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Assessment of Some Selected Beverages and Fresh Edible Vegetables as Nutritional Source of Vitamin C (Ascorbic Acid) by Cyclic and Square Wave Voltammetry

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Abstract- Cyclic voltammetry (CV) and square wave voltammetry (SWV) were used for characterizing and determining Vitamin C concentration in some selected beverages and fresh edible vegetables. The oxidation peak for Vitamin C occurred at 400 mV (vs Ag /AgCl) for both CV and SWV both at glassy carbon working electrode. The influence of the operational parameters for the analytical signal was investigated. The effect of pH was also studied for both methods where pH2 and pH3 were selected as working pH for CV and SWV, respectively. Calibration graphs were set that showed a linear dependence between the peak current and Vitamin C concentration within the range of 0.008 – 0.08 mM. The Vitamin C content determined ranged between 5.26 mg/100 mL juice for Lactuca sativa and 28.3 mg/100 mL for Capsicum annum juices and 7.62 mg/100ml for lettuce and 31.1 mg/100 ml for green pepper juices obtained by squeezing fruit. The effect of temperature on vitamin C content of some selected vegetables was determined to ascertain the effect of heat on the vitamin C contents of these vegetables. The amount of Vitamin C in these vegetables was degraded when the temperature was increased from 25 - 65 ^{0}C.

Keywords- *Vitamin C, cyclic voltammetry, square wave voltammetry, glassy carbon electrode, fresh edible vegetables and beverages.*

I. INTRODUCTION

Ascorbic acid, a water-soluble vitamin (Vitamin C), is important in forming collagen, a protein that gives structure to bones, muscles and blood vessels. It is one of the most ubiquitous vitamins ever discovered (Gazdik, et al., 2008). Besides playing a paramount role as an antioxidant and free radical scavenger, it has been suggested to be an effective antiviral agent (Gazdik, et al., 2008). The human body is unable to synthesize vitamin C due to the loss of the gene responsible for the synthesis of gulonolactone oxidase which is necessary in the conversion of glucose to ascorbic acid (Behpour M.; Golestaneh, M., 2010). The only way human can get ascorbic acid is via food. But the daily needs of vitamin C for a human are not clear yet (Yilmaz et al., 2008) The amount of vitamin C required in a healthy diet varies with age and gender. According to Health Canada, children ages 1-3 require 15 mg/day, adult females 75 mg/day, and adult males need 90 mg/day (Matei &Magearu, 2004). It is not possible to relate servings of fruits and vegetables to an exact amount of vitamin C, but the WHO dietary goal of 400g/day (WHO Technical Report Series, 1990), aimed at providing sufficient vitamin C to meet the 1970 FAO/WHO guidelines—that is, approximately 20–30mg/day—and lower the risk of chronic disease. The WHO goal has been roughly translated into the recommendation of five portions of fruits and vegetables per day (Williams, 1995). The current US recommended daily allowance (RDA) for ascorbic acid ranges between 100-120mg/ per day for adults (Gazdik et al., 2008). A Vitamin C deficiency can have a number of detrimental health effects, scurvy being the most serious and well known. Other animals such as primates, guinea pigs are also unable to synthesize vitamin C naturally and can also develop scurvy (Gazdik et al., 2008; Yilmaz et al., 2008 & Fei et al., 2005). Since vitamin C is water soluble, excess levels are not a major health concern because it is readily excreted from the body. Vitamin C functions as a vital electron donor and is an important antioxidant (Esch et al., 2010).

Ascorbic acid can be mostly found in fruits, vegetables and beverages. The main sources of ascorbic acid are citrus fruits, hips, strawberries, peppers, tomatoes, cabbage, spinach, soft drinks, wine and others. Among animal sources, liver and kidney have highest content of the compound, but very low when compared with plant sources (Yilmaz et al., 2008). It is a labile substance which easily undergoes degradation caused by enzymes liberated from the raw material during technological processes, too high temperature, access to air and presence of heavy metals (Azeez et al., 2010). Additionally, ascorbic acid added during the manufacturing level to processed foods may be a major source of variation in some market foods (Dogan et al., 2006). This is why estimation of ascorbic acid in food stuffs represents an indicator to access the level of ascorbic acid before, during and after processing and to also control the quality of finished products. However, there have been difficulties in quantifying ascorbic acid due to its instability in aqueous solutions. The instability of ascorbic acid (I) is due to its oxidation to dehydroascorbic acid (II), which is a reversible reaction, as shown in figure (1) and subsequently

to 2,3diketoL-gulonic acid (III) (Nezamzadeh et al., 2007). The later reaction is irreversible. This oxidation reaction has been used for voltammetric determination of ascorbic acid (Pisoschi et al., 2008).

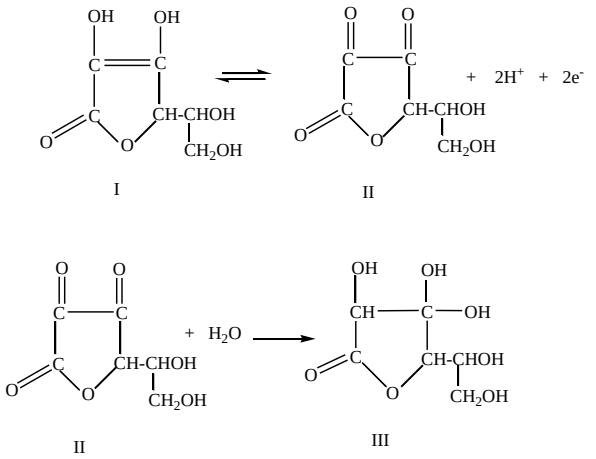

Figure 1. The instable ascorbic acid oxidation to dehydroascorbic acid and subsequently to 2,3diketoL-gulonic acid (Pisoschi et al., 2008).

