly added in food as an antioxidant and stabilizer. The vitamins from B group are water soluble and played a significant role in human metabolism. In addition, it is important to consider the amount of the microelements that are necessary to meet our daily needs as nutrients. The research was made on refreshing beverages fortified with vitamins. The analysis was made on 20 different beverages in 2014, 2015 and 2016. The quantitative determination of the water soluble and fat-soluble vitamins in the refreshing beverages was performed by using by HPLC-DAD method. The vitamin C was determined by iodometric method. In the examined samples were usually present the following water soluble vitamins: B_1 (from 0.18 to 0.3 mg/100ml), B_3 (from 2.40 to 3.20 mg/100ml), B_5 (from 0.8 to 1.04 mg/100ml), B_6 (from 0.20 to 0,37 mg/100ml) and the vitamin C (from 9.06 to 16.41 mg/100ml), and more rarely were present vitamin B_2 (from 0.22 to 0.28 mg/100ml) and the liposoluble vitamin E (from 0.7 to 1.33 mg/100ml).

Keywords: refreshing beverages, microelements, HPLC method.

Introduction
Consumer demand is becoming more fastidious when choosing a beverage, of which they expect besides good sensory properties, to also have health benefits (http://hexagonnutrition.com/published-articles/03.06.2017). In order to meet the consumer's needs and to give contribution in improving their health, it is necessary to use all the advantages of the technology and science in order to produce "novel" food that will be in function for human health. This type of food may contain different macronutrients and micronutrients, which can reduce the risk of certain diseases (Mandic, 2007). According to the Rulebook on requirements regarding the quality of soft drinks (Official Gazette of the R. of Macedonia No. 15/2013) "Soft drinks" are products obtained by a special technological procedure from drinking water, natural mineral water or natural spring water in which can be added: aromas, sugars, sweeteners, starch hydrolysates, fruit juice, concentrated fruit juice, fruit syrup, plant extracts, fruit extracts, tea

