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Quantitative estimation of ascorbic acid levels in citrus fruits at variable temperatures and physicochemical properties

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Abstract

Ascorbic acid levels in matured ripe and unripe sweet oranges (*Citrus sinensis*), lemons (*Citrus lemonum*) and grapefruits (*Citrus grandis*) were investigated using redox titration involving oxidation-reduction conversion of ascorbic acid. The analyses were carried out under refrigeration temperature, ambient temperature and intense sunlight to ascertain the extent of the effects of temperature. Other parameters probed were ripening stage, time of exposure and specie. The results suggest that the ascorbic acid levels in the unripe fruits were higher than the ripe ones but generally decreased upon increase in temperature, ripening and time of exposure. Orange has the highest ascorbic acid content (59.00±0.3 - 50.10±0.1 and 49.50±0.3 - 43.00±0.1 mg/100 g juice) followed by lemon (50.10±0.1 - 41.30±0.2 and 41.00±0.1 - 35.20±0.2 mg/100 g juice) and finally by grapefruit (44.80±0.2 - 36.40±0.2 and 34.20±0.3 - 24.00±0.3 mg/100 g juice) both in the unripe and ripe fruits at the various temperature conditions. Since the ascorbic acid content of citrus fruits depends on a lot of factors a few of which include climatic/environmental conditions, soil chemical composition, specie, maturity state and temperature, it is pertinent to explore the ascorbic acid levels in these citrus fruits with respect to a particular geographical location so as to establish information on the value of these fruits as raw materials for neighbouring outfits using ascorbic acid especially the Fruit Juice Industries.

Key words: Ascorbic acid, Citrus fruits, Orange, Lemon, Grapefruit, Juice.

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1. Introduction

Citrus fruits among other uses are sources of food and medicine. Fruits are divided into climacteric and non-climacteric classes based on their respiration pattern during ripening. In non-climacteric category are the grapefruit, lemon, orange, melon, pineapple and the strawberry. In these fruits the respiratory pattern show slow drift downwards after detachment from the parent plant while climacteric classes undergo a distinct ripening phase e.g. bananas, pears and avocados [1]. Orange, lemon and grapefruit have the same morphology, fruit development and maturation except in the percentage composition of ascorbic acid and sugars [2]. Ascorbic acid also known as Vitamin C, L-ascorbic acid and the Antiscorbutic Vitamin, when pure is a white crystalline water-soluble vitamin found especially in citrus fruits and vegetables. It is synthesized by most organisms from glucose but man and other primates and various other species must obtain it from their diet [3]. Ascorbic acid is the most abundant vitamin in orange, lemon and grapefruit [4]. The peel is especially rich in ascorbic acid. There is a considerable variation in the ascorbic acid content of juice of different fruit [4]. The citrus species are medium sized tree of the rutacene family. They are evergreen trees that give fruits of different forms and sizes (from round to oblong) which are full of fragrance, flavour and juice. Citrus fruits have a rough, robust and bright colour (from green to yellow) skin or rind known as epicarp which covers the fruits and protects them from damages [5]. The glands contain the essential oils that the fruit its typical citrus fragrance. The endocarp is rich in soluble sugars and contains significant amounts of ascorbic acid, pectin, fibres, different organic acids and potassium salt which gives the fruit its characteristic citrus flavour [5,6].

Ascorbic acid is required in the synthesis of collagen in connective tissues, neurotransmitters, steroid hormones, carnitine, and conversion of cholesterol to bile acid and enhances iron bio-availability [7]. Ascorbic acid is a great antioxidant and helps to protect the body against pollutants [8,9]. It is also a biological reducing agent linked to prevention of degenerative disease such as cataracts, certain cancers and cardiovascular diseases [10]. It promotes healthy cell development, proper calcium absorption, normal tissue growth and repair such as healing of wounds and burns and strengthening the wall of the capillaries. Ascorbic acid is needed for healthy gums, to help protect against infection and assisting with clearing up infection; and is thought to enhance the immune system and help reduce cholesterol levels and high blood pressure [11]. A deficiency of ascorbic acid in the body results in scurvy, a disease characterized by sore, spongy gums, loose teeth, fragile blood vessels, swollen joints and anaemia [12]. A shortage of ascorbic acid may also result in haemorrhages under the skin and a tendency to bruise easily, poor wound healing, oedema and weakness [13]. Lack of energy, poor digestion, bronchial infection and colds are also indicative of an under-supply of ascorbic acid [14]. Many of the deficiency symptoms can be explained by a deficiency in the hydroxylation of collagen, resulting in defective connective tissues [15]. Ascorbic acid is widely used as antioxidant in frozen fruits, canned meat, beverages, beer and other food items. It is used medically as drug and in pharmaceutical industry for the manufacture of drugs rich in ascorbic acid [10]. Ascorbic acid is used in the treatment of scurvy and prickly heat and thus required in the tropics in relatively larger quantities so as to ensure among other things the normal functioning of the hyperactive sweat glands. Its application in animal feed is fast growing as it supplements the Vitamin C content of the feed [10]. Apart from the citrus fruits, few other sources of ascorbic acid are green leafy vegetables, berries, guavas, tomatoes, melons, papayas and white potatoes [13]. Ascorbic acid content of citrus fruits is never constant but varies with some factors which include climatic/environmental conditions, maturity state and position on the tree, handling and storage, ripening stage, specie and variety of the citrus fruit as well as temperature [2]. It is in the light of this that the quest to have knowledge of the ascorbic acid content of some selected citrus fruits in South Eastern Nigeria was born with respect to temperature, ripening stage, specie and time. Routine measurement of ascorbic acid in fruit juices is therefore imperative for substantiating nutritional claims and this can be conveniently accomplished by quantitative method of analysis as shown in this paper.