Several analytical methods have been used to determine ascorbic acid content of food stuffs. Conventional (Volumetric) methods with an oxidant solution such as dichlorophenolindophenol, potassium iodate, bromate and using N-bromosuccinimide. Volumetric techniques suffer from lack of specificity and inability to determine total ascorbic acid thus limiting their use to samples not containing reducing agents. Fluorimetric methods provide more selective possibilities of ascorbic acid determination but the need to strictly control pH of the environment in which ascorbic acid is being determined and high cost of the required equipment are the short comings of this method *(*Okiei *et al.*, 2009*).*

High performance liquid chromatography (HPLC) is a successful method for ascorbic acid determination when selectivity and specificity are needed (Okiei et al., 2009). HPLC with electrochemical detection has turned out to be a selective and sensitive method for ascorbic acid assessment in foodstuff and biological fluids (Azeez et al., 2010).

A potentiometric biosensor (Ogunlesi et al., 2010) for ascorbic acid determination was made by ascorbate oxidase immobilization on a polymeric matrix, fixed on a graphite-epoxy composite electrode. The results reported in literature regarding the determination of ascorbic acid by cyclic voltammetry are not numerous. Nevertheless, cyclic voltammetry has been previously used for antioxidant content assessment, and in particular low-molecular-weight antioxidants, including ascorbic acid; this technique has turned out to be a convenient methodology, validated for the quantification of low-molecular weight antioxidant capacity of tissue homogenates, blood plasma, or plant extracts (Ahmed et al., 2005).

In this study cyclic and square wave voltammetry were used for qualitative characterization and quantitative determination of vitamin C (ascorbic acid) from some selected beverages and fresh edible vegetables. This can help people know vegetables and beverages are good nutritional sources of vitamin C, by what factors can vitamin C be degraded while preparing them for feeding and how can they prevent themselves from scurvy. The purpose of the present study was to assess the level of vitamin C in some selected beverages and fresh edible vegetables locally grown in Amhara Region Adet Agricaltural Center- Ethiopia and also to observe the effect of home cooking temperature on the this Vitamin.

II. EXPERIMENTAL PART

A. Reagents and Chemicals

- 99% L-ascorbic acid (Blulux)
- 98 % Sodium phosphate monobasic (Blulux)
- 85-88% Phosphoric acid (Blulux) and 98% NaOH(Blulux)
- Distilled water
- 98% Na2EDTA.2H2O (Blulux) to prevent ascorbic acid oxidation

B. Samples selected for analysis

Fresh tomato (solanum *inculneatum*), green pepper (piper *abyssinicum*), cabbage leaves (*Brassica oleracea*), lettuce leaves (*Lactuca sativa*), garlic (*Allium satinumc*) and carrot (*Daucus carota*) obtained from Adiet Agricultural Center.

Canned tomato paste (*rotana*), wine, pepsi and mirinda that were bought from local market.

C. Instrumentation

A BAS100 electrochemical analyzer and three electrode systems consisting of glassy carbon (3 mm diameter) as working electrode, Ag/AgCl as reference and platinum wire as auxiliary electrode, pH meter 3305 and analytical beam balance were used.

1) Polishing working electrode

Before each measurement the glassy carbon working electrode was polished with aluminum oxide powder (*Pharmacos LTD*), and washed with distilled water. For each measurement, the potential was scanned between -200 mV and +1000 mV with sensitivity of 100 μA.

D. Working procedure and analysis of samples by cyclic and square wave voltammetry

1) Preparation of supporting electrolyte

0.1 M phosphate buffer solution was prepared daily with 0.1 mM $Na_2EDTA.2H_2O$, and 0.1 M sodium phosphate monobasic and adjusted to the proper pH with phosphoric acid and NaOH.

2) Calibration Curve

A stock solution of 0.08 mM was prepared by dissolving 3.5226 mg of ascorbic acid in 250 cm^3 of 0.1M phosphate buffer (pH 2 for CV and pH 3 for SWV) dosed with 0.1mM EDTA solution. Other standard solutions, 0.008 mM, 0.016

mM, 0.024 mM, 0.032 mM, 0.048 mM, 0.064 mM and 0.08 mM were prepared from stock solution by serial dilution with phosphate buffer to the final volume of 50 ml. 15 cm^3 of each standard ascorbic acid solution was put into the electrochemical cell and the voltammogram was recorded. All measurements were made at room temperature.

3) Sample extraction

10 g of the sample was minced and blended with 100 cm^3 of the phosphate buffer. The homogenized sample was filtered through filter paper. 10 cm^3 of the filtrate was transferred to the electrochemical cell and the voltammogram was recorded. All measurements were made at room temperature.

4) Effect of heat on vitamin C content of some vegetables

100 ml water was boiled at a specified temperature (25 to 65 ^{0}C) in 100 ml beaker using temperature adjuster (IKA C-MAG HS 4). 10 g of each sample was put into the boiled water for 20 min. The vitamin C content of tomato (*solanum inculneatum*), green pepper (*piper abyssinicum*), and garlic (*Allium satinumc*) was determined by cyclic and square wave voltammetry to ascertain the effect of heat on the vitamin C content of these vegetables.

5) Sample analysis

Sample was analyzed using single standard addition method. The accuracy and validity of the proposed methods was further ascertained by performing recovery studies via standard addition method and percentage difference.

6) Quality control

- Since ascorbic acid is light sensitive it was treated in a closed room in the absence of light and light resistant bottles were used.
- Phosphate buffer was prepared daily to protect its degradation.

III. Result and Discussion

A. Cyclic Voltammetric behavior of AA

Figure 2 shows the cyclic voltammograms for phosphate buffer (a) and the oxidation of 8 mM ascorbic acid (b) in 0.1 M phosphate buffer supporting electrolyte at pH 2. The buffer solution gave no response while anodic peak current was observed at 400 mV in the voltammogram of the ascorbic acid solution. No cathodic peak current was found indicating an irreversible heterogeneous charge transfer due to the absence of electro activity on the reverse scan during cyclic voltammetry (Sun & Jiao, 2005).

1) Optimization of the solution pH

The electrochemical behavior of ascorbic acid was dependent on the pH value of the aqueous solution. Therefore, the electrochemical behavior of ascorbic acid in buffered solutions has been studied at different pH values from 1 to 6 by cyclic voltammetry as shown in Figure 3.

