QUANTITATIVE ANALYSIS OF ASCORBIC ACID CONTENTS OF SOME SELECTED FRUITS FOR PREGNANT AND LACTATING WOMEN IN IJANIKIN, LAGOS STATE

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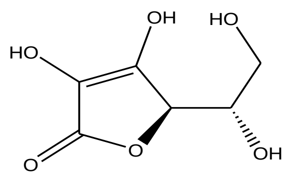
Abstract
Ascorbic acid in vitamin C is a water soluble antioxidant, which is found to be essential co-factor for stimulating the production and maturation of stable collagen molecules in human bodies. It's also responsible for increasing the amount of collagen-specific messenger RNA (mRNA). Ascorbic acid is also essential for hydroxylation of proline and lysine. This study determines the ascorbic acid contents of selected citrus fruits and that of a commercially available vitamin C product. The process involved direct treatment of ascorbic sorbose with potassium tetra-oxomanganate (vii), and that of direct filtration methods with standard iodine. It was discovered that the values obtained from direct filtration were considerably low compared with that of back titration with sodium thiosulfate. It was established that there was appreciable amounts of ascorbic acid in the sampled citrus fruits. The experiments in this study recommended the need to include citrus fruits in diets as antioxidant protection, immune system support, collagen production, healing wounds, iron absorption, and for growth and repairs in human bodies.
Keywords: Quantitative, Ascorbic Acid, Antioxidant, Fruits

Introduction

The good health and physical well-being of humans are ensured by consuming balanced diets, which contains a number of accessory factors termed vitamin (A, B, C, D, E and K),together with the other classes of food substance. The water soluble vitamins C, was discovered by a Nobel laurcate – Albert Szent – Gyorghly in 1937. He initially isolated the compound from plant fruit juices and animal glands and confirmed its anti-scorbutic factor that prevents scurvy. Albert Szent- Gyorghly earlier described vitamins C as hexuronic acid (Lykkesfeldt, 2020). He described the sugar like vitamin as making the body work better so that it is stronger and healthier. He emphasized further that, it is stored in the adrenal and pituitary glands, in the kidney, liver, ovary, eye and other organs. It is released during heavy exercise and intense strain or stress. Ashor, et al, (2019) stated that the chemistry of pharmacology of vitamin C is complex and has unfortunately rarely been taken into account when designing clinical studies in testing its effects on human health (Ashor, et al, 2019).

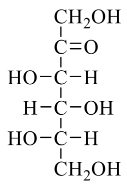
Another Nobel Prize winner Pauling (1974) while writing on the Vitamin C, highlighted that the ascorbic acid present in Vitamin C, is responsible for its prevention of scurvy and cold. He recommended that 1gm of Vitamin C daily, would short the frequency of colds by 45% and reduce the total days of illness by 60 percent. In a corresponding research carried out by Cameron and Pauling (1976) revealed that, a daily dose of Vitamin C had improved the lives of one hundred (1000) cancer patients in Scotland. Marik (2018) claimed to have being using Vitamin C therapy in treating viral diseases including hepatises, mononucleosis, herpes-simplex, measles and pneumonia. Intravenous or moth in-take modes of administrating Vitamin C are normally used. Massive doses of Ascorbic-acid had also been used to cure hard drug effect including the influence or heroin methadore, barbiturates and cocaine (Hansen, et al., 2014).

Vitamin C have been discovered to be functional in the minimization of the environment pollution effects of Carbonmonoxide, Cadmium Mercury and Lead-poisoning. It is pertinent to note that Vitamin C is used in the maintenance of collagen, i.e. protein required for the formation of connective tissues in the skin, ligaments and bones. It also plays a major role in healing of wound and burns, as if facilitate the formation of connective tissue in the scar. Van Gorkom, et al. (2018) discussed that it could also be used for the formation of red blood cells and prevention of hemorrhage. In addition, ascorbic acid present in Vitamin C aids the metabolism of tyrosine, amino acid and phenylamine. It convert folic acid into very active folinic acid. It also prevent the oxidation of thiamine (Vitamin B), Vitamin A, E and B_2 (Riboflavin), folic acid and Pantothenic acid. Ascorbic acid is a carbohydrate derivative present in vitamin C. It is a white crystalline powder, soluble in water and sensitive to oxidation. Literature revealed that the X-ray analysis of the structure of L-ascorbic acid is a flat molecule represented below:

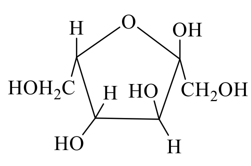


Structural Formula of Ascorbic Acid

Ascorbic acid is a highly strong reducing agent. It even reduces hexoses sugar which it chemically resembles. It is a water soluble substance, and cannot be stored in the body, consequently it must be frequently synthesized for it to be available for the body metabolism. In the synthesis of ascorbic acid, the commercially technical dextrose is hydrogenated to sorbitol, which is biologically oxidized to sorbose. Next, diacetone sorbose is oxidized to diacetone glosomic acid. The mothyl oster of the acid is then reacted with sodium methylate to give sodium salt of ascorbic acid. Sorbose is a penthahydroxy ketone. In solution, it exists as a reversible mose-line of an open-chain form or cyclic form (Davies et al., 1991).

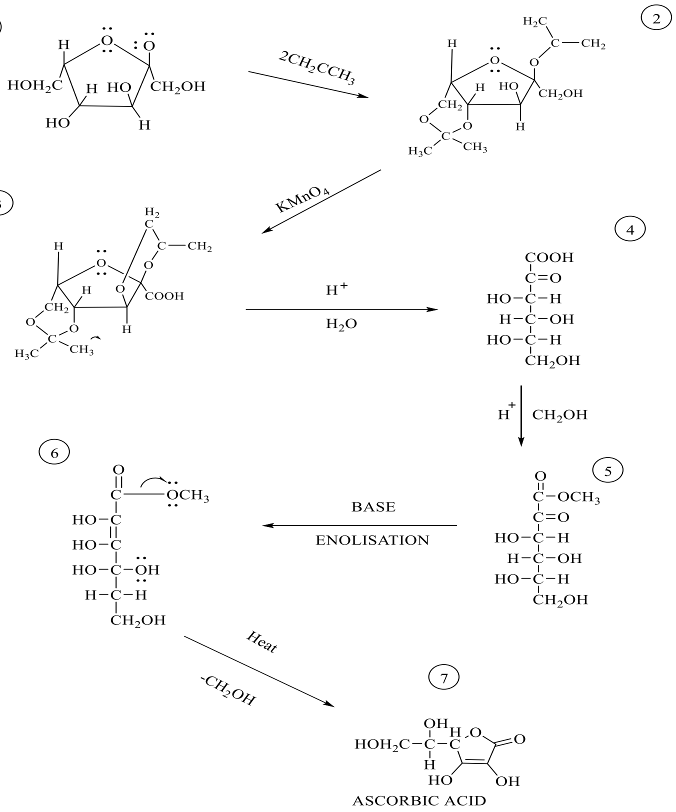


Open-Chain Sorbase



Cyclic Hemiketal Sorbase

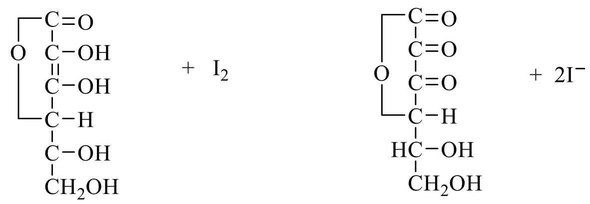
The synthesis of ascorbic acid is influenced through the oxidation of sorbose by $KMnO_4$ However, the direct treatment of sorbose with $KMnO_4$, causes the oxidation of all its alcohol groups, during the process the carbon bonds break and the whole molecule falls apart. Meanwhile, the cyclic form reacts with acetone in the presence of acid to form a tricyclic hemiketal. The OH groups of the cyclic hemiketal are property positioned for the reaction to occur (IUPAC, 2025) viz:



As shown in the equations above, compound has only one free – CH_2OH groups that is need for oxidation. All the others are protected in Ketal linkages. Oxidation of two (2) with $KMnO_4$ gives three (3); while the hydrolysis of (3) with aqeous acid gives (4). The carboxyl group of (4) reacts with methyl alcohol in the presence of an acid to produce a methyl ester, i.e (5) compound (5) then enolises when treated with a base to give (6) and the latter, when heated finally cyclicises to Ascorbic acid (Crawford & Crawford, 1980).

Methodology

The methodology for the experiment was developed for determining the ascorbic acid content of selected fruits and that of commercial Vitamin C tablet (EMZOR BRAND), using the "Direct Filtration" method with standard iodine. This determination depends upon the quantitative oxidation of ascorbic acid to dehydro-ascorbic acid by iodine in acid pollution.



Materials

1. Extracted Orange juice, Grape fruits juice and pineapple juice.
2. Commercial Vitamin C table (EMZOR Brand)

iii. 0.100 mol dm^3 iodine in aqueous potassium iodide.

This is prepared by dissolving 6.35g of solid iodine in a solution of 20g potassium iodide, in about 20cm^3 of water. Then transferring the solution quantitatively into a 250cm^3 volumetric flask and make up to the mark with water.

iv. 1% starch solution. This is prepared by measuring 1g of starch and mixing it with 100cm^2 of water and warm.

v. 2 mol dm^3 of Tetraoxosulphate (VI) acid. This prepared this;

Calculations

Specific gravity of H_2SO_4 = 1.84 gcm^{-3}

Purity value of H_2SO_4 = 98 g mol^{-1}

100 cm3 of H_2SO_4 = 1.84 gcm^{-3} x 1000cm^{-3} = 1840g dm^{-3}

But Molar Concentration = $\frac{Mass\ Conc.}{Molar\ mass} = \frac{1840\ dm^{-3}}{98mol^{-1}} = 18.78\ moldm^{-3}$

Since H_2SO_4 is 97% pure

Molar Con. = $\frac{97}{100} \times 18.78 = 18.22\ moldm^{-3}$

By applying dilution law: $C_1V_1=C_2V_2$,

Then, 2 $moldm^{-3} gives\ 2 \times 1000 = 18.22\ \times v_2$

$V_2 = \frac{2\times100}{18.22} = 109.70cm^3$

1000 cm^3 of water would be used to dilute 109.70 cm^{3}of conc H_2SO_4

Procedure

The experiment was carried out by measuring 50cm3 of the fruits juice into a titration flask, then add 10cm^3 of the 2 mol dm^3 H_2SO_4. Titration the contents of the flask with iodine solution, using starch solution as indicator (at the start of the titration). The end-point was when the contents of the flask acquired a blue-black colour.

Average volume of is used during the titration with Orange juice = 1.20cm^3

Average volume of is used during the titration with Grape juice = 0.85cm^3

Average volume of is used during the titration with Pine Apple Juice = 0.75cm^3

Average volume of is used during the titration with Vitamin C solution = 8.10cm^3

Calculations

Let the volume of 0.100 mol dm3 of 1_2 (aq) used in oxidation be V: cm^3. Then the number of 1_2(aq) used to oxidise ascorbic acid will be

$$\frac{V}{1000} \text{ x } 0.01$$

From equations 1 mol of Ascorbic acid require 1 mol of 1_2 (aq) then the number of moles of ascorbic acid present in the substance used will be A moles.

i. Massive of Ascorbic Acid ($C_6H_8O_6$) = (6 x 12) + (8 x 1) + (16 x 6) = 176g. The mass of ascorbic acid present = Mol of Ascorbic acid X mass of 1 mole + A X 176g.