extracts, cereals and other products, with or without the addition of carbon dioxide, mineral salts or vitamins. Soft drinks are available everywhere in the world, almost in the same forms, bottles, cans, paper laminated packaging, cups and many other forms of packaging (Ashurst, 2005). The properties of soft drinks are mainly related to the properties of the constituent components. Nutrition and energy value originates mainly from the sugar content. The biological significance is determined based on the content of minerals, vitamins, enzymes and amino acids. The presence of these ingredients is quite variable and primarily is conditioned by the composition of the basic and auxiliary raw materials, as well as from the very way of production. Vitamin C is the most frequently added vitamin and it must be labeled in the product declaration (Каракашова, 2008). The vitamins have a different chemical nature, they are essential for the human organism and need to be ingested through the diet because they cannot be synthesized in the body. The vitamins are active even in minimal quantities, usually they are present in different amounts in food products, and each of them is absolutely necessary for proper growth and evolution of the organism and maintenance of good health. The vitamins, according to solubility are divided into: water-soluble vitamins (hydro soluble) and fat-soluble vitamins (liposoluble) (Ѓорѓев и сор., 2008). Fat-soluble vitamins (vitamin A, D, E and K) are absorbed into the fats from food and stored in various tissues. In contrast, vitamins that are soluble in water (vitamins from group B and vitamin C), are not stored in the body in significant amounts (Katalinić, 2007). There are various reasons and goals for fortification of the soft drinks. By fortification of soft drinks there is the possibility of simultaneously overcoming a deficiency of more than one micronutrient. A wide variety of beverages are suitable for fortification, including fruit juices, fruit nectars, vegetable juices, fortified water, carbonated drinks, energy drinks and sports drinks. The quantity of the nutrients to be added to the fortified food corresponds to an amount generally recognised as both safe and effective by the Food and Drug Administration or the Recommended Daily Allowances-RDA. Soft drinks are usually fortified by more vitamins in order to gain greater health impact. On a weight basis, as a percentage of the total dry beverage formulation, these additional nutrients can make up from about 0 to 20 %, in terms of total dry matters. The choice of nutrients used to fortify beverages is based on a number of factors such as the chemical form of the micronutrient in terms of its bioavailability, the effects on the organoleptic characteristics of the particular beverage and cost (http://hexagonnutrition.com/published-articles/ 3.6.2017). Soft beverages can be preserved mainly in two ways: by heating and by adding chemical preservatives, as additives. The primary processing ie. the production of semi-products (juices, concentrates, puree, etc.), is performed usually separately or in another part of the factory, and most often from another producer. The technological procedure depends on the final product that we want to produce and is mainly performed in two phases. The first phase takes place in the department for syrup making, in tanks, with stirrers. Sugar syrup is prepared as a concentrated (cca 60%) sugar solution and contains 30 to 50 % final volume of water. It is prepared by cold or hot procedure or with a temperature higher than 60 ºC (by air removing and inactivating the eventual enzymes, getting more stable, better quality syrups). The syrup is pasteurized at 80 ºC, for 2 minutes, in a plate pasteurizer and it must be filtered. The fruit components are added to the sugar syrup and then follows the addition of regulators of acidity, colors, aroma, preservatives, vitamins and other ingredients. Mixing sugar syrup with other ingredients can be carried out in tanks with stirrers. The components that are previously dissolved separately are added and mixed in the tanks (Levaj, 2013). The content of the additives should not exceed the allowable quantity prescribed in the Rulebook on additives that can be used for food production (Official Gazette of the R. of Macedonia No. 31/2012, Amendments No. 114/2013). The fortification of the beverages with vitamins is planned in such a way so that the nutritive ingredients for fortification result in a desired quantity, solubility, bioavailability, pH value, temperature, light and stability of individual ingredients, overall stability, colour and flavour of the finished beverage. As a beverage becomes fortified, the pH of the beverage rises and therefore the beverage becomes more alcaline. To counter this, an acidifying additive is typically added to the beverage in order to keep the pH of the beverage lower. As the beverage is more acidic, it becomes more resistant to microbial growth. In most cases, the pH of fruit drinks and juices is below 4.5 and the heat treatment is required in the pasteurization. Some loss of the vitamins, thiamin, folic acid and ascorbic acids occurs as a result of the heating. The vitamin stability is affected most by the heat, the moisture, the pH and the light; but, given their chemical heterogeneity, vitamin losses in different foods vary considerably during both processing and storage of the final product. The most unstable vitamins are C, A, D, B_1 and B_{12} (http://hexagonnutrition.com/published-articles/ 03.06.2017). Since certain vitamins can be partially degraded during storage, their initial amount should be slightly higher than the declared, and therefore the risk of excessive intake must be taken into account (Bonner et al., 1999). The second phase is a continuous process, for homogenization, pasteurization, filling and finalization. Pasteurization is performed only if no preservatives are added, and can be performed before and after filling the beverages in a bottle. The temperature is in the range of 75-100 ºC and the heating time is determined for each product separately (Vereš, 2003). A combined preservation, by chemicals and pasteurization is also possible, and is performed before the filling: in a plate pasteurizer, if the beverage does not contain fruit particles, at a temperature of 85 to 90 ºC, for 30 to 60 seconds, or in a tubular pasteurizer if the beverage contains fruit particles. Preservatives are added to the soft drinks in a certain concentration immediately before filling, but only in the permissible concentrations, in accordance with national legislation (usually up to 120 mg/L). K-Sorbate and Na-benzoate are the most commonly used preservatives. The process of filling takes place in an aseptic block for filling (in sterile conditions) on a line with 3 processes: sterilization of bottles, filling and closing. (Levaj, 2013).