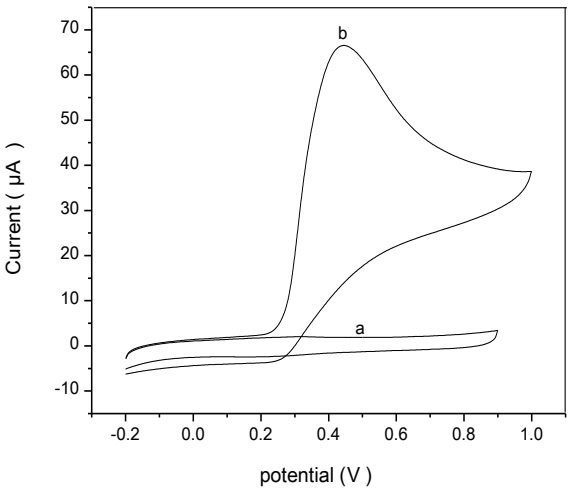

Figure 2. Cyclic voltammograms for 8 mM ascorbic acid in 0.1 M phosphate buffer (pH 2) at a scan rate of 100 mV/s.

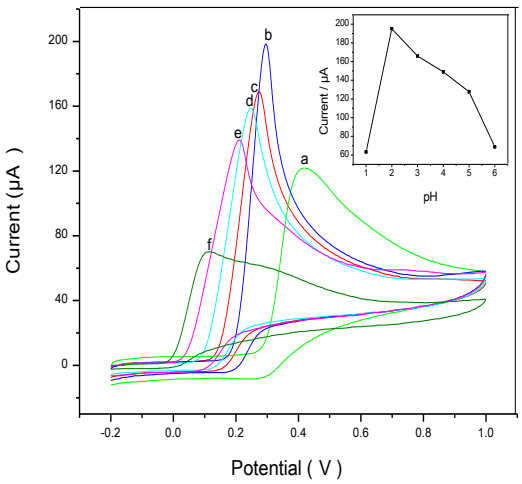

Figure 3. Cyclic voltammogram of 8 mM ascorbic acid in 0.1 M phosphate buffer containing 0.1 mM $Na_2EDTA.2H_2O$ for pH values of (a) 1, (b) 2, (c) 3, (d) 4, (e) 5 and (f) 6 at a scan rate of 100 mV s^{-1}. Inset: plot of peak current *vs.* pH.

Variation in the electrolyte pH results variations in the formal potential of ascorbic acid. The results indicated that the peak potential, E_p, shifted to more negative values with increasing pH. Such a behavior suggests that the participation of protons in the electrode process and the acidic dissociation of ascorbic acid occur at or before the rate determining step (Raoof et al 2006; Goyal et al. 2006). The anodic peak current increased with an increase in pH up to 2.0, and then regularly decreased up to pH 6 as shown in the inset of Figure 3.2. Owing to its efficiency of oxidation, pH 2.0 was chosen as optimal pH.

2) Effect of varying scan rate

The effect of varying scan rate (ν) on the cyclic voltammograms of ascorbic acid in phosphate buffer supporting electrolyte was studied. The peak current is found to be linearly proportional to the square root of scan rate as shown in the inset of Figure 4, with its linear regression equation of given by equation 1:

$$I_p = 24.00v^{1/2} + 41.42 \quad (1)$$

With its correlation coefficient of 0.999 the result illustrates that the process of ascorbic acid oxidation is diffusion controlled irreversible oxidation process (Zhao1et al., 2006).

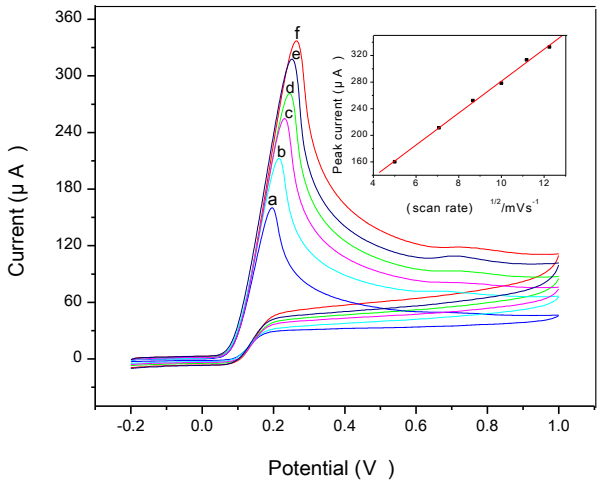

Figure 4. Cyclic voltammograms of 8 mM ascorbic acid in 0.1 M phosphate buffer solution (pH 2.0) at various scan rates: (a) 25, (b) 50, (c) 75, (d) 100, (e) 125, and (f) 150 mV s^{-1}. Inset: plot of peak current *vs* square root of scan rate ($v^{1/2}$)

The peak potential shifted to more positive values as the scan rate increased as shown in Figure 5, in agreement with the irreversible electrochemical behavior observed for ascorbic acid oxidation (Raoof et al., 2007). In order to obtain information on the rate-determining step a Tafel slope, b, was determined using Equation 2 for a totally irreversible diffusion controlled process (Xavier et al., 2011).

$$E_p = (b/2)\log v + \text{constant} \quad (2)$$

The slope of E_p *vs* logν plot was found to be 86.6 mV with its linear regression equation expressed by Equation 3:

$$E_p = 86.609\log v + 71.53 \quad (3)$$

and correlation coefficient of 0.992, thus b = 2×86.6=173.2 mV. This slope value indicates a one-electron transfer to be rate limiting step (Xavier et al., 2011; Razmi & Harasi, 2008).

