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Vitamin C determination in foods. Titrimetric methods – A review

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Abstract

Vitamin C or ascorbic acid is essential for human life and is required for a range of physiological functions in the human body. Ascorbic Acid (AA) is a natural and powerful water-soluble antioxidant.

The importance of vitamin C in keeping the human organism in good health has been extensively documented. Human beings cannot produce vitmin C on its own, as it does not possess the required enzymes (L-gulonolactoneoxidase), similar to other primates, guinea pigs, rats and bats.

In food analysis, the correct method for the determination of vitamin C should measure the combined content of ascorbic acid and dehydroascorbic acid to determine the total content of vitamin C. There are a large number of methods permitting direct analysis of ascorbic. The purpose of this work paper is to present different titration methods used for determination of vitamin C

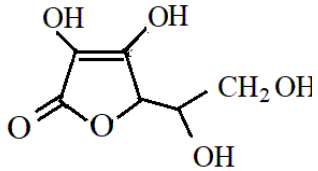
Key words: ascorbic acid, titrimetric method, spectrophotometric method, food

1. Introduction

Health is very important for humanity. In order to survive, people must be healthy. Vitamins have an important role for human health. Ascorbic acid, rather known as vitamin C, is an essential nutrient that acts as a terrific antioxidant and involves many physiological functions from collagen synthesis to immune defense and iron absorption. It is found in natural forms in many foods, especially in fruits and vegetables, and is often added to foods as preservative or nutritional supplement.

Vitamin C is called ascorbic acid (AA) and its chemical formula is $C_6H_8O_6$. Dehydroascorbic acid (DHAA) is also a form of vitamin C because it displays high biological activity along with AA. Dehydroascorbic acid exhibits the properties of vitamin C, as it is readily reduced in the body to ascorbic acid [1,2,3,4].

The L enantiomer is the biochemically and physiologically active form of ascorbic acid. Ascorbic acid is a water-soluble compound with a strong antioxidant character required by the human body. Ascorbic acid is a very important reducing agent in biochemical processes [2,5] being easily oxidated to dehydroascorbic acid (DHAA).



AA

Figure 1. The structure formula of ascorbic acid.

The name vitamin C is attributed to ascorbic acid and dehydroascorbic acid, as both forms

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display high biological activity. AA is known for its reductive properties, being easily oxidated to DHAA. Dehydroascorbic acid displays the properties of vitamin C, as it is easily reduced in the organism to ascorbic acid [3,5].

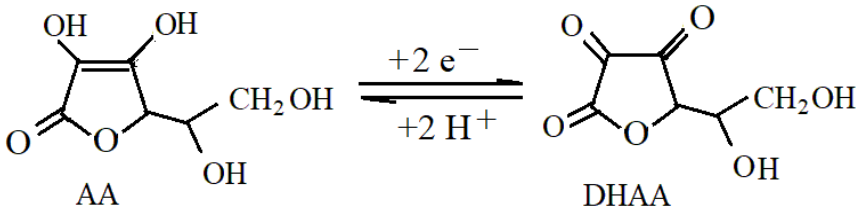

Figure 2. The structures of L-Ascorbic acid (L-AA) and Dehydroascorbic acid (DHAA)

As time passes, the AA content in products sharply decreases, and they become increasingly degraded. Some properties of ascorbic acid are displayed in table 1 [6,7,8].

Table 1. Properties of L-ascorbic acid

Chemical name	L-ascorbic acid
Empirical formula:	$C_6H_8O_6$
Molar mass ($g \cdot mol^{-1}$):	176.13
Appearance:	Crystalline powder White to slightly yellow
Solubility	
in water:	33 g/100 mL;
in ethanol:	2 g/100 mL;
	Freely soluble in water, sparingly soluble in alcohol, insoluble in ether diethyl ether, petroleum ether, chloroform and benzene
Taste:	Strong acidic taste
Odour:	Odourless
Density ($g \cdot cm^{-3}$)	1.65
pH:	≈ 3 (5 mg/mL); ≈ 2 (50 mg/mL)
Acidity (pKa):	4.17 (pKa1); 11.6 (pKa2)
Melting point:	190°C – 192°C

In plants and most animals, vitamin C is synthesized from glucose in the presence of the enzyme L-gulonolactone oxidase. In humans and primates the catalytic enzyme (L-gulono-lactone oxidase) is missing. Because humans (as well as other primates and guinea pigs) are unable to synthesize ascorbic acid, they need to take vitamin C from the diet [2,3,9]. Ascorbic acid synthesis occurs in various organs of animals, in amphibians, reptiles and fish it occurs in the kidneys, while in mammals the site of synthesis is in the liver [9].