Material and methods

In this research were analyzed different non-carbonated soft drinks fortified with vitamins from domestic and foreign production, which were delivered for regular quality control during 2014, 2015 and 2016 years, at the Laboratory for food quality testing, Institute of Public Health, Skopje. A total of 75 samples were analyzed, which differ according to the composition of the basic raw materials and the technology of production. The analysis has been made of hydrosoluble vitamins (Thiamine B_1, Riboflavin B_2, Niacin B_3, Pantothenic acid B_5, Pyridoxine B_6 and vitamin C) and of vitamin E, as a liposoluble vitamin, by applying a liquid chromatography HPLC-DAD method, with prior appropriate methods of extraction for the vitamins from the samples, by organic solvents. For the hydrosoluble vitamins of group B, the extraction was performed with acetic acid (1 : 4), and detected by using HPLC method, column Supelcosil LC – 8DB, 250 x 4 mm, mobile phase: hexan-1-sulfonic acid-sodium salt, water, HPLC purity, 99-100 % acetic acid, methanol and triethylamine, pH = 3.2; flow: 2.0 mL/min.; T = 35°C; λ = 275 nm. For detection of the Pantothenic acid B_5, another mobile phase was used: a mix of two water solutions of KH_2PO_4, in different concentration, with pH value 2.5, by adding of concentrated phosphoric acid. The vitamin E was extracted with methanol, and determined by applying a gradient HPLC method, using a column Rp8, 250 mm x 5 µm, mobile phase: A methanol; B acetonitrile: methanol : water HPLC purity (63 : 33 : 4 v/v), by gradient: 0.5 min.: 60 % A : 40 % B; 0.5 min. 70 % A : 30 % B; 0.5 min. 80 % A : 20 % B; 0.5 min. 90 % A : 10 % B; 8 min. 100 % A. Vitamin C determination was performed by using a solution of 0.1 N I_2 and 1 % starch solution as an indicator. The statistical processing of the results was made according to the obtained values, in order to make a comparison between the years and during each year for those samples of products that were repeated. The obtained values for vitamins were compared with the values declared on each of the products.

Results and discussion

Based on performed analysis of total 75 samples, during the years of research, 20 different products of soft drinks were determined, according to the producer; the basic raw material from which they were produced; the type and the fruit share, individually or mixture; the production by using pasteurization or combination of pasteurization and preservatives. According to the production origin of the product, it was determined that 80 % of the analysed samples were from domestic production, and 20 % were from abroad.

In the Table 1 are presented a list of products, where each product has been replaced with a mark, according to the trade names of the producer for the soft drinks, the fruit share in the product, the content of dry matter as an important parameter for the quality of the soft drinks and the manner of production, i.e. the application of the technological pasteurization or combination pasteurization and preservatives. Regarding the fruit share, it can be concluded that 15 % of the products were with 50 % fruit share; 5 % with a fruit share of 40 %; 10 % with a 30 % fruit share; 5 % with a fruit share of 20 %; 45 % with a fruit share of 12 %; 5 % with a fruit share of 11 % and 15 % with a 10 % fruit share. In terms of the content of soluble dry matter, it has been determined that 55 % of the examined soft drinks contain 12 %; 5 % contain 11 % soluble dry matter; 25 % contain 10 % and 15 % contain 7 % soluble dry matter.

According to the technological procedure of production, 35 % of the soft drinks were produced by a pasteurization procedure, while 65 % were produced by applying a pasteurization and preservatives, as Na-bezoate and K-sorbate, in concentrations in accordance with national regulations. After the performance of the analysis and determination of the content of hydrolytic vitamins from the group B (B_1, B_2, B_3, B_5, B_6), vitamin C, and vitamin E as liposoluble vitamins, the obtained results are presented graphically in Figures 1, 2, 3. According to results it can be concluded that the vitamin C was one of the most common vitamins that is present in most of the beverages, primarily as an antioxidant, but also to increase the biological value of the product itself. The presence of the vitamin C was established in 65 % of the products, of which in 8 % of the analysed samples the presence of the vitamin C was not confirmed. In the examined samples, the content of the vitamin C was in range from 9.06 ± 0.9 mg/100 g in the soft drink marked S, with fruit share of 10 %, to 16.41 ± 1.05 mg/100 g in the soft drink marked N, with fruit share of 12 %. It can be notice that the vitamin C was the only present vitamin in beverages with a fruit share of 50 %.

Table 1. Products of soft drinks, marked according to the producer, the fruit share, content of dry matters and the technology of production