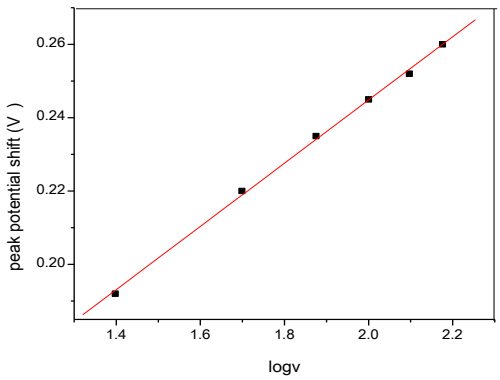

Figure 5. Effect of varying scan rates on peak potential shift of 8 mM ascorbic acid in 0.1 phosphate buffer at pH 2.0

a) Effect of varying ascorbic acid concentrations

Voltammograms show that peak current increases linearly with increasing concentration of ascorbic acid from 0.008 to 0.08 mM as shown in Figure 6. The calibration graph of various ascorbic acid concentrations immersed in 0.1 M phosphate buffer, pH 2.0 was determined as shown in the inset of Figure 6. The linear regression equation is given in Equation 4:

$$I_p = 3728.05C_{AA} + 16.79 \quad (4)$$

where I_p represents the value of the current intensity, from which the background value was subtracted and C_{AA} is the ascorbic acid concentration) and r = 0.997. This shows that ascorbic acid can be quantitatively measured by cyclic voltammetry.

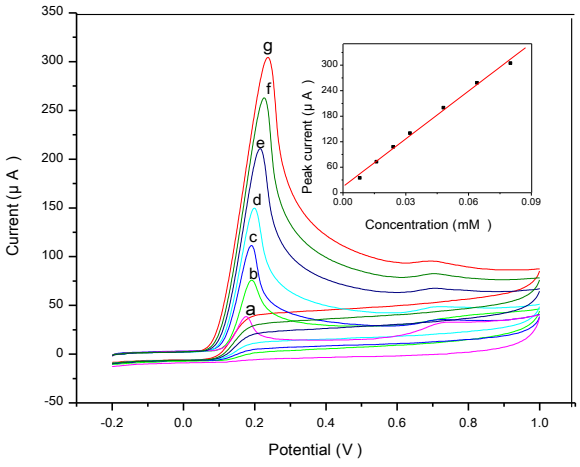

Figure 6. Cyclic voltammograms of ascorbic acid in 0.1 M phosphate buffer supporting electrolyte at pH 2.0 for concentrations of 0.008 (a), 0.016 (b), 0.024 (c), 0.032 (d), 0.048 (e), 0.064 (f), and 0.080 mM (g) at a scan rate of 100 mVs^{-1}. Inset: Calibration graph for ascorbic acid at various concentrations

B. Application of the electrochemical method to ascorbic acid determination

1) Validation of the proposed procedure

The repeatability of the measurement was calculated from 7 independent runs of ascorbic acid solution. The linear regression equation is given in Equation 5.

$$I_p = 3728.5C_{AA} + 16.79 \quad (5)$$

where C_{AA} is in mM with R = 0.997, and P <0.0001.

Validation of the procedure for the quantitative determination of the ascorbic acid was examined via evaluation of the limit of detection (LOD), limit of quantification (LOQ), and repeatability. LOD and LOQ were calculated from the peak current using the following equations (Kalanur et al., 2008):

$$LOD = 3\ s/m, \quad (6)$$

$$LOQ = 10\ s/m \quad (7)$$

Where s is the standard deviation of the peak currents (n=7) and m is the slope of the calibration curve Figure 6. LOD and LOQ were obtained as 0.0889 mM and 0.0296 mM, respectively with p <0.0001.

2) Application to real sample analysis

In order to verify the accuracy of the method for ascorbic acid determination in vegetables and beverages, the standard addition method was applied to lettuce analysis. 10 g of the sample was minced and blended with 100 cm^3 of the phosphate buffer. The homogenized sample was filtered through filter paper. 10 cm^3 of the filtrate was transferred to the electrochemical cell. Its voltammogram was registered and its peak height (hx) was measured. After adding a single aliquot (Va = 6 ml) of standard solution having concentration of Cst, a new voltammogram was registered and a new peak height (ha) was also measured. Sample concentration (Cx) was obtained using Equation 8 (Protti et al., 2001).

$$Cx = \frac{hx}{(ha - hx)} \frac{CstVa}{Vx} \quad (8)$$

Figure 7 shows cyclic voltammogram of (a) sample of lettuce and sample containing acid standard solution. The shapes and positions of the onset potential, peak potential, and the increase in peak current are similar to the voltammogram of the standard ascorbic acid.

3) Determination of degree of recovery for lettuce (Lactuca sativa) and red wine

10 cm^3 of the sample of red wine was mixed with 5 $cm^3$of phosphate buffer (pH 2).

15 cm^3 of the homogenized sample was transferred to the electrochemical cell. Its voltammogram was registered and its peak height (hx) was measured. After adding a single aliquot (Va = 6 ml) of standard ascorbic acid solution having concentration of Cst, a new voltammogram was registered and a new peak height (ha) was also measured as shown in Figure 8. The degree of recovery was determined by Equation 9 using a standard addition or spike procedure.

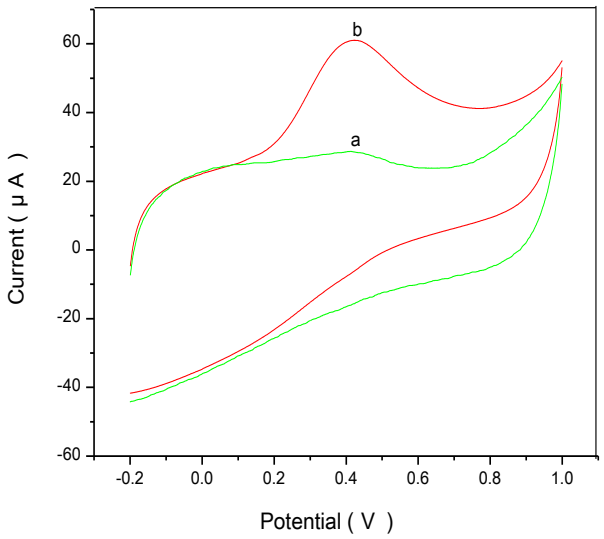

Figure 7. Cyclic voltammogram of (a) sample of lettuce and (b) a + 6 ml of 8 mM ascorbic acid standard at pH 2 and scan rate of 100 mVs^{-1}.

$$R_A = \frac{Q_A(O + S) - Q_A(O)}{Q_A(S)} \quad (9)$$

where $Q_A(S)$ is the quantity of analyte A added (spike value) and $Q_A(O + S)$ the quantity of A recovered from the spiked sample and $Q_A(O)$ from the original sample.