Ascorbic acid is found in natural forms in many foods, especially in fruits and vegetables, and is often added to foods as a preservative or nutritional supplement. There are various fruits with a significant content of vitamin C, among them we can mention the following: citrus fruits, tropical fruits, berries, apples, pears, quince, sea buckthorn, kiwi and many others. Citrus fruits (oranges, lemons, limes, and grapefruit) are among the best-known sources of vitamin C, for instance a medium orange usually supplies about 70 mg of vitamin C [5]. Berries (strawberries, raspberries, blueberries, and blackberries) are some of the richest sources of ascorbic acid. About 89 mg is provided by a cup of strawberries [10].

Top vegetable sources include bell peppers, especially red ones, broccoli, Brussels sprouts, and kale. One cup of raw bell peppers contains about 120 mg of vitamin C [10]. Potatoes and Tomatoes are generally consumed food items that provide vitamin C. A medium potato contains around 20 mg [6,11]. Many breakfast cereals, fruit juices, and plant-based milk are fortified with ascorbic acid to enhance nutritional value [12]. Vitamin C is frequently added to processed foods for its preservative and antioxidant properties. It prevents browning in fruits and vegetables and improves the shelf life of products [13]. This vitamin is sensitive to heat, light, and oxygen. Cooking methods such as boiling or steaming can lead to significant losses (up to 50-70%), while microwaving or steaming helps retain more nutrients [14]. The recommended dietary allowance (RDA) for vitamin C varies by age, sex, and physiological status, thus for adult males the RDA is 90 mg/day, for adult females the recommended dose is 75 mg/day; smokers need an additional 35 mg/day due to increased oxidative stress [15].

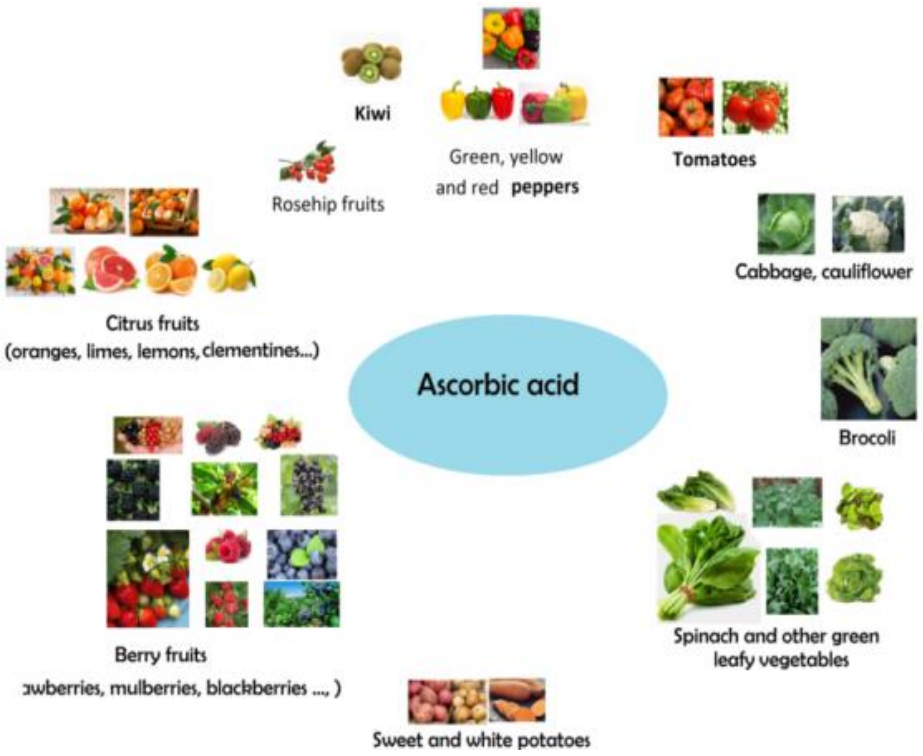

Figure 3. Different sources of vitamin C

2. Vitamin C Analysis Methods – Overview

Food analysis represents an important research area in current chemistry, mainly due to the continuous emergence of new foods, supplements, nutraceuticals, etc., requiring the availability of fully validated analysis methods.