Mark	Fruit share	Dry matters	Produced by
A	min. 50 %	min. 12 %	pasteurization
B	min. 50 %	min. 12 %	pasteurization
C	min. 50 %	min. 12 %	pasteurization
D	min. 40 %	min. 12 %	pasteurization
E	min. 30 %	min. 12 %	pasteurization
F	min. 30 %	min. 12 %	pasteurization
G	min. 20 %	min. 12 %	pasteurization
H	min. 12 %	min. 12 %	pasteurization and preservatives
I	min. 12 %	min. 10 %	pasteurization and preservatives
J	min. 12 %	min. 10 %	pasteurization and preservatives
K	min. 12 %	min. 10 %	pasteurization and preservatives
L	min. 12 %	min. 11 %	pasteurization and preservatives
M	min. 12 %	min. 10 %	pasteurization and preservatives
N	min. 12 %	min. 12 %	pasteurization and preservatives
O	min. 12 %	min. 12 %	pasteurization and preservatives
P	min. 12 %	min. 12 %	pasteurization and preservatives
Q	min. 11 %	min. 10 %	pasteurization and preservatives
R	min. 10 %	min. 7 %	pasteurization and preservatives
S	min. 10 %	min. 7 %	pasteurization and preservatives
T	min. 10 %	min. 7 %	pasteurization and preservatives

According to the performed analyzes, it can be concluded that in the samples of soft drinks, the most commonly used vitamins were from group B: B_1, B_2, B_3, B_5, B_6, and for each of them has been determined presence in different combinations and concentrations in the products. According to the obtained results from the examined samples, the following concentrations were determined: vitamin B_1 was within from 0.18 ± 0,01 mg/100 g in the soft drink marked N, with 10 % fruit share, to 0.30 ± 0.05 mg/100 g in the sample of soft drink marked E, with 30 % fruit share; the values for vitamin B_2 were within the range from 0.23 ± 0.01 mg/100 g in the soft drink marked E with 30 % and H with 12 % fruit share, to 0.28 ± 0.02 mg/100 g in beverage marked M with 12 % fruit share; the lowest value of vitamin B_3, 2.40 ± 0.10 mg/100 g was determined in the soft drink sample, marked E with 30 % fruit share and the highest value 3.20 ± 0.10 mg/100 g was determined in sample marked F, with fruit share 30 %; for the vitamin B_5 has been determined the lowest concentration of 0.80 ± 0.05 mg/100 g in the sample N, with fruit share 12 %, and the highest value 1.04 ± 0.08 mg/100 g in the soft drink sample marked E, with 30 % fruit share; the obtained values for vitamin B_6 were within the range from 0.2 ± 0.01 mg/100 g in the sample R with fruit share 10 %, to 0.37 ± 0.04 mg/100 g, determined in the soft drink sample marked F, with fruit share 30 %. It has been determined that the vitamins from B group were present in 70 % of the analyzed products, of which, the most commonly present in all of the products was the vitamin B_6. It was estimated that the vitamins B_1, B_3 and B_5 were present in 71.42 % of products, of which, for the vitamin B_1 in 9.52 % and the vitamin B_5 in 5 % of the sample products, in certain years, was not detected their presence. The vitamin B_2 was determined in 35.71 % of the products where the vitamins from group B were present, from which for 36.36 % of the samples in certain years, its presence was not detected.

