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Durim Alija

University of Tetova, durim.alija@unite.edu.mk

Eljesa Ziberi

University of Tetova

Gafur Xhabiri

University of Tetova

Xhezair Idrizi

University of Tetova

Namik Durmishii

University of Tetova

See next page for additional authors

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Presenter Information

Durim Alija, Eljesa Ziberi, Gafur Xhabiri, Xhezair Idrizi, Namik Durmishii, and Ismail Ferati

Control of Vitamin C (ascorbic acid) in non-alcoholic and fresh drinks

Durim Alija¹, Eljesa Ziberi¹, Xhezair Idrizi¹, Ismail Ferati¹

¹Faculty of Food Technology and Nutrition, University of Tetova, Tetovo,
Macedonia

durim.aliya@unite.edu.mk

Abstract. This paper deals with the method of determining vitamin C in non-alcoholic and fresh drinks, also deals with its importance in the human body and elaborates the problematic consumption of vitamin C in the daily dose of adult people. Lack of vitamin C manifests itself with: pain and rapid muscle fatigue, increased infections, anemia, osteoporosis, hemorrhage, etc. According to the highest amount of vitamin C in 100 g, there are: pepper 128 mg, 113 mg cabbage, 90 mg kiwi, 59 mg strawberries, 53 mg lemon, 51 mg spinach, 38 mg grapefruit, 23 mg tomatoes. Ascorbic acid and its sodium, potassium and calcium salts are widely used as an antioxidant food additive. Relevant food additives from ascorbic acid (E-numbers) are: E300 - ascorbic acid, E301 - sodium ascorbate, E302 - calcium ascorbate, E303 - potassium ascorbate.

Keywords: vitamin C, daily need, lack of vitamin C, ascorbic acid, food additives, E-numbers

INTRODUCTION

Vitamin C attracts attention of the research community and consumers as a nutrient with a broad biological activity and importance for human health. It is a white, crystalline, odourless, water-soluble compound. Main sources of vitamin C are fruits and vegetables (green and red peppers, collard greens, broccoli, spinach, tomatoes, potatoes, strawberries, oranges, etc.). Vitamin C supports the absorption of iron and the formation of collagen. Vitamin C is often added to foods not only as a nutrient (to make up for processing losses) and an antioxidant ^[1], but it is added also in order to prevent the browning of fresh and canned fruits and vegetables.

Vitamin C is often added as a fortificant to fruit juices, fruit-flavoured drinks, juice added soda waters, dry cocktails or beverages, cereal-based products, and milk. General principles for the addition of essential nutrients to foods are given by the Codex Alimentarius Commission. The Codex definition of fortification is „the addition of one or more essential nutrients to a food whether or not it is normally contained in the food, for the purpose of preventing or correcting a demonstrated deficiency of one or more nutrients in the population or specific population groups“ [2].

¹ Kirby, C. J. - Whittle, C. J. - Rigby, N. - Coxon, D. T. - Law, B. A.: Stabilization of ascorbic acid by microencapsulation in liposomes. International Journal of Food Science and Technology, 26, 1991

² Food fortification: technology and quality control. Report of an FAO technical meeting held in Rome, 20-23 November '95

Ascorbic acid is recognized to be one of the most heat sensitive nutrients in foods, therefore, it is a marker of the loss of other nutrients^[3]. The aim of this article is to review the papers on ascorbic acid degradation in fortified foods at various storage temperatures. For the sake of comparing the published data and finding relevant trends, the rate constants of degradation are calculated.

Recommended intakes

Intake recommendations for vitamin C and other nutrients are provided in the Dietary Reference Intakes (DRIs) developed by the Food and Nutrition Board (FNB) at the Institute of Medicine (IOM) of the National Academies (formerly National Academy of Sciences)^[4]. DRI is the general term for a set of reference values used for planning and assessing nutrient intakes of healthy people. These values, which vary by age and gender⁴, include:

- Recommended Dietary Allowance (RDA): average daily level of intake sufficient to meet the nutrient requirements of nearly all (97%–98%) healthy individuals.
- Adequate Intake (AI): established when evidence is insufficient to develop an RDA and is set at a level assumed to ensure nutritional adequacy.
- Tolerable Upper Intake Level (UL): maximum daily intake unlikely to cause adverse health effects^[4].