2. Material and Methods

Ripe and unripe citrus fruits which included sweet oranges (*Citrus sinensis*), lemons (*Citrus lemonum*) and grapefruits (*Citrus grandis*) were harvested from Umudike farms in Abia State, Nigeria. 150 matured fruits were used for the analysis; 30 unripe and 20 ripe each of oranges, lemons and grapefruits. The fruits were stored in a dark cupboard at room temperature and were being withdrawn gradually until the end of the analysis. Unripe fruits collected from the cupboard after the first day of the study (two days) showed evidence of ripening while the ripe ones became more ripened. The degree of ripening in both the ripe and unripe fruits increased as the length of time of the analysis lingered. The analysis lasted for six days.

2.1. Extraction of Fruit Juice

The juice was squeezed out of the fruits manually until most of the juice was collected in a clean glass beaker and the residual portion of the juice squeezed through layers of muslin cloth. The extract was then filtered through another clean layer of muslin cloth to eliminate certain impurities. A portion of the juice was kept in a refrigerator overnight (18 hours) prior to the analysis, another portion was exposed to intense sunlight for the same length of time and the third portion was left at ambient temperature. Ascorbic acid contents of these portions were promptly determined. No further treatment was given to the extracts before using them immediately for the analysis.

2.2. Preparation of Reagent Solutions

A weight of 15 g metaphosphoric acid pellets (HPO_3) was dissolved in warm (60˚C) distilled water using successive portions until the acid completely dissolved and was transferred to a 500 ml stoppered bottle. The solution was allowed to cool to room temperature and 40 ml glacial acetic acid was added followed by sufficient distilled water to make 450 ml. The solution was stored in a refrigerator and was being used each time for analysis. HPO_3 slowly changes to H_3PO_4 but this solution satisfactorily remains for 7-10 days when stored in a refrigerator. A weight of 30 g potassium iodide crystals was dissolved in 100 ml distilled water in a conical flask to give a solution of 30% potassium iodide. The solution was then transferred into a stoppered reagent bottle. A weight of 2.5 g $CUSO_4.5H_2O$ was dissolved in a beaker using distilled water. The solution was transferred to a 100 ml volumetric flask qualitatively by rinsing the beaker with successive portions of distilled water until it was made up to the 100 ml mark. The solution became exactly 0.01M copper sulphate ($CUSO_4.5H_2O$) solution. It was then transferred to a stoppered reagent bottle.

2.3. Determination of Ascorbic Acid

This was done using the method of the Association of Vitamin Chemists as described by Kirk and Sawyer [16]. 5 g of the sample was dispersed in 50 ml of EDTA/TCA solution and homogenized. The homogenate was filtered using a filter paper and more of the extractant was used to wash the residue in the filter paper until 100 ml filtrate was obtained. A 20 ml portion of the filtrate was measured into a conical flask and 10 ml of 30 % potassium iodide solution was added to it, mixed well and then followed by four drops of 1 % starch solution. The mixture was titrated against 0.01 M $CuSO_4$ solution until a blue-black colour appeared. A reagent blank was also titrated using 20 ml of distilled water. The vitamin C content was calculated based on the relationship that 1 ml $CuSO_4$ = 0.88 mg vitamin C. Therefore,

$$\text{Vitamin C mg/100g} = \frac{100}{W} \times 0.88 \times (T-B) \times \frac{VT}{VA}$$

Where,

W = weight of sample
T = titre value of sample
B = titre value of blank
VT = total extract volume
VA = volume of extract titrated

3. Results and Discussion

The method employed for ascorbic acid determination was based on the oxidation-reduction conversions of ascorbic acid. When copper sulphate solution was titrated against a mixture of potassium iodide, ascorbic acid extract and starch indicator, the potassium iodide reacted with copper sulphate forming cupric iodide (CUI_2). The excess iodide ions in the solution oxidised the ascorbic acid and when the oxidation was completed, the iodide ions would react with starch indicator to form a blue-black colour at the endpoint.