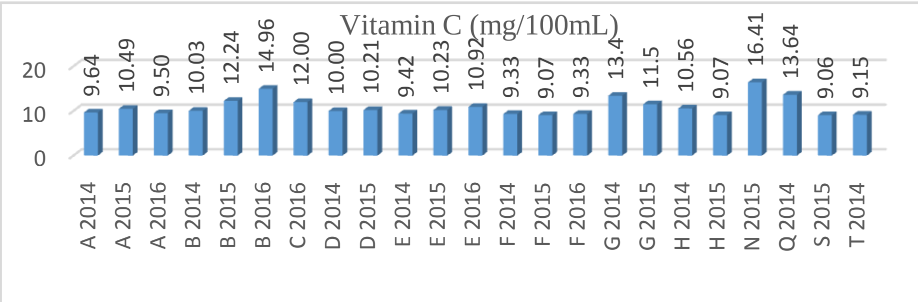

Figure 1. Content of the Vitamin C in the examined samples of the soft drinks

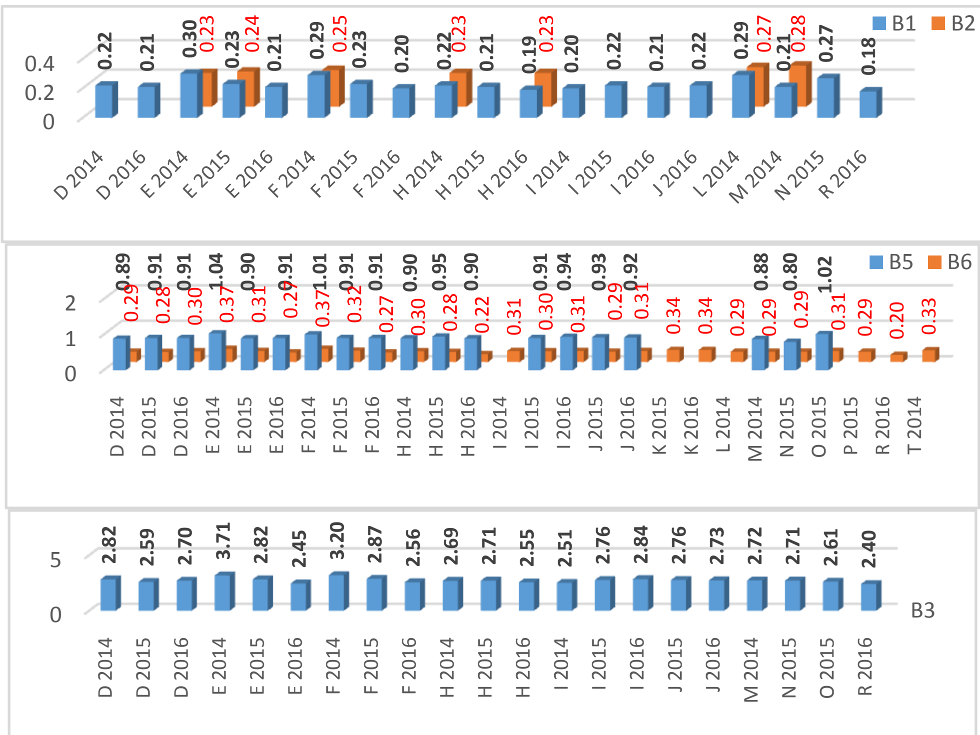

Figure 2. Vitamins B_1, B_2, B_3, B_5 and B_6 in the examined samples of soft drinks

Based on this research, the vitamin E has the lowest value of 0.7 ± 0.02 mg/100 g determined in the soft drink marked T, with 10 % fruit share, while the highest value of 1.33 ± 0.07 mg/100 g was determined in the soft drink sample marked N, with fruit share of 12 %. It was estimated that the vitamin E has been present in 40 % of the products, of which in 35.71 % of the samples its presence was not determined.

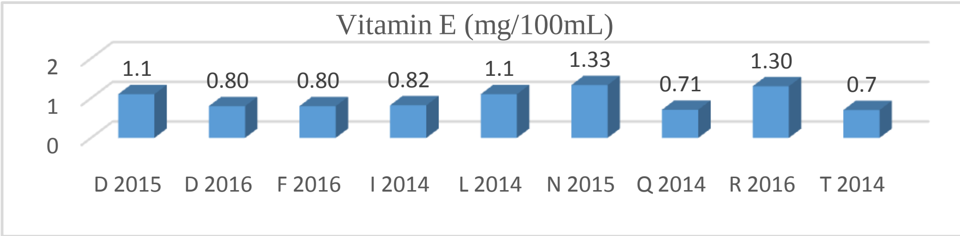

Figure 3. Vitamin E in the examined samples of soft drinks

The results for the vitamin content in the samples of various soft drink products, examined during years of research, were processed by using an ANOVA variance analysis, to determine the effect of the factor year on the quantity of vitamins in the products. Based on the statistical processing of the obtained results, it has been assesed that there were no statistically significant differences in the content of vitamins in the samples of the products examined several times during the year and in different years, but was noticed the absence of certain vitamins in certain years. A comparison was made for the obtained values with the declared values of the packaging of the products, where it is notable that in some products the presence of the vitamin A, the vitamin D, the folic acid, the biotin and the vitamin B_{12} was not determined, despite their declared value, what can be due to their low concentrations in the products or the possibility of interference during analysis with another component of the matrix or the laboratory capability to properly extracti and detecti them using the existing equipment. According to the obtained values for the vitamins, the comparison was made with those declared, and the greatest deviation of the presence of the vitamin E in the products was noticed, 40 % of the products declared its presence, but it was not detected during the analysis. For the other products, the values obtained for the vitamin E 57.14 % of products statistically significantly differ from the values on the declaration, which is due to several factors, but above all that this vitamin is liposoluble and during the technological process of fortifying the juices, it is necessary to add appropriate carriers and stabilizers. In terms of the other analysed hydrosoluble vitamins from group B and the vitamin C, it was assessed that the obtained values were not statistically significantly different from the declared values on the products.