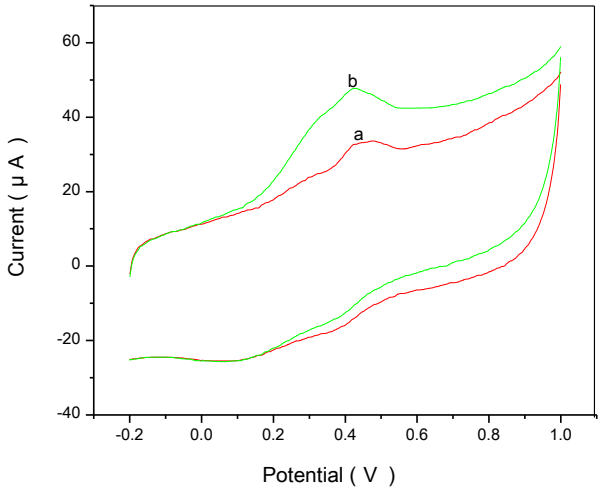

Figure 8. Cyclic voltammogram of (a) sample of red wine and (b) a + 6 ml of 8 mM ascorbic acid standard at pH 2 and scan rate of 100 mVs^{-1}.

Figure 8 shows the cyclic voltammogram of red wine sample (a) and sample containing ascorbic acid standard solution (b). The onset potential, peak potential, offset potential and the increase in peak current are similar to the voltammogram of standard ascorbic acid.

TABLE I. DETERMINATION OF DEGREE OF RECOVERY FOR LETTUCE (*LACTUCA SATIVA*) AND RED WINE

Sample	Concentration before addition of standard to 100 ml filtrated of solution	Concentration after addition 84.54 mg AA to 100ml of filtrated solution	%recovery
Lettuce	9.26 mg/100 ml	93.8 mg/100 ml	99.99
Red wine	23.90 mg/100ml	108.44 mg/100ml	100.00

The degree of recovery for lettuce and wine was determined from Figure 7 and 8, respectively. It is very close to the ideal value 100% as shown in the Table 1.

4) Cyclic voltammetric study of the effect of temperature on vitamin C content of garlic, tomato and green pepper

The stability of vitamin C in sample depends on many factors. Cooking temperature is one such factor which dramatically affects vitamin C content. The rate of oxidation of L- ascorbic acid to the unstable L- dehydroascorbic acid increases as the temperature increases. This increasing rate of oxidation speed-up the rate at which vitamin C activity is lost.

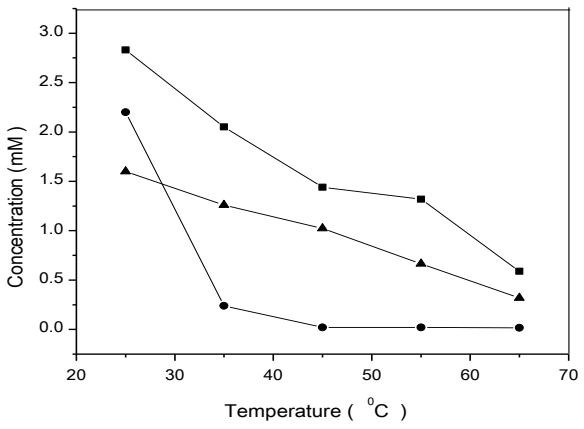

Figure 9. Effect of temperature on vitamin C content of garlic (●), tomato (▲) and pepper (■) cooked for 20 min each in 25, 35, 45, 55 and 65 ^{0}C boiled water.

Vitamin C content of garlic, tomato and green pepper was decreased as the temperature was increased as shown in Figure 9. During cooking, the vitamin C content gradually decreases at a rate depending on cooking temperature. The more rapid decrease of ascorbic acid concentration at the beginning of the cooking can be attributed to the immediate reaction/degradation of an amount of ascorbic acid with cooking temperature (Polydera et al., 2003).

C. Square wave voltammetric behavior of ascorbic acid

Figure 10 shows the square wave voltammograms of the phosphate buffer (a) and the oxidation of ascorbic acid (b) in 0.1 M phosphate buffer supporting electrolyte at pH 3.0.

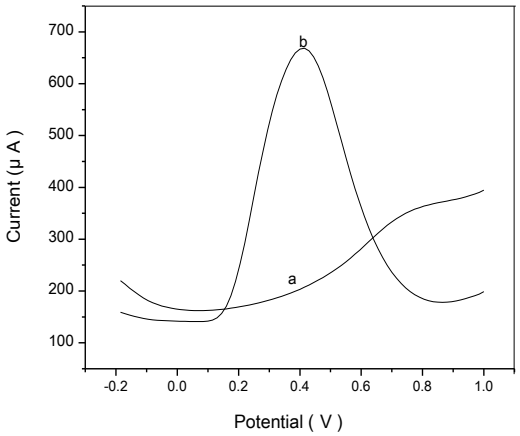

Figure 10. Square wave voltammograms for (a) 0.1 M phosphate buffer supporting electrolyte (b) a + 8 mM ascorbic acid at pH 3.0. Experimental conditions: f = 60 Hz, ΔE_p = 100 mV, ΔE_s = 15 mV.

The buffer solution gave no response while anodic peak current was observed at 400 mV in the voltammogram of ascorbic acid solution as shown in (a) and (b) of Figure 10, respectively. No cathodic peak current was found indicating an irreversible heterogeneous charge transfer due to the absence of electro activity on the reverse scan during cyclic voltammetry.

1) Optimization of the solution pH

The influence of pH on the electrochemical oxidation of ascorbic acid was investigated in the range 1− 6 as shown in Figure 11. In all cases the concentration of the phosphate buffer was maintained at 0.1 M and voltammograms were obtained using 8 mM ascorbic acid.

The peak current for the electrochemical oxidation of ascorbic acid was strongly affected by the solution pH as shown in Figure 11. The inset of maximum peak current was observed at pH 3. Therefore, all subsequent measurements were performed at pH 3. This pH is also suitable for better stability of ascorbic acid.