The determination of vitamin C in various samples, such as foods, supplements or pharmaceuticals, can be performed by a variety of analytical methods (chemical, spectrophotometric, electrochemical, chromatographic methods). The choice of method depends on the type and complexity of the sample, the level of precision desired and the equipment available. There are several methods to accurately quantify the concentration of AA from natural samples, food, biological fluids and pharmaceutical formulations. Regarding the methods reported in the literature, the following classification can be made:

1. Titrimetric method
2. UV spectrophotometric method
3. Electrochemical methods
   i) Voltammetric method
   ii) Potentiometric method
   iii) Amperometric method
4. Enzymatic method
5. Fluorometric method
6. Chemiluminometric method
7. Chromatographic methods
   i) Liquid chromatography (LC)
      a. High performance liquid chromatography (HPLC)
      b. Ultra performance liquid chromatography (UPLC)
   ii) Gas chromatography (GC)
8. Capillary electrophoresis

Vitamin C (ascorbic acid) is a crucial nutrient in human diets, and its accurate quantification in foods and supplements is essential for quality control and regulatory compliance. Several analytical methods are used for determining vitamin C, ranging from simple titrimetric techniques to advanced chromatographic and spectrophotometric methods.

The most commonly used analytical methods are presented below. In this work, the tritirimetric and spectrophotometric methods used for the determination of vitamin C in food will be presented.

3. Titrimetric Method

Titrimetric methods, also known as volumetry, are analytical techniques that involve measuring the volume of a titrant of known concentration required to react completely with a substance in the sample. These methods are classified according to the nature of the chemical reaction

between the titrant and the analyte.

Titrimetric methods for analyzing vitamin C are simple, rapid, cost-effective, economical, and adaptable to routine analyses. These methods are especially suitable for foods and supplements containing measurable quantities of vitamin C without excessive interference from other reducing agents. These methods are based on the redox properties of vitamin C, which acts as a reducing agent. The choice of method depends on the chemical reactions involved and the sensitivity required. The most commonly used approaches are: iodometric titration – based on reduction of iodine by ascorbic acid; and redox titration with 2,6-dichlorophenolindophenol (DCPIP) – based on the reduction of a redox dye by ascorbic acid.

Chemical dosage methods of vitamin C in foods (or in any product) are based on the reducing property of ascorbic acid. Quantitative determination method of Vitamin C are generally accomplished volumetrically and the titration solutions are oxidants.

Traditionally used titrimetric methods are based on the use of an oxidizing solution such as dichlorophenol indophenol (DCPIP), potassium iodate or potassium bromate, methylene blue [16,17,18,19]. These compounds/substances react with vitamin C and depending on the volume of solution used, vitamin C is quantitatively determined.

3.1. Iodometric and iodimetric titration

The method is based on the redox reaction between ascorbic acid and iodine. Ascorbic acid reduces iodine (I_2) to iodide (I^-) until all ascorbic acid in the sample is oxidized The reaction endpoint is determined using a starch indicator, which forms a blue-black complex with iodine.[6,16].

Iodimetric method

The conversion of I_2 to iodide (I^-) is a reversible reaction. This redox system has a reduction potential of around 0.54 V [19]. Substances with a lower reduction potential than iodine readily lose electrons and act as reducing agents relative to iodine (I_2). Iodimetric titrations are those titrations in which iodine is used to determine the concentration of a reducing agent. In this method, ascorbic acid is a reducing agent and the ascorbic acid solution is titrated with an iodine solution using starch as an indicator. In this titration ascorbic acid is oxidized to dehydroascorbic acid and I_2 is reduced to (I^-).

$$\underset{\text{AA}}{C_6H_8O_6} + \underset{\text{iodine}}{I_2} \rightarrow \underset{\text{DHAA}}{C_6H_6O_6} + \underset{\text{iodide } (I^-)}{2HI}$$

When complete oxidation of AA to DHAA occurs, the excess iodine reacts with the starch (indicator) and forms a blue-black starch-iodine complex, indicating the endpoint of the redox titration. The iodimetric method is simpler than the potassium iodate method [19,20].

Determination of vitamin C content, by direct titration with iodine, was carried out by Suntornsuk L. *et al.* in 2002, from fresh and freeze-dried herbal juices (of guava, lemon, sweet pepper and passion fruit). This technique showed excellent linearity over the concentration range tested (100-500) of vitamin C content found in juice sample with good precision and recovery [6,21,22].

These methods are simple and inexpensive and the minimum equipment is required for analysis. A limitation of this method is given by the interference of other reducing agents present in the sample matrix.

Iodometric titration

Iodometric method can also be used to determine vitamin C ($C_6H_8O_6$). There are many oxidizing (oxidants) agents (e.g. potassium iodate, potassium permanganate and potassium dichromate) that have higher reduction potential values than iodine.