Table 1. Ascorbic acid contents of three unripe citrus fruits under different temperature conditions.

Temperature (˚C)	Ascorbic Acid Content in mg/100g Juice		
	Orange	Lemon	Grapefruit
Refrigeration	59.00±0.3	50.10±0.1	44.80±0.2
Ambient	58.60±0.1	49.40±0.2	44.20±0.1
Sunlight	57.20±0.2	48.00±0.1	43.10±0.3

Table 2. Ascorbic acid contents of three ripe citrus fruits under different temperature conditions.

Temperature (˚C)	Ascorbic Acid Content in mg/100g Juice		
	Orange	Lemon	Grapefruit
Refrigeration	49.50±0.3	41.00±0.1	34.20±0.3
Ambient	48.90±0.4	40.30±0.2	33.60±0.3
Sunlight	48.00±0.2	38.40±0.3	32.00±0.4

Table 3. Ascorbic acid contents of three unripe citrus fruits under different temperature conditions.

Temperature (˚C)	Ascorbic Acid Content in mg/100g Juice		
	Orange	Lemon	Grapefruit
Refrigeration	56.50±0.2	47.00±0.1	41.00±0.3
Ambient	56.00±0.3	46.00±0.2	40.40±0.3
Sunlight	55.00±0.1	44.60±0.4	39.20±0.1

Table 4. Ascorbic acid contents of three ripe citrus fruits under different temperature conditions.

Temperature (˚C)	Ascorbic Acid Content in mg/100g Juice		
	Orange	Lemon	Grapefruit
Refrigeration	47.00±0.4	37.10±0.3	30.00±0.3
Ambient	46.50±0.2	37.80±0.4	29.70±0.1
Sunlight	45.40±0.2	36.70±0.1	28.70±0.3

Table 5. Ascorbic acid contents of three unripe citrus fruits under different temperature conditions.

Temperature (˚C)	Ascorbic Acid Content in mg/100g Juice		
	Orange	Lemon	Grapefruit
Refrigeration	53.30±0.2	42.40±0.1	37.50±0.3
Ambient	52.00±0.2	42.00±0.4	37.00±0.1
Sunlight	50.10±0.1	41.30±0.2	36.40±0.2

Table 6. Ascorbic acid contents of three ripe citrus fruits under different temperature conditions.

Temperature (˚C)	Ascorbic Acid Content in mg/100g Juice		
	Orange	Lemon	Grapefruit
Refrigeration	44.50±0.1	36.50±0.2	26.10±0.2
Ambient	43.90±0.3	36.00±0.3	26.70±0.1
Sunlight	43.00±0.1	35.20±0.2	24.00±0.3

All data are means ± standard deviations of triplicate determination

The same experimental procedure was applied for both the ripe and unripe fruits at the three different temperature conditions i.e. at refrigeration temperature, ambient temperature and under intense sunlight. It is observed from the results (Tables 1-6) that ascorbic acid content of each of the citrus species differs under different temperature conditions. At refrigeration temperature, highest ascorbic acid content was observed followed by ambient temperature and lastly by sunlight temperature. At refrigeration temperature, the content of the juice was preserved and so underwent little or no biochemical transformation. At ambient temperature, the juice became prone to oxidation. Oxygen is the most destructive element in juice causing degradation of ascorbic acid [17]. At increased temperature, the juice was more susceptible to oxidation and the effects of temperature and consequently experienced more degradation of ascorbic acid. All types of fruit juices are inherently unstable and rapidly undergo microbial attack by organisms already present in the fruit, which gained access to the product during ripening. They are also subjected to enzymatic and non-enzymatic changes. The longer the juice stays in the atmosphere, the more the degradation hence the lesser the ascorbic acid content. Also, as the temperature increased, the ascorbic acid content decreased. However, one of the major sugars found in juices, fructose, can also cause ascorbic acid breakdown. Conversely, higher levels of citric and malic acids stabilize ascorbic acid [17]. The results of these investigations have corroborated the fact that citrus juices must be stored at proper cool temperatures with oxygen barriers for best retention of ascorbic acid levels.

