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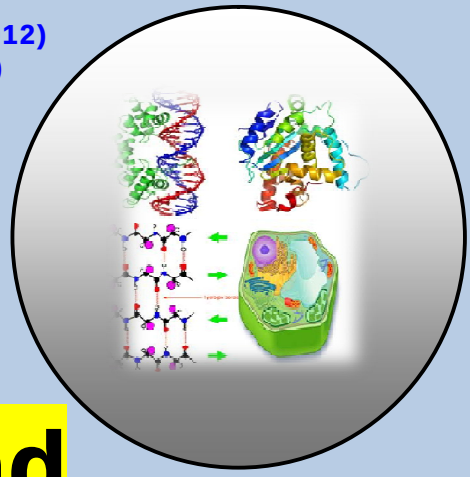

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RESEARCH PAPER

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Quantitative Determination of Ascorbic Acid in Orange Collected from Kochi Local Market by Using Cyclic Voltammetry

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ABSTRACT

Vegetable and fruits are reported to decrease the risk of degenerative diseases and have a protective effect against oxidative stress. The oxidative stress is initiated by the production of free radicals (unstable molecules) in our body. Therefore in turn these radicals can start chain reactions; it can cause damage or death to the cell. Antioxidants protect billions of cells in the body from free radical's damage. Ascorbic acid or vitamin c is one of the antioxidants mostly found in fruits. The cyclic voltammeter method was applied for assessing ascorbic acid concentration in orange juice. The oxidation peak for ascorbic acid appears at about 652mv (versus Ag/Agcl) on a platinum working electrode. The equation of calibration graph was; Y= 2.592X+7.103, R = 0.997 ("Y" is measured current in µA and X is the analyte concentration, mm). The determined ascorbic acid concentration was 3.35Mm %R.S.D=1.96%.

Keyword: Free radicals, Antioxidant, Ascorbic acid, Electrode and Cyclic voltammetery.

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INTRODUCTION

Epidemiological evidence shows an association between decreased risk of cardiovascular disease and a diet rich in fruits and vegetables. Vegetables and fruits are also reported to decrease the risk of *degenerative diseases* and could have a protective effect against oxidative stress. These effects may be related to natural antioxidants, including phenolic acids, flavonoids, vitamin E, and vitamin c which have the ability to scavenge free radicals and protect cells from the damage caused by free radicals (*Salamih, A et al*).

Vitamin C is also known as ascorbic acid, is a valuable food component because of its antioxidant and therapeutic properties. It helps the body in forming connective tissues, bones, teeth, blood vessels and plays a major role as an antioxidant that forms part of the body defense system against reactive organic compounds and free radicals, thereby preventing tissue *damage*. It is widely used in the treatment of certain disease such as scurvy, common cold, anemia, hemorrhagic disorders, wound healing as well as infertility (*Okiei, M. et al)*. According to *Hoyle, C.et al* Increasing awareness of the importance of antioxidants in the diet has led to greater importance placed on the consumption of foods containing antioxidants. Ascorbic acid, vitamin c is important as a dietary component and as a food preservative. The dietary value of orange and lemon or their juice is long established, having been determined by evidence based medicine in the eighteenth century to be antiscorbutic. The beneficial effects of vitamin c are not restricted to its antiscorbutic activity as there is now evidence to show that it is protective against neuro degeneration and may ameliorate some cardiovascular disease and have anti cancer properties.

Traditional methods for ascorbic acid assessment involve titration with an oxidant solution dichlorophenol indophenols, potassium iodate or bromate. Although titrimetric methods are simple to use in the determination of vitamin c, difficulties are encountered with commonly used titrants and interferences often occur with coloured samples. Direct spectrophotometric determination of ascorbic acid in the UV region is prone to matric effect since many organic compounds in complex samples may also exhibit ultraviolet absorbance. Thus there is a need to adopt a procedure that will accurately determine the levels of vitamin c. Chromatographic methods, particularly HPLC with electrochemical detection, has turned out to be a selective and sensitive method for ascorbic acid (AA) assessment in food stuffs and biological fluids.