Let the mass of substance used be Bg, then % of Ascorbic acid present $= \frac{A \times 176}{B}$

Table I: Summary of values obtained from direct titration calculation

Substance Analaysed	Average Vol. of 1_2 used in Titration	No. of Moles of Ascorbic Present	Mass of Ascorbic Acid Present	Percentage of Ascorbic Acid Present
Orange Juice	1.20cm3	0.00012 Mol	0.921g	0.042%
Grape Juice	0.85cm3	0.000085Mol	0.015g	0.030%
Pineapple Juice	0.76cm3	0.000076 Mol	0.013g	0.026%
Vitamin C	8.10cm3	0.00081Mol	0.143g	0.28%

Determination of the Ascorbic Acid content of the selected fruits and Commercial Vitamin C in Table-I (Emzor Brand) using the “Back-Titration” method with standard iodine. In this method, excess iodine is generated by the reaction between iodate (V) and iodide ions in acid solution. Some of the generated iodine reacts with the ascorbic acid, according to the equation: $C_6 H_8 O_{6\,(aq)}$ ------------ $C_6 H_8 O_{6\,(aq)}$ + 2HI (aq) and the excess iodine is back titrated with standard sodium trioxosulphate solution. The results obtained from this method are generally more reliable than those of the direct method, as less iodine is lost to the surroundings when it is generated in the reaction.

Materials

i. A 0.020 mol dm^{-3} aqueous solution of potassium iodate (v) (i.e 4.28g dm^{-3} of KIO_3)

ii. A 0.120 mol dm^{-3} aqeous solution of sodium trioxosulphate (IV) solution (i.e 29.76g dm^{-3} of $Na_2S_20_y$ $5H_2O$).

iii. 2 mol dm^{-3} of Tetraoxosulphate (VI) acid.

iv. Solid Potassium iodine.

v. Extracted Orange juice, Grape fruit and Pineapple juice.

vi. Vitamin C table (EMZOR BRAND).

vii. 1% Starch solution.

Procedure

2 grammes of solid potassium iodide was weighed into a solution 25 cm^3 of water and 25cm^3 of H_2SO_4, and 25 cm^3 of Potassium iodate (v) solution. This was swirled and titrated against sodium trioxosulphate (IV) solution using the starch indicator.

The experiment was repeated with other samples of juice and vitamin C table. The Titrate values obtained at end point were given below;

ii. Average volume of $Na_2S_2O_3.5H_2O$ used when orange juice was involved in the mixture for titration ------ 19.65 cm^3

iii. Average volume $Na_2S_2O_3.5H_2O$ used when Grape fruit juice was involved in the mixture for titration for titration 21.18 cm^3

iv. Average volume pf $Na_2S_2O_3$, 5H2O used when Pineapple was involved in the mixture for titration 21.75cm^3

v. Average volume of $Na_2S_2O_3$, $5H_2O$ used when Vitamin C tablet were used in the mixture for titration 8.90cm^3.

Calculations

Let the volume of 0.120 mol dm3 $Na_2S_2O_3$ used to reduced excess I_2 **(aq)** ve Vt cm^{-3}, then the number of mol of thiosulphate used = $\frac{Vt \times 0.12}{2 \times 1000}$ and since 2 mol of $S_2O^2{}_3$ **(aq).**

1 mol of 12 (aq) then, the number of mol of excess iodine;

$$\frac{Vt \times 0.12}{2 \times 1000}$$

The iodine formed in the reaction was result of 25cm^3 of 0.023 mol dm^{-3} KIO_3 (aq) reacting with $KI_{(aq)}$ used for this reaction.

$$= \frac{25 \times 0.02}{1000}$$

And since 1 mol of KIO_3 (aq) 3 mol of I_2 (aq), then the number of mol of iodine generated:

$$= \frac{3 \times 25 \times 0.02}{1000} = 0.0015 \text{ mol}$$

Hence, the number of moles of iodine required to oxidise the ascorbic acid = (0.0015 – B) mol

From the equation between ascorbic acid and iodine, 1 mol of I_2 (aq) reacts with 1 mol (176g) of Ascorbic acid, hence, hence (0.0015 – B)

Mol of I_2 (aq) will react with (0.0015 – B) x 176g of Ascorbic acid.