The results indicated that the peak potential, E_p, shifted to more negative values with increasing pH. Such a behavior suggests that the participation of protons in the electrode process and the acidic dissociation of ascorbic acid occur before the rate determining step (Peeter, 2006-2007).

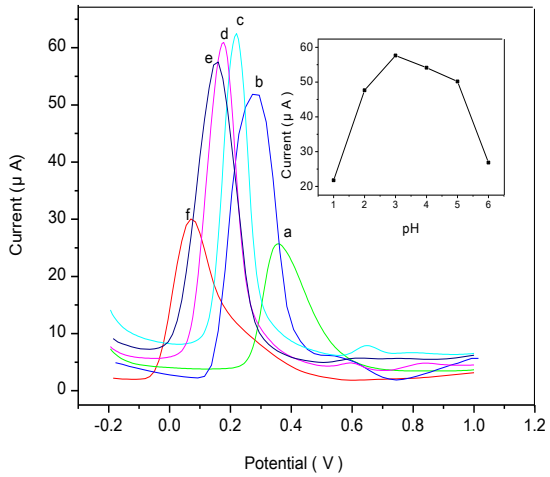

Figure 11. Square wave voltammogram of 8mM ascorbic acid in 0.1M phosphate buffer containing 0.1 mM $Na_2EDTA.2H_2O$ for pH values of (a) 1, (b) 2, (c) 3, (d) 4, (e) 5 and (f) 6. Inset: Plot of peak current *vs* pH. Experimental conditions: f = 60 Hz, ΔE_p = 100 mV, ΔE_s = 15 mV.

2) Effect of square wave voltammetric parameters

The electroanalytical method for the determination of ascorbic acid was developed using square wave voltammetry, which is an effective and well-established pulse voltammetric technique suitable for determination of organic compounds (Levent et al., 2009; Parham et al., 2008). The response obtained by square-wave voltammetry was dependent on parameters such as frequency (f), pulse height (ΔE_p) and scan increment or step potential (ΔE_s), which have a combined influence on the peak current. Hence, they were analyzed in order to optimize the experimental setup for ascorbic acid determination. The square wave parameter optimization was carried out in solutions of 8 mM ascorbic acid in 0.1 M phosphate buffer of pH 3 containing 0.1 mM $Na_2EDTA.2H_2O$.

a) Effect of pulse amplitude

For the investigation of the influence of the pulse amplitude on the analytical signal as shown in Figure 12, we varied this parameter between 25 and 150 mV, at 15mV step potential and 60 Hz frequency.

The value of the measured current intensity increased with the applied pulse amplitude. An optimum value of 100 mV was chosen for further studies and for real sample analysis. Greater values of the pulse amplitude were not employed, in order to avoid the decrease of resolution.

b) Effect of step potential

The influence of step potential, which determines the amount of potential changes between two data points in the experiment, was investigated between 5 and 20 mV at fixed f and ΔE_p as shown in Figure 13.

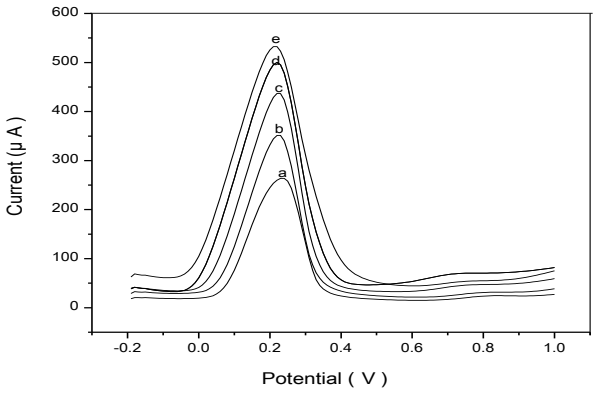

Figure 12. Influence of the pulse amplitude on the analytical response at ascorbic acid determination by square wave voltammetry ; (a) 25 mV, (b) 50 mV, (c) 75 mV , (d) 100 mV, (e) 125 mV and (f) 150 mV ; experimental conditions: ΔE_s = 15 mV, f= 60 Hz.

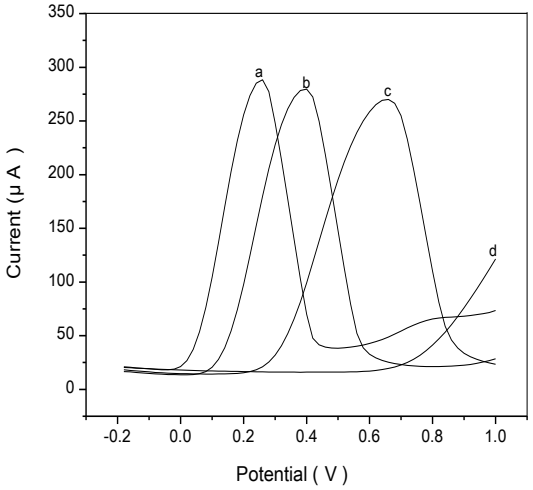

Figure 13. Influence of the step potential on the analytical response at ascorbic acid determination by square wave voltammetry; (a) 5 mV, (b) 10 mV, (c) 15 mV, and (d) 20 mV. Experimental conditions: ΔE_p = 100 mV, f= 60 Hz.

The peak height increased up to 15 mV because the effective scan rate was increased, but at higher values of step potential, the peak heights decreased. Accordingly step potential of 15 mV was chosen for further study. The potential shift is due to the increase in scan rate as a result of increasing step potential.

c) Effect of Frequency

The effect of square wave frequency on peak current and peak potential of ascorbic acid was studied in the range of 15 - 90 Hz at constant ΔE_p and ΔEs as shown in Figure 14. The peak current was found to increase linearly with square wave frequency as shown in the inset of Figure 14 and the relation between i_p and f can be represented by the equation 10:

$$i_p = 1.66f + 129.34 \quad (10)$$

The correlation coefficient for the expression was 0.998.