Table 1 lists the current RDAs for vitamin C⁴. The RDAs for vitamin C are based on its known physiological and antioxidant functions in white blood cells and are much higher than the amount required for protection from deficiency^[5]. For infants from birth to 12 months, the FNB established an AI (Adequate Intake) for vitamin C that is equivalent to the mean intake of vitamin C in healthy, breastfed infants.

Table 1: Recommended Dietary Allowances (RDAs) for Vitamin C

<i>AGE</i>	<i>MALE</i>	<i>FEMALE</i>	<i>PREGNANCY</i>
<i>0-6 months</i>	40 mg	40 mg	
<i>7-12 months</i>	50 mg	50 mg	
<i>1-3 year</i>	15 mg	15 mg	
<i>4-8 year</i>	25 mg	25 mg	
<i>9-13 year</i>	45 mg	45 mg	
<i>14-18 year</i>	75 mg	65 mg	80 mg
<i>19+ year</i>	90 mg	75 mg	85 mg
<i>SMOKERS</i>	Individuals who smoke require 35 mg/day more vitamin C than nonsmokers.		

³ Esteve, M. J. - Frigola, A. - Martorell, L. - Rodrigo, C.: Kinetics of green asparagus ascorbic acid heated in a high-temperature thermoresistometer

⁴ Institute of Medicine. Food and Nutrition Board. [Dietary Reference Intakes for Vitamin C, Selenium, and Carotenoids](#)

⁵ Jacob RA, Sotoudeh G. Vitamin C function and status in chronic disease.

1.3 Sources of Vitamin C

Fruits and vegetables are the best sources of vitamin C (see Table 2) [6]. Citrus fruits, tomatoes and tomato juice, and potatoes are major contributors of vitamin C to the American diet. Other good food sources include red and green peppers, kiwifruit, broccoli, strawberries, Brussels sprouts, and cantaloupe (see Table 2). Although vitamin C is not naturally present in grains, it is added to some fortified breakfast cereals. The vitamin C content of food may be reduced by prolonged storage and by cooking because ascorbic acid is water soluble and is destroyed by heat [7]. Steaming or microwaving may lessen cooking losses. Fortunately, many of the best food sources of vitamin C, such as fruits and vegetables, are usually consumed raw. Consuming five varied servings of fruits and vegetables a day can provide more than 200 mg of vitamin C.

Table 2: Selected Food Sources of Vitamin C [8]

<i>Fruits, vegetables & drinks</i>	<i>Mg/serving</i>	<i>Percent(%) DV*</i>
Red pepper	95	158
Orange juice, cup	93	155
Orange, 1 medium	70	117
Grapefruit juice, cup	70	117
Green pepper	60	100
Broccoli	51	85
Strawberries, fresh	49	82
Tomato juice	33	55
Spinach cooked	9	15

*DV = Daily Value. DVs were developed by the U.S. Food and Drug Administration (FDA) to help consumers compare the nutrient contents of products within the context of a total diet. The DV for vitamin C is 60 mg for adults and children aged 4 and older. The FDA requires all food labels to list the percent DV for vitamin C. Foods providing 20% or more of the DV are considered to be high sources of a nutrient.

VITAMIN C INTAKES AND STATUS

According to the 2001–2002 National Health and Nutrition Examination Survey (NHANES), mean intakes of vitamin C are 105.2 mg/day for adult males and 83.6 mg/day for adult females, meeting the currently established RDA for most nonsmoking adults [9]. Mean intakes for children and adolescents aged 1-18 years range from 75.6 mg/day to 100 mg/day, also meeting the RDA for these age groups. Although the 2001–2002 NHANES analysis did not include data for breastfed infants and toddlers, breastmilk is considered an adequate source of vitamin C. Use of vitamin C-containing supplements is also relatively common, adding to the total vitamin C intake from food and beverages. NHANES data from 1999–2000 indicate that approximately 35% of adults take multivitamin supplements (which typically contain vitamin C) and 12% take a