Table II: Summary of the values obtained from the calculations based on the "Back – Titration"

Substance	Vol. of $S_2O_2{}^3$ used to reduce excess I_2	Number of Moles of Excess I_2	Mol of Mol of 12 required to oxidise Ascorbic Acid	Mass of Ascorbic Acid Present	Percentage of Ascorbic Acid Present
Orange juice	19.65cm^3	0.00118 mol	0.00032mol	0.056g	0.224%
Grape fruit Juice	21.18cm^3	0.00127 mol	0.00023mol	0.040g	0.016%
Pineapple Juice	21.75cm^3	0.00130 mol	0.00020mol	0.035g	0.14%
Vitamin C Table	8.90cm^3	0.000534mol	0.00097mol	0.17g	8.5%

Table III: Comparison of the values obtained for the mass of ascorbic acid during the "direct titration" and the "back titration".

Substance	Direct Titration		Back Titration	
	Mass in gram Analysed	Mass in Milligram	Mass in gram	Mass in Milligram
Orange juice	0.021g	21mg	0.056g	56mg
Grape fruit juice	0.015g	15g	0.040g	40mg
Pineapple juice	0.013g	13mg	0.035g	35mg
Vitamin C Table	0.143g	143mg	0.17g	170mg

Discussion of Findings

Result contained from the "direct titration" and the "Back Titration" were found on Tables I and II above respectively. Table III gave a comparison of these values for each of the selected fruits. These figures as found for the "Direct Titration" were 21mg, 15mg, 13mg and 143mg for Orange fruit, Grape fruit and Pineapple fruit and Vitamin C table respectively. Further calculations obtained from the "back titration" gave 56mg, 40mg, 35mg and 170mg for Orange fruit, Grape fruit, Pineapple fruit and Vitamin C also in the same order of arrangement, which is to confirm that the fruits used for the experiment contain considerable amount of ascorbic acid for our daily dietary needs (Crawford & Crawford, 1980). This was augment by Hansen et.al. (2014), where it was established that the deficiency of vitamin C would affect human cognitive functioning.

The experiments in this study also showed that the values obtained from the "direct titration" were considerably low compared with those of the "Back Titration". At this juncture, the researcher would like to highlight that, during the 'Back-Titration" exercise, the ascorbic acid reacted with iodine in the preliminary reaction, before titration with standard sodium thiosulphate solution. It was the excess iodine generate from the preliminary reaction, which was back titrated with sodium thiosulphate. Hence, there were negligible amount of iodine lost to the surroundings. Therefore, the results of the "Back titration" becomes more reliable. That notwithstanding, the direct method have shown that an appreciate amount of the ascorbic acid was present in the fruits (Ashor, et al., 2019). Thus, justifying the inclusion of the back-titration as a method of analysis in the research.

Conclusion

In recognition of the roles of some accessory factors such as vitamins in the Human body's growth and development, the National Agency for Food, Drug Administration and Control (NAFDAC) have the "Recommendation daily Allowance (RDA) for the Consumption of Ascorbic acid as between 20mg and 50mg. Also pregnant and lactating women have the RDA given as 60mg and 80mg respectively. Consequently, the results obtained from the experiment (see Table II), complimented earlier work by Cameron and Pauling (1976), and confirmed the need to include the fruits in diets, especially for pregnant and lactating women.

Recommendations

a. The most important recommendation in the study is to obtain vitamin C from varied fruits and vegetables.

b. Ascorbic acid provide natural sources of antioxidants, formation of collagen, developing immune system (in reducing disease risks) and preventing iron deficiencies.

c. Helps lower blood pressure.

d. Fruits and vegetables with rich ascorbic acid for diets includes – oranges, kiwi fruits, bell peppers, broccoli, kale, strawberries and spinach.

e. Vitamin C is recommended for pregnant and lactating women.

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