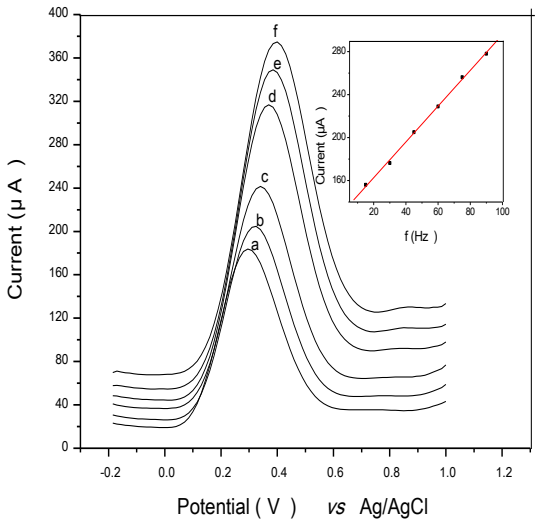

Figure 14. Square-wave voltammogram of 8 mM ascorbic acid in 0.1 M phosphate buffer solution (pH 3.00) at various frequencies: (a) 15, (b) 30, (c) 45, (d) 60, (e) 75, and (f) 90 Hz. Inset: plot of peak current *vs* frequency. Experimental conditions: ΔE_p = 100 mV, ΔE_s = 15 mV

The peak current increased with the frequency due to the increase in the effective scan rate but the peak shape and baseline were distorted at frequencies higher than 60 Hz and therefore 60 Hz was selected as optimum frequency for further study. This was attributed to the greater contribution of the capacitive current at higher frequencies (Parham et al., 2008).

The peak potential also shifted to more positive potential with increase in square wave frequency and the plot of E_p *vs* log f was linear and its correlation coefficient was 0.999 as shown in Figure 15. The variation of E_p with logf obeys the Equation 11:

$$E_p \text{ (mV)} = 128.81\log f + 143.3 \quad (11)$$

These observations are in agreement with the properties of irreversible electrochemical process which is diffusion controlled (Parham et al., 2008).These results support the inferences obtained from cyclic voltammetry studies.

3) Effect of varying ascorbic acid concentrations

Under the obtained optimum conditions (pH =3, *f* = 60 Hz, ΔE_p = 100 mV, ΔE_s = 15 mV), the calibration graph for determination of ascorbic acid was obtained in the concentration range of 0.008 - 0.08 mM. The voltammograms for different concentrations of ascorbic acid are shown in Figure 16. The regression equation is represented by Equation 12 as shown in the inset of Figure 16.

$$I_p = 10838.03C_{AA} + 26.16 \quad (12)$$

with a correlation coefficient of 0.991 as shown in the inset of Figure 16.

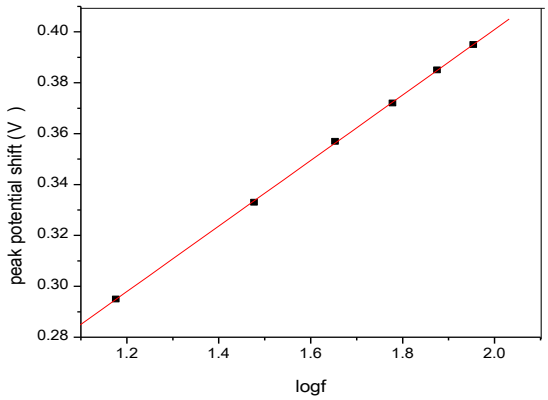

Figure 15. Effect of square wave frequency on the peak potential shift of 8 mM ascorbic acid at pH 3. Experimental conditions: ΔE_p = 100 mV, ΔE_s = 15 mV.

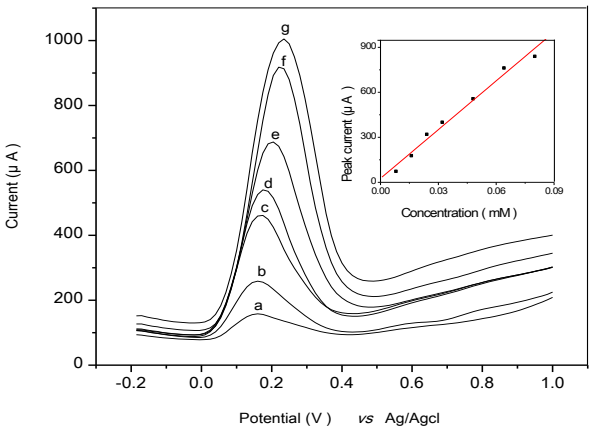

Figure 16. Square wave voltammograms of ascorbic acid in 0.1 M phosphate buffer supporting electrolyte at pH 3.0 for various concentrations: (a) 0.008, (b) 0.016, (c) 0.024, (d) 0.032, (e) 0.048, (f) 0.064, and (g) 0.080 mM. Inset: A calibration plot of peak current *vs* concentration under the optimized parameters.

The peak current increased when concentration was increased indicating diffusion controlled irreversible oxidation process and ascorbic acid can be determined using square wave voltammetry.

D. Application of the OSWV method for ascorbic acid determination

1) Validation of the proposed procedure

The repeatability of the measurement was calculated from 7 independent runs of ascorbic acid solution. LOD and LOQ were obtained as 0.0115 mM and 0.0385 mM, respectively with p <0.0001 from the calibration graph as shown in the inset of Figure 16.

2) Application to real sample analysis

In order to verify the accuracy of the method for ascorbic acid determination in vegetables and beverages, the standard addition method was applied to tomato analysis. 10 g of the sample tomato was minced and blended with 100 cm^3 of the phosphate buffer. The homogenized sample was filtered through filter paper. 10 cm^3 of the filtrate was transferred to the electrochemical cell. Its voltammogram was registered and its peak current (hx) was measured.