The results of this investigation have shown that the citrus fruits analysed contained reasonable amounts of ascorbic acid and would be economically valuable as industrial raw materials. However, the ascorbic acid content of the selected unripe fruits was found to be greater than that of the ripe ones. Also, the ascorbic acid decreased upon ripening, temperature increase and time which are attributed to degradation caused by heat and oxidation. Sweet orange contained the highest amount of ascorbic both in the ripe and unripe groups followed by lemon and finally by grapefruit. In the manufacture of packaged or canned fruit juices, the value of the raw material may be governed by the amount of the required ingredient it contains; a quantitative analysis is performed to establish the proportion of ascorbic acid in it. The final product is subject to quality control to ensure that its ascorbic acid content is up to the required standard. This research provides information on the ascorbic acid contents of sweet orange, lemon and grapefruit grown in South Eastern Nigeria precisely at Umudike, Abia State. This would help localized industries using ascorbic acid to know the richness of ascorbic acid in these citrus fruits when they are used as raw materials. It has been reported that high nitrogen fertilizer rates can lower ascorbic acid levels in citrus fruits and that proper potassium levels are needed for good ascorbic acid levels [2]. Additionally, climate, especially temperature-total available heat affects ascorbic acid levels. Areas with cool nights produce fruits with higher ascorbic acid levels while hot tropical areas produce citrus fruit with lower levels of ascorbic acid. Environmental conditions that increase the acidity of citrus fruits also increase ascorbic acid levels. The position of citrus fruits on the tree also affects ascorbic acid levels [2]. All these and more contributed to the reasons behind the erratic values of ascorbic acid contents of citrus fruits all over the world.

4. Conclusion

This research therefore makes recommendable the sweet oranges, lemons and grapefruits grown within Umudike area to industries as raw materials owing to their high contents of ascorbic acid both at ripe and unripe stages. They could also be prepared in exportable forms for industries outside Nigeria. A necessary advice would be to encourage the cultivation of these plants since environmental friendliness that would boost ascorbic acid content in them are available and also employ storing and processing conditions that would not undermine the quality of the produce.

References

[1] K.J. Thurlor. and I.D. Lumley. (1989). Fruit classification. International Journal of Plant Sciences. 13(5) 85-91.

[2] G.D. Holcombe. (1992). Fruit growth and development, Applied Botany, 1st ed. Livingstones Publisher, Churchill, pp. 46.

[3] J.E. Alibone. (2000). Livestock feeds and feeding. Nutrition Abstracts and Reviews. 72(12) 651-659.

[4] A. Ralph. and D.A. Bender. (2000). Vitamin C, Journal of Human Nutrition and Dietetics. 2(2) 75-81.

[5] D.E. Okwu and I.N. Emenike. (2007). Nutritive value and mineral content of different varieties of citrus fruits. Journal of Food Technology. 5(2) 105-108.

[6] G.D.P. Roger. (2002). Encyclopedia of medicinal plants, education and healthy library. Editorial Safeliz S.L. Spalm. 1,153-154; 265-267.

[7] K.M. Robert. , K.G. Daryl. , A.M. Peter. and W.R. Victor. (2000). Structure and functions of water-soluble vitamins, Harper’s Biochemistry.25th ed., McGraw-Hill New York, pp. 640-641.

[8] D.E. Okwu. (2004). Phytochemicals and vitamin content of indigenous spices of South Eastern Nigeria. Journal of Sustainable Agriculture and Environment. 6: 30-34.

[9] D.E. Okwu. (2005). Phytochemicals, vitamins and mineral contents of two Nigerian medicinal plants. International Journal of Molecular Medicine and Advance Sciences. 1(4) 375-381.

[10] N. Gary. (2004). The antioxidant vitamin-vitamin C. Journal of Raw Materials and Research. 1(1) 16-20.

[11] R.H. Larsen. (1997). Vitamin C: Your ultimate health insurance. International Journal of Alternative and Complementary Medicine. 15(8) 19-26.

[12] N. Piff. , K. Law., B. Mauro and J. Norris. (1998). Vitamins and minerals. American Journal of Family Health. 1(1) 32-35.

[13] C.F. Enloe, R. Vanalter and J.R. Mulligan. (1985). Vitamin C for yourself. Nutrition Today. 20(1) 20-25.

[14] A.R. Ronzio and A. Robert. (1992). Ascorbic acid. The Encyclopedia of Nutrition and Good Health, vol. 1, MacGraw-Hill New York, pp. 106.

[15] C.J. Henry., M. Alfawaz. , J.E. Blundell and A.W. Buttriss. (2004). Vitamin C and Scurvy. International Journal of Food Sciences and Nutrition, 55(1), 24-28.
[16] A. Kirk and P. Sawyer. (1998). Pearson's food composition and analysis, 4, 204-206.
[17] T. Atinmo., T. Ogunta., A.U. Okorie and T.M. Kamalu. (1981). Nutrition and good health. Nigerian Journal of Nutritional Sciences. 2(2) 80-85.