⁶ U.S. Department of Agriculture, Agricultural Research Service. 2011. USDA National Nutrient Database for Standard Reference, Release 24

⁷ Weinstein M, Babyn P, Zlotkin S. An orange a day keeps the doctor away: scurvy in the year 2000

⁸ Also in the 6th Footnote

⁹ Moshfegh A, Goldman J, Cleveland L. What We Eat in America, NHANES 2001-2002: Usual Nutrient Intakes from Food Compared to Dietary Reference Intakes

separate vitamin C supplement. According to 1999–2002 NHANES data, approximately 29% of children take some form of dietary supplement that contains vitamin C [10].

Vitamin C status is typically assessed by measuring plasma vitamin C levels [11]. Other measures, such as leukocyte vitamin C concentration, could be more accurate indicators of tissue vitamin C levels, but they are more difficult to assess and the results are not always reliable.

Vitamin C and health

Due to its function as an antioxidant and its role in immune function, vitamin C has been promoted as a means to help prevent and/or treat numerous health conditions. This section focuses on four diseases and disorders in which vitamin C might play a role: cancer (including prevention and treatment), cardiovascular disease, age-related macular degeneration (AMD) and cataracts, and the common cold.

Health risks from excessive Vitamin C

Vitamin C has low toxicity and is not believed to cause serious adverse effects at high intakes. The most common complaints are diarrhea, nausea, abdominal cramps, and other gastrointestinal disturbances due to the osmotic effect of unabsorbed vitamin C in the gastrointestinal tract [12]. Other reported effects of high intakes of vitamin C include reduced vitamin B₁₂ and copper levels, accelerated metabolism or excretion of ascorbic acid, erosion of dental enamel, and allergic responses. However, at least some of these conclusions were a consequence of assay artifact, and additional studies have not confirmed these observations.

The FNB has established ULs for vitamin C that apply to both food and supplement intakes (Table 3) [13]. Long-term intakes of vitamin C above the UL may increase the risk of adverse health effects. The ULs do not apply to individuals receiving vitamin C for medical treatment, but such individuals should be under the care of a physician [14].

Table 3: Tolerable Upper Intake Levels (ULs) for Vitamin C

AGE	MALE	FEMALE	PREGNANCY
1-3 year	400 mg	400 mg	
4-8 year	650 mg	650 mg	
9-13 year	1200 mg	1200 mg	
14-18 year	1800 mg	1800 mg	2000 mg
19+ year	2000 mg	2000 mg	2000 mg

¹⁰ Picciano MF, Dwyer JT, Radimer KL, Wilson DH, Fisher KD, Thomas PR, et al. Dietary supplement use among infants, children, and adolescents in the United States, 1999-2002

¹¹ Bates CJ. Bioavailability of vitamin C. *Eur J Clin Nutr* 1997;51 (Suppl 1):S28-33.

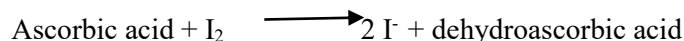
¹² Francescone MA, Levitt J. Scurvy masquerading as leukocytoclastic vasculitis: a case report and review of the literature

¹³ Also in the 4th Footnote

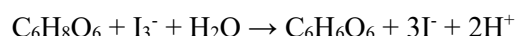
¹⁴ Also in the 4th Footnote

METHOD OF CONTROLLING VITAMIN C

Importance of vitamin C for our health leads us to explore the methods of detection of Vitamin C in food. One way to determine the amount of vitamin C in food is by redox titration using iodine. As the iodine is added during the titration, the ascorbic acid is oxidized to dehydroascorbic acid, while the iodine is reduced to iodide ions.



As it is demonstrated in the below example:



As long as vitamin C is present in the solution, the triiodide is converted to the iodide ion very quickly. However, when all the vitamin C is oxidized, iodine and triiodide will be present, which react with starch to form a blue-black complex. The blue-black color is the endpoint of the titration.