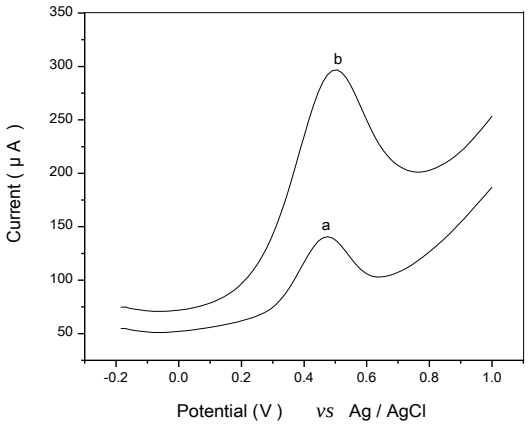

Figure 17. Square voltammogram of (a) sample of tomato and (b) a + 8 mM AA standard at pH 3, Experimental conditions: f 60 Hz; ΔE_s 15 mV; ΔE_p 100 mV.

After adding a single aliquot (Va = 6 ml) of standard solution having concentration of Cst, a new voltammogram was registered and a new peak current (ha) was also measured. Sample concentration (Cx) was obtained using equation 8. Figure 17 shows square wave voltammograms sample of tomato (a) and a + 6ml of 8 mM standard ascorbic acid solution (b) under the optimized parameters. The shapes, baselines and positions of the peak potentials and the increase in peak current are similar to the voltammogram of the standard ascorbic acid.

3) OSWV determination of degree of recovery for fresh tomato sample

TABLE II. DETERMINATION OF DEGREE OF RECOVERY FOR TOMATO (*SOLANUM INCULNEATU)* (N= 3).

Sample	Concentration before addition of standard to 100 ml filtrated of solution	Concentration after addition 84.54 mg AA to 100ml of filtrated solution	%recovery
Tomato	48.06mg/100ml	132.60 mg/100ml	100

The degree of recovery for tomato was determined from Figure 17, as shown in Table 2, with an excellent recovery value of 100%. The method was applied for the other vegetable samples in similar way.

TABLE III. ASCORBIC ACID CONCENTRATIONS IN SOME FRESH EDIBLE VEGETABLES AND BEVERAGES AS DETERMINED BY CYCLIC VOLTAMMETRY AND SQUARE WAVE VOLTAMMETRY (N=3).

Sample		Concentration of AA from CV mg/100ml	Concentration of AA from SWV mg/ 100ml	% Difference
Common Name	Botanical Name			
Garlic	*Allium satinumc*	22.1	30.00	30.30
Pepper	*Capsicum annu*	28.3	31.10	9.40
Lettuce	*Lactuca sativa*	5.26	7.62	36.60
Tomato	*Solanum inculneatm*	16.00	3.50	37.97
Tomato Paste	*Rotana*	8.47	12.48	38.28
Carrot	*Daucu carota*	5.83	8.729	39.82
Cabbage Leaves	*Brassica oleracea*	5.95	9.188	42.77
Mirinda	-	6.17	12.14	65.20
Pepsi	-	8.328	12.94	43.37
Red Wine	-	13.57	20.62	41.24

As shown in Table 3, the results obtained by square wave voltammetry were greater than the results obtained by cyclic voltammetry for all samples. They are also in a good agreement with the data reported in literature regarding the acid content of vegetables and wine: the vitamin C content of tomato determined by cyclic and differential pulse voltammetry is 18.63 and 19.24 mg/100 g sample at carbon past electrode, respectively (Pisoschi et al., 2011). The ascorbic acid content of wine obtained by cyclic and differential voltammetry is 15.05 and 16.22 mg/ 100 ml sample, respectively (Parham et al., 2008).The ascorbic acid content of garlic obtained by cyclic voltammetry is 38.68 mg/ 100 g (Okiei ascorbic et al., 2009). The ascorbic acid content of carrot, cabbage and lettuce leaves obtained by cyclic voltammetry is 9.28, 23.05, and 22.27 mg/100 g, respectively (Ogunlesi et al., 2010). The content of AA determined using HPLC in garlic and cabbage are 39.65 ± 11.90 and 34.29 ± 5.83 mg/100g, respectively, but 14.68 ± 4.99 mg/100g for tomato (Shimada, Y. and Ko, S., 2006).

4) OSWV study of the effect of temperature on vitamin C content of garlic, tomato and green pepper

The level of vitamin C in garlic, tomato and green pepper was highly affected by the applied temperature as shown in Figure 18.

When the temperature increased from 25 to 65 ^{0}C, the level of vitamin C of these vegetables decreased continuously. Garlic was affected more.

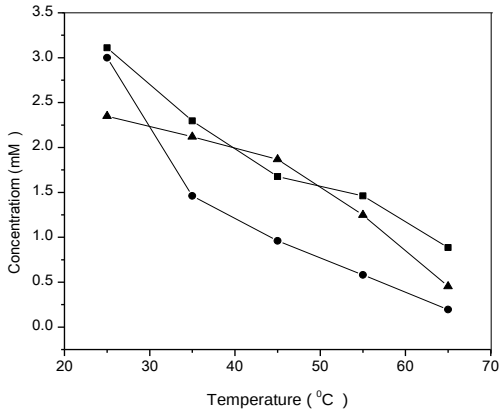

Figure 18. Effect of temperature on vitamin C content of garlic (●), tomato (▲) and green pepper (■) cooked for 20 min each in 25, 35, 45, 55 and 65 ^{0}C boiled water.

IV. CONCLUSION

The level of vitamin C determined from each vegetable and beverages was in the range of 5.26 mg/100 ml for lettuce to 28.3 mg/100 ml for green pepper for cyclic voltammetry and 7.62 mg/100 ml for lettuce and 31.1 mg/100 ml for green pepper juices obtained by squeezing vegetable for square wave voltammetry technique. The lowest and the highest values of vitamin C were obtained from lettuce and green pepper, respectively. The results obtained by square wave voltammetry were better than the results obtained by cyclic voltammetry. So, SWV is better than CV for ascorbic quantification. Thus, effect of temperature was studied to ascertain the effect of heat on the vitamin C content of garlic, tomato and green pepper. The amount of Vitamin C in these vegetables was degraded when the temperature was increased from 25 - 65 ^{0}C by both techniques. Garlic was highly affected with percentage loss of 99.29% and 93.47% using CV and SWV, respectively. Hence fresh vegetables have higher level of vitamin C than cooked vegetables.

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