Fluorimetric methods were also used for ascorbic acid estimation (*Aurelia, M.et al)*. A recently developed voltammetric methods allow rapid, simple, selective and sensitive determination of low molecular weight antioxidants and vitamins (e.g., ascorbic acid) and drugs, without the necessity of time consuming separation. Cyclic voltammetery is a unique technique for the electro chemical characterization of compounds by providing data about their oxidation/ reduction potentials. Besides simplicity and rapidness, this technique is based on the chemico-physical properties to the molecules and can be widely used in evaluating antioxidant in oil and food stuff. However many plant's and animal's product contain organic compounds and those organic compounds undergo oxidation reaction in our body to form free radicals, unstable molecule or atoms, they can oxidize nucleic acids, proteins and lipids to initiate degenerative diseases like cardiovascular disease and cancer*(Cosio, M.S.et al)*. Thus, this study was focused on the quantitative determination of ascorbic acid [AA] in orange juice especially on the level of ascorbic acid in the fruit collected from Kochi local market.

MATERIAL AND METHODS

Materials and Reagents

The following reagents (chemicals) were used during the experiment. L- Ascorbic acid (nice, India, laboratory reagent, 99%), potassium chloride (Qualikems), Glucose (Finkem), tartaric acid (Kemie Labs, 98%). All those chemicals have analytical grade quality.

Apparatus and instrument

Cyclic voltammetery experiments were carried out using Bas- Epsilon EC-version 1.40.67 voltammetric analyzer (Bio analytical systems, USA). A conventional three electrode setup was used with pt disc as working electrode , platinum wire counter electrode (Bas, MW 1032) and Ag/Agcl electrode (Bas, MF 2079) used as reference electrode, Electrochemical cell and electrode polishing alumina were used during the experiment.

Sampling methods

Orange sample was collected from Kochi local market found in Jimma town by random sampling method. In laboratory the orange juice was obtained by fruit pressing. The three average sized orange fruits were peeled and the juice was obtained by pressing. Then the juice was filtered using micro filter, and the clear juice was subsequently analyzed. Solid Kcl was added as supporting electrolyte into the clear fruit juice in order to obtain a concentration of 0.34 mol $^{-1}$ KCl.

Procedure

The cyclic voltammetery experiments were carried out by using Bas-Epsilion Ec-version 1.40.67 voltammetric analyzer (Bio- analytical systems, USA). A three electrodes setup was used equipped with Pt electrode as working electrode, a platinum wire counter electrode (BAS, MW1032) and Ag/Agcl electrode (BAS, MF 2079) as a reference electrode.

100 m mol L^{-1} ascorbic acid stock solutions was prepared by dissolving it in 0.34 M Kcl electrolyte from the prepared stock solution of different ascorbic acid concentrations expressed as, (0.1, 0.75, 2, 8, 10) mmolL^{-1}. These standard solutions were used for calibration graph. The volume of each standards and the analyzed sample was 50 mL. Before each determination, the Pt working electrode was cleaned mechanically, by polishing it by alumina. The potential window for each measurement was -100 to 1000 mv, with scan rate of 50 mv/s. A study of interference for ascorbic acid determination in orange fruit juice by cyclic voltammeter was performed at Pt working electrode. The 1M of interferent (glucose or tartaric acid) was added to the analyzed sample, and the final volume of analyzed sample was 50 mL. All the investigations were performed by using the working procedure stated above.

RESULT AND DISCUSSIONS

The concentration of ascorbic acid was determined by cyclic voltammetery in orange juice using platinum (Pt) working electrode. Several voltammograms were obtained for different ascorbic acid concentrations of standard solutions (figure 1). As the voltammograms showed in figure1, no reduction peak appears for ascorbic acid. This confirms that electrochemical oxidation of ascorbic acid is an irreversible process. The peak corresponding to ascorbic acid (AA) oxidation appeared at about 652 mV.

The calibration graph (figure 2) shows a linear range obtained between 0.1 and 100 mM ascorbic acid (Y= 2.592X +7.103, R = 0.997, where Y represents the value of the current intensity as μA and X is the analyte concentration as mM. The value of calculated for the relative standard deviation (R.S.D) was 1.96% (Ascorbic acid = 3.35mM).