The value of the reduction potential is directly proportional to the strength of the oxidizing agent, Thus, potassium permanganate, potassium iodate, and potassium dichromate are stronger oxidizing agents than iodine (I_2).

$$\text{Oxd agnt} + KI \rightarrow I_2 + \text{Rd Oxd agent}$$

where:

Oxd agt – oxidizing agent,

Rd Oxd agt – reduced form of oxidizing agent

The iodometric method uses iodine as an oxidant, which is achieved from the action of iodine on potassium iodide. Ascorbic acid solution (extract) contains a strong acid (such as sulfuric acid or hydrochloric acid) and potassium iodide (KI). Potassium iodate is added to this extract. Potassium iodate reacts with KI to release I_2. The iodine produced reacts with ascorbic acid ($C_6H_8O_6$) to form

dehydroascorbic acid ($C6H_8O_6$) and iodide ions (I^-).

$$KIO_3 + 5KI + 6H^+ + \rightarrow I_2 + 6K + 3I_2 + 3H_2O$$

$$C_6H_8O_6 + I_2 \rightarrow C_6H_6O_6 + 2I^- + 2H^+$$

The potassium iodide is always added in excess to ensure completion of reaction and to dissolve the iodine. After complete utilization of ascorbic acid, the excess I_2 remaining in the solution can be titrated with sodium thiosulphate solution using starch as an indicator [16,19]. Starch forms an intense blue colored complex with I_2 and at the end point of the titration the blue colour disappears.

$$I_2 + 2Na_2S_2O_3 \rightarrow NaI + Na_2S_4O_6$$

By the amount of sodium thiosulphate used in the titration the remaining I_2 after reaction with ascorbic acid can be calculated. Thus iodometry is an indirect titration for estimating the concentration of ascorbic acid [23]

3.2. Dichlorophenolindophenol titration method

This method is also called the dye titration method. The principle of this method is a titration of ascorbic acid with dichlorophenolindophenol. 2,6-dichlorophenolindophenol (DCPIP) is a redox dye, used as the titrant.This dye titration method, (AOAC 2012, 2016) uses the reducing power of the vitamin, and employs 2,6-dichlorophenolindophenol as the redox indicator for the ascorbic acid determination. The principle of this method is a titration of ascorbic acid with dichlorophenolindophenol [19]. Ascorbic acid reduces DCPIP to a colorless compound, and the endpoint is observed as the first appearance of a pink color in acidic conditions

The most commonly used titration methods are iodometric and 2,6-dichlorophenolindophenol (DCPIP) redox titration.

In the reaction between Vitamin C (AA) and DCPIP, the color changes from blue to colorless. The extraction of vitamin C from the food (samples) is carried out with meta-phosphoric acid (MPA) and the pH adjusted to 1.2. The food extract is then titrated against 2,6-dichlorophenol indophenol, which is a redox reaction, In this titration ascorbic acid in the extract is oxidized to DHAA and the dye is reduced to a colourless compound. The end point of titration is a pink colour due to the excess unreduced dye in acid solution. If the extracts are intensely colored, repeated extraction with ether is performed to facilitate easy detection of the end point [19].

They react in a 1:1 manner, so if a known quantity of DCPIP solution reacts with the plant tissue extract, the quantity of DCPIP used gives a direct measure of the quantity of ascorbic acid present [19,24].

DCPIP titration is suitable for fresh juices and multivitamin supplements that do not contain significant quantities of copper or iron. However, highly colored extracts from fruits and vegetables, for example, can mask color changes at the end point [25]. The advantages and limitations of titrimetric methods are briefly presented in Table 2.

Table 2. Advantages and limitations of titration methods

Feature	Iodometric Titration	DCPIP Titration
Sensitivity	Moderate	High
Specificity	Moderate	High
Sample complexity	Simple	Simple to moderately complex
Equipment Requirements	Basic lab setup	Basic lab setup
Interference Susceptibility	High	Low

Iodometric and DCPIP titrations are indeed widely used for the estimation of vitamin C in foods and pharmaceuticals due to the robustness of the methods. These techniques are satisfactory for routine quality control analyses, but for more difficult matrices, other approaches have been investigated.

4. Conclusion

Ascorbic acid (vitamin C), a water-soluble compound, is a particularly important vitamin for humans. Its deficiency could have consequences manifested by health problems. Since the human body cannot produce it, this vitamin is taken only through food, or different other intake products. Various natural and prepared products (food supplements, foods – prepared or natural) are available for human consumption.

Titrimetric methods for analyzing vitamin C are simple, rapid, cost-effective, economical, and adaptable to routine analyses.

There are different methods available for the analysis of ascorbic acid. However, there is

still a need to find noble, rapid, reliable, accurate, cost-effective and easy-to-implement methods to determine vitamin C.

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