This titration procedure is appropriate for testing the amount of vitamin C in vitamin C tablets, juices, and fresh, frozen, or packaged fruits and vegetables. The titration can be performed using just iodine solution and not iodate, but the iodate solution is more stable and gives a more accurate result.

The goal of this laboratory exercise is to determine the amount of vitamin C in samples, such as fruit juice.

EXPERIMENTAL PROCEDURE

For right results from this experimental procedure, we need to prepare the solutions very accurately. The key solutions for this experimental are:

3.2 Starch indicator solution: (0.5%).

To prepare the starch indicator we need to weigh 0.50 g of soluble starch and add it to 50 mL of near boiling water in a 100-mL conical flask. Stir to dissolve and we use it cooled.

IODINE SOLUTION

For iodine solution we weigh 5 g of potassium iodide and 0.268 g potassium iodate into a 200 mL of distilled water. Then we add 30ml of 3M sulfuric water. Finally, we transfer iodine solution to a 500ml volumetric flask, making sure to rinse all traces of solution into the volumetric flask using distilled water.

VITAMIN C STANDARD SOLUTION

Dissolve 0.250 g vitamin C (ascorbic acid) in 100 ml distilled water. Dilute to 250 ml with distilled water in a volumetric flask. Label the flask as your vitamin C standard solution.

Beside the accordingly making solutions, it's important to make the right Standardization of Solutions. For standardization of solution we need to add 25.00 ml of vitamin C standard solution to a 125 ml Erlenmeyer flask. Then we add drops of 1% starch solution. Finally, we note the amount of iodate solution used for titration.

*From the titration of the standard vitamin C solution, these data were obtained:

1. from the first titration we spend **24ml** iodate solution
2. from the second titration we spend **22ml** iodate solution

3. from the third we spend **20ml** iodate solution
Now we calculate the average value of iodate solution:

$$V = \frac{24ml+22ml+20ml}{3} = 22ml \text{ iodine solution was used to titrate the standard vitamin C solution}$$

TITRATING JUICE SAMPLES

1. We add 25.00 ml of juice sample to a 125 ml Erlenmeyer flask.
2. We have titrated until the endpoint is reached. (Adding iodine solution until you get a color that persists longer than 20 seconds.)
3. We have repeat 3 times the same titration.

TITRATING REAL FRUIT

1. We have added 10.00 ml of Real Lemon into a 125 ml Erlenmeyer flask.
2. For this titrate we have used three measurements.

OUR SAMPLES TESTED:

Titration juice samples:

From juice we have samples from two local producers:

1. Fructal multivitamin 200ml
2. Bravo orange 500 ml

Titration real fruit samples:

From juice fresh fruit we have taken samples from two types:

1. Lemon juice
2. Orange juice

How we have calculated Vitamin C

1. We have calculated the ml of titrant used for each flask. Take the measurements obtained and average them.

$$\text{average volume} = \text{total volume} / \text{number of trials}$$

2. Then we determine how much titrant was required for your standard. Example with 6 ml spent of iodine solution in titration:

$$\frac{22ml \text{ iodine solution}}{0.250 g \text{ of Vitamin C}} = \frac{6.00 ml \text{ iodine solution}}{X ml \text{ of Vitamin C}}$$

$$22X = 1.5$$

$$X = 0.068g \text{ Vitamin C in that sample}$$

3. Also, we must have finally calculation in mind for the volume of our sample, so we can make other calculations, such as grams per liter. For a 25-ml juice sample, for example:

$$\frac{0.068g}{25 ml} = \frac{0.068g}{0.025L} = 2.72 g/L \text{ of Viramin C in that sample}$$

RESULTS:

Data processing:

From standard vitamin C titration are obtained these data:

1. From the first titration we spent:
 - I. 3.3 ml iodine solution
 - II. 3.4ml iodine solution
 - III. 3.5ml iodine solution
2. From the se second titration we spent:
 - I. 3.1 ml iodine solution
 - II. 2.9 ml iodine solution
 - III. 3.3 ml iodine solution
3. From the third titration we spent:
 - I. 5.0 ml iodine solution
 - II. 5.1 ml iodine solution
 - III. 5.5 ml iodine solution
4. From the fourth titration we spent:
 - I. 4.3 ml iodine solution
 - II. 4.7 ml iodine solution
 - III. 4.8 ml iodine solution