Conclusions

Based on the research, we came to the following conclusions:

Of the total of 75 examined samples, over the three years, it was determined that there were 20 products of soft drinks, assessed according to the producer, the basic raw material, the type and the fruit share and of the technology of production. It was determined that 80 % of the analysed samples were from domestic production, and 20 % were from abroad. Regarding the fruit share, it can be concluded that 15 % of products were with 50 % fruit share; 5 % with a fruit share of 40 %; 10 % with a 30 % fruit share; 5 % with a fruit share of 20 %; 45 % with a fruit share of 12 %; 5 % with a fruit share of 11 % and 15 % with a 10 % fruit share. The vitamin C was present in 65 % of the products and is the only present vitamin in beverages with a fruit share of 50 %. It has been determined that the vitamins from B group were present in 70 % of the analysed products, of which, the most commonly present in all products was the vitamin B_6. It was estimated that the vitamin E has been present in 40 % of products, of which in 35.71 % of samples its presence was not detected. There were no statistically significant differences in the content of the vitamins in the samples of the products examined several times during the year and in different years, but the absence of certain vitamins in certain years was noticed. For the vitamin E it was noticed that for 40 % of the products its presence was declared, but not detected during the analysis. For analysed hydrosoluble vitamins, group B and the vitamin C, the obtained values were not statistically significantly different from the declared values on the products. Factors that affect the stability of the vitamins in the product are: the technological procedure, the form in which the vitamins are added, their quantity, the presence of other additives and the possibility of interaction with them, but of particular importance are also the conditions of storage and the storage of the final products (temperature, light and shelf life). The vitamins need to be added in higher quantities because it is considered that the declared amount of the given vitamin should be sustained by the end of the prescribed shelf life for the given product.

References

1. Ashurst P. R., (2005), Chemistry and Technology of Soft Drinks and Fruit Juices, Second Edition, by Blackwell Publishing Ltd, Ashurst and Associates Consulting Chemists for the Food Industry, Hereford, UK;
2. Beverage Fortification, Hexagon nutrition, naturally yours, (http://hexagonnutrition.com/published-articles/ 03.06.2017);
3. Bonner G, Warwick H., Barnardo M. and Lobstein T., (1999), Fortification examined How added nutrients can undermine good nutrition, The Food Commission (UK) Ltd, 94 White Lion Street, London N1 9PF;
4. Vereš M., (2003), Principi konzervisanja namirnica; Poljoprivredni fakultet, Beograd;
5. Ѓорѓев Д., Кендровски В., Ристовска Г., Димитровска З., (2008), Хигиена на храна и исхрана, Катедра за хигинеа, РЗЗЗ, Медицински факултет, УКИМ, Скопје;
6. Каракашова Љ., (2008), Производство на безалкохолни пијалаци, интерна скрипта, Факултет за земјоделски науки и храна, УКИМ, Скопје;
7. Katalinić V., (2007), Kemijsko-tehnološki fakultet, Sveučilište u Splitu, Temeljno znanje o prehrani, Priručnik;
8. Levaj B., (2013), Tehnologija voća i povrća, II dio, Prehrambeno-biotehnološki fakultet Sveučilišta, Zagreb;
9. Mandic M. L., (2007), Znanost o prehrani, Hrana i prehrana u cuvanju zdravlja, Izdavac Sveucilište J.J.Strossmayera u Osijeku, Prehrambeno tehnološki fakultet;
10. Official Methods of Analysis (AOAC), (1995), 16th Eds. USA p.780;
11. Правилникот за адитивите што се употребуваат во производството на храна (Службен весник на РМ бр. 31/2012); Правилник за изменување и дополнување на правилникот за адитивите што се употребуваат во производството на храната (Службен весник на РМ бр. 114/2013);
12. Правилникот за барањата во однос на квалитетот на освежителните безалкохолни пијалаци (Сл. Весник на Р.М. бр. 15/2013);
13. (http://www.canterbury.ac.nz/media/documents/science/outreach/vitaminc_iodine.pdf - 15.09.2017).