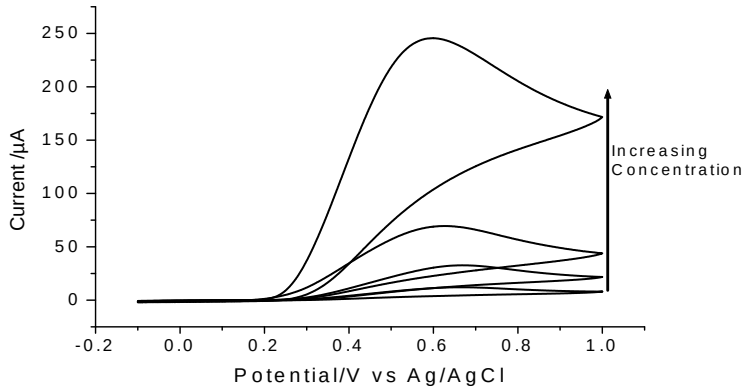

Figure 1. Cyclic voltammograms obtained for different ascorbic acid concentrations expressed as mM (2mM, 8mM, 10mM, and 100mM).

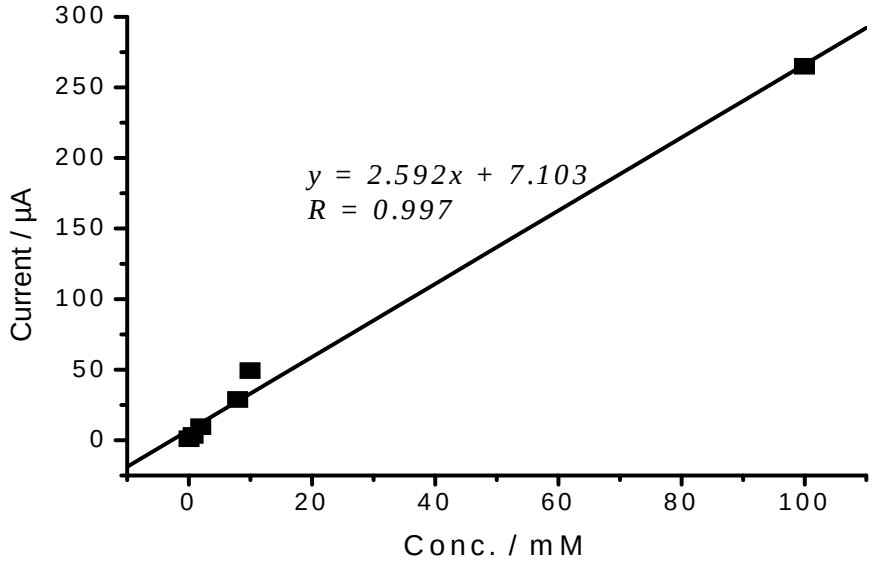

Figure 2. Calibration graph obtained at ascorbic acid determination by cyclic voltammetery at a Pt working electrode.

As the calibration graph, in figure 2 shows the linear dependence of the concentration of ascorbic acid with the peak current. In other word the peaks of voltammograms described in figure 1 are directly related with concentration of standards. World health organization /WHO/ recommended that the daily intake of ascorbic acid is 200-500 mg/day *(Cosio, M.S.et al)*. This means the intake of excess or less AA is not advisable. Based on this study the quantity of AA in orange from Kochi local market is good for health.

Interference study

The influence of some interferents commonly present in fruit juices, namely Glucose and tartaric acid were investigated. The influence of these compounds on the analytical current peak height is presented in table 1

Table 1. Study of interference at ascorbic acid determination performed on two common chemical species found in citrus juice.

Sample with interferent, sample without interferent	Interferent/analyte molar ratio	Current response	Influence on the analytical peak height
Glucose	150	15.8765NA	Less than 1%
Tartaric acid	150	16.205NA	1ess than 3%
Sample	-	15.7965NA	-

As can be seen from table 1 glucose and tartaric acid have no significant influence on the analytical signal up to the interferent to analyte molar ratio of 150/l As some literature indicates that the permitted relative deviation is less than ± 5 implies no interference in the determination of ascorbic acid by cyclic voltammetery.

In order to decrease the risk of degenerative diseases due to free radical's cell damage, the consumers of organic foods should adopt the habit of consuming vegetables and fruits diet because these are rich sources of antioxidants. Vitamin C is one of many antioxidants. Antioxidants are nutrients that block some of the damage caused by free radicals. Free radicals are made when your body breaks down food or when you are exposed to tobacco smoke or radiation. The buildup of free radicals over time is largely responsible for the aging process.