TITRATION CALCULATIONS

For the first titration (Fructal multivitamin)

$$\frac{22ml \text{ iodine solution}}{0.250g \text{ Vitamin C}} = \frac{3.4ml \text{ iodine solution}}{X \text{ ml Vitamin C}}$$

$$22X = 0.85$$

$$X = 0.038g \text{ Vitamin C in that sample}$$

$$\frac{0.038g}{25 ml} = \frac{0.038g}{0.025L} = 1.5 g/L \text{ of Viramin C in that sample}$$

For the second titration (Bravo Orange)

$$\frac{22ml \text{ iodine solution}}{0.250g \text{ Vitamin C}} = \frac{3.1ml \text{ iodine solution}}{X \text{ ml Vitamin C}}$$

$$22X = 0.77$$

$$X = 0.035g \text{ Vitamin C in that sample}$$

$$\frac{0.035g}{25 ml} = \frac{0.035g}{0.025L} = 1.4 g/L \text{ of Viramin C in that sample}$$

For the third titration (Lemon juice)

$$\frac{22\text{ml iodine solution}}{0.250\text{g Vitamin C}} = \frac{5.2\text{ ml iodine solution}}{X\text{ ml Vitamin C}}$$
$$22X = 1.3$$
$$X = 0.059\text{g Vitamin C in that sample}$$

$$\frac{0.059\text{g}}{10\text{ ml}} = \frac{0.059\text{g}}{0.010\text{L}} = 5.9\text{ g/L of Vitamin C in that sample}$$

For the fourth titration (Orange juice)

$$\frac{22\text{ml iodine solution}}{0.250\text{g Vitamin C}} = \frac{4.6\text{ ml iodine solution}}{X\text{ ml Vitamin C}}$$
$$22X = 1.15$$
$$X = 0.052\text{g Vitamin C in that sample}$$

$$\frac{0.052\text{g}}{10\text{ ml}} = \frac{0.052\text{g}}{0.010\text{L}} = 5.2\text{ g/L of Vitamin C in that sample}$$

DISCUSSION

To achieve results for quantity of vitamin C in food we could use also the developed method who proved us with accuracy in vitamin C determination in fruit juice. Although other volumetric methods are more sensitive than developed methods for determining ascorbic acid, volumetric methods can be used with very good results to analyze ascorbic acid in fruit juice. Classical volumetric method to determinate the concentrations of ascorbic acid in fruit juice determined is in good agreement with the data reported in literature regarding the content of ascorbic acid in citrus fruits. The reported values for lemon are 5.9 g/L juice fruit, for multivitamin juice the result was 1.5g/L, in bravo juice the result was 1.4g/L. Other results were content of 5.2 g/L in fresh orange juice. With the result we wanted to prove that with volumetric method we can successfully make analyzes in food industry, for assessing the vitamin C content in natural fruit juice and soft drinks. This method is easy to implement and enables companies to have an internal control of vitamin C in soft drinks in or case. The results prove why, recently, this technique has been more and more preferred to the previously applied methods, as it is characterized by accuracy, rapidity, good specificity, and sensitivity, and also by the simplicity of the required equipment and procedure.

CONCLUSION

Knowing the importance of daily vitamin C content in our body, we decided to analyze two types of products we consume every day. We have analyzed fresh citrus fruits and soft drinks. Ultimately, Vitamin C is arguably one of the most important vitamins for health benefits and life. Its various functions prove it to be a versatile substance that acts to support many aspects of the body. With our study we wanted to compare the real content of Vitamin C in soft drinks and the label of the product. So, with the analyzed of fresh juice we wanted to know the real content of vitamin C in fresh fruits from our stores. Also, our goals were to see how much Vitamin C contain the juice with concentrate base and those one from fresh fruits.

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