Free radicals may play a role in cancer, heart disease, and conditions like arthritis. The body is not able to make vitamin C on its own, and it does not store vitamin C. It is therefore important to include plenty of vitamin C-containing foods in your daily diet. People who are eating vegetables and fruits should also consider the risk of insufficient and excess intake of antioxidants such as ascorbic acid

CONCLUSION

Fruits are generally available in such areas, but problems related to vitamin c deficiency are common. Therefore this result will provide a suitable guide to the population in their choice of fruits with high levels of vitamin C such as orange. Adequate consumption of fruits with high vitamin C content can result in improved health by reducing diseases such as diabetes, heart diseases and cancer etc.

In this method for ascorbic acid determination shows that cyclic voltammetery can be successfully used part of quality management in food industry, for assessing the vitamin c content in natural fruits. The result proved that the developed method (cyclic voltammetery) for ascorbic acid determination is characterized by accuracy, rapidity; specificity and sensitivity since AA undergo irreversible reaction.

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REFERENCES

Salamih, A., Faraz, M., Abdolmajid, B.M., Kimia, T. and Farzad, K. 2012. A simple electrochemical method for the rapid estimation of antioxidants potentials of some medicinal plants. Shaheed. Beheshti University. 11(1) 117-121.

Okiei, M., Ogunles, L., Azeez, V., Obakachi, M. and Osunsanmi, G. 2009. The voltammetric and Titrimetric determination of Ascorbic Acid levels in Tropicals fruit samples. *Int. J. Electrochem Sci.,*4, 276-287. Hoyle, C.H.V. and Santos, J.H. 2010. Cyclic voltammetric anlaysis of antioxidant activity in citrus fruits from South East Asia. *Int. Food Research Journal,* 17, 937-746.

Aurelia, M.P., Aneta, P., Gheorghe, P.N. and Aurel, P. 2011. Determination of ascorbic acid content of some fruit juices and wine by voltammetry performed at Pt and earbon paste electrodes. *Molecules,* 16 1349-1365. Cosio, M.S., Buratti, S., Mannino, S. and Benedetti, S. 2006. Use of an electro chemical method to evaluate the antioxidant activity of herb extracts from the family. *Food Chem*. 97, 725-731.

Pallmieri, B. and Sblndorio, V. 2007. Oxidative stress tests: over view or reliability and use. *Eur. Rev. Med. Sch.* 11, 383-399.

Noriko, N. and Niro. W. 2001. Cancer and oxidative stress, *TMAJ*, 44(12), 535-539.

Finkel, T. and Holbrook, N.J. 2000. Oxidants, oxidative stress and the biology of aging. *Nature*, 408,239-247.

Murray, B., Botham, K. and Rodwell, W. 2009. Harper's Illustrated biochemistry, 28th ed.; *Mc Graw Hill: lange*, P 492.

Ve, I. and Song, H. 2008. Antioxidant vitamins intake and the risk of coronary heart disease. Meta analysis of cobort studies. *Eur. J Cardiovascular Prev. Dahabi*, 16, 26-34.

List of antioxidants in food: http:// www buzzle. Com/antioxidant rich foods (Accessed on 19 January, 2013).

Harbowy, M.E. and Bolentine, D.A. 1997. *Tea Chemistry Crist. Rev. Plant Sci.*, 16,415-422.

Mc Cord, J.M. and Fridovich, I. 1988. *Free Radical Biology and Medicine*, 5,363.

Shi, H.L., Noguchi, N. and Niki, E. 199. *Biofactors,* 9,141.

Erdurak-kilic, C.S., Uslu, B., Dogan, B., Ozkan, S.A. and Coskun, M. 2006. Anodic Voltammetric behavior of ascorbic acid its selective determination in pharmaceutical dosage forms and some Rosa species of Turkey. *J. Anal Chem*, 61, 1113-1120.

Gima, G.A., Rall, W.T. and Nies, A.S. 1991. Good man and Giman's. The pharmacological basis of therapeutics. *Mc Graw. Hill*, Singapore, 1547.

Aurelia, M.P., Andrei, F.D. and Slawomir, K. 2008. Ascorbic acid determination in commercial fruit juice samples by cyclic voltammetry. *J. Auto Met Man Chem*. 93, 7651. Abdol, A, B., Nafiseh, S., Behrooz, J. and Mohammed, R.O. 2010. Determination of L-Ascorbic Acid in plasma by voltammetric method. *Med. Sci.*, 9(2), 123-128.

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