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Electrochemical determination and comparison of ascorbic acid in freshly prepared and bottled fruit juices: A cyclic voltammetric study

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ABSTRACT

Voltammetric techniques have been considered as important methods among the analytical techniques used for the identification and determination of trace concentrations of many biological molecules such as L-ascorbic acid (AA). In this study, ascorbic acid content of some freshly prepared and bottled fruit juices were determined and compared by cyclic voltammetry using carbon paste working electrode. Various factors such as the effect of ascorbic acid concentration on the response of CPE, pH of phosphate buffer solution and some common instrumental parameters on the response characteristics of the electrodes were investigated. From the calibration graph, the relationship between the peak height and ascorbic acid concentration within carbon paste working electrode was investigated. The equation of the calibration graph was found to be: I_p (μA) = 5.034 (mM) + 1.919, R^2=0.998, SD= 2.138, n=10. The LOD and LOQ for the developed method were determined to be 0.0221 mM & 0.0735 mM respectively. The degree of recovery for some freshly prepared and bottled fruit juices was calculated by adding standard solution of ascorbic acid to the analyzed fruit juices and the results were found to be in the range between 93.35% - 105.29%.

Keywords: Cyclic voltammetry, ascorbic acid, bottled juices, carbon paste electrode

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INTRODUCTION

Ascorbic acid, commonly known as vitamin C, is an essential nutrient that plays a vital role in protecting the body from infection and disease. It is needed for the formation of collagen, the protein that makes up connective tissue, and is essential to muscles, bones cartilages, blood vessels, capillaries, tissues, skin and teeth [1, 2]. Popular literature suggests that large doses (one gram or more) may prevent or cure the common cold or influenza [3, 4]. Some excellent sources of vitamin C include: Kiwi fruit, orange juice, cantaloupe, cranberry juice, grapefruit, strawberries, watermelon, raspberries, green peppers, cauliflower, broccoli, collard greens, potato, tomato, sweet potato, and red peppers [5, 6]. Low vitamin C levels have been associated with high blood pressure, increased heart attack risk, increased risk for developing cataracts, and a higher risk for certain types of cancer, Stunted Growth, Slow-healing wounds & fractures, Scurvy, tooth decay, Improper Bone Development, Loss of appetite, Weakened Cartilages, Poor Collagen Production, Skin hemorrhages and impaired digestion are some of the common deficiency symptoms for vitamin C [7]. High levels of ascorbic acid in the human body can cause adverse effects; it may lead to gastric irritation, and the metabolic product of vitamin C (oxalic acid) can cause renal problems. That is why ascorbic acid content of foodstuffs and beverages represents a relevant indicator of quality which has to be carefully monitored, regarding its variation during manufacturing and storage [8-10]. Thus accurate and specific determination of the nutrients content of fruits is extremely important to

understand the relationship of dietary intake and human health. Numerous analytical techniques are available for the determination of vitamin C in different matrices. Some of the techniques include: direct titration [11, 12], fluorometric methods [13], chromatographic methods [14-16], Electrochemical [17]. However, some of these methods are time-consuming, some are costly, some need special training operators, or they suffer from the insufficient sensitivity or selectivity [6]. Due to its selectivity and sensitivity, an electrochemical method to determine of ascorbic acid has been a subject of considerable interest [10, 12]. Because of low costs of the required equipment as well as the simplicity of the employed procedures, voltammetry appears to offer an attractive alternative method to determine ascorbic acid, particularly during the routine quality control of some food products [13]. Cyclic voltammetry (CV) is a versatile tool that allows the electrochemical characterization of a wide variety of materials. It offers a rapid location of redox potentials of the electroactive species [18]. Carbon paste electrodes have been used as the working electrode in cyclic voltammetry experiments aimed to the identification, characterization and quantification of antioxidants, including ascorbic acid, phenolic and polyphenolic compounds, glutathione and synthetic antioxidants [19]. The use of carbon-paste matrix, besides renewability by a simple polishing, offers several other advantages including easy preparation, uniform distribution of the catalyst into the paste, better reproducibility and stability, adequate robustness in aqueous solutions, low background current, wide potential window and versatility [9, 10]. Recent advances in the food and pharmaceutical industries and a need for nutritional assessment have necessitated the development of a selective, simple and accurate method to determine of ascorbic acid [11]. This study was carried out with aim of determination and comparison of ascorbic acid contents of some natural freshly prepared and commercial bottled fruit juices by cyclic voltammetry using carbon paste electrode as well as to study the influences of some factors such as pH of buffer solution, concentration of ascorbic acid, and the common instrumental parameters on the response characteristics of the CPE.

EXPERIMENTAL SECTION

2.1 Instrumentation

Voltammetric experiments were carried out with a BAS (Bioanalytical Systems); CV-50W voltammetric analyser, which was connected to a DELL desktop computer used for electrochemical measurements and treating data. Cyclic Voltammetry with three electrode systems: carbon paste electrode as working electrode, saturated Ag/AgCl electrode used as reference electrode and a platinum electrode as an auxiliary electrode were used in all the voltammetric measurements. 353 ATC pH-meter was used to read the pH of the buffered solution. Digital (110g/0.1mg) balance model LA 114 was used for mass measurements, and ultra-8V centrifuge machine was also used in analyzing samples to obtain clear solutions.

2.2 Chemicals and reagents

All the chemicals used for the experimental purposes were of analytical grade and used without further purification. The chemicals used for this experimental purpose include: Ascorbic acid (NICE, India), graphite powder (BDH, England), distilled water, KH_2PO4 (NICE, India), K_2HPO4 (FINKEM), paraffin oil (Nice, India), NaOH (Scharlau), H_3PO_4 (FINKEM), potassium iodide (Scharlau), iodine (NICE, India) and starch indicator solution. Buffer solutions with pH= 4, pH=7, and pH=10 (Bululux Labratories) were used to calibrate the pH-meter. Phosphate buffer solutions with pH ranging between 2 and 9 were prepared from 0.1M K_2HPO_4 and 0.1M KH_2PO_4 and the desired pH values were adjusted using NaOH and H_3PO_4 as appropriate. Since ascorbic acid solutions were unstable, the sample and reference solutions were made freshly and keep away from light to avoid oxidation prior to any test. Standard solutions of ascorbic acid with concentration ranging between 0.07 mM and 20 mM were obtained by diluting 0.1M stock solution of ascorbic acid with the respective volumes of 0.1 M phosphate buffer (supporting electrolyte) solution.

2.3 Real sample analysis

Various fruits (sweet orange juice, mango juice, pineapple juice and tomato juice) were bought from fruit shops available in Mekelle city, Ethiopia and freshly prepared fruit juices were obtained by fruit pressing. The fruit samples were first washed with water; the juice from each fruit was squeezed out, and filtered. Then, the obtained juice was centrifuged until a clear sample was obtained, which was subsequently analyzed. Bottled fruit juices were obtained from supermarket, and the juices were first filtered and then centrifuged before analysis. The ascorbic acid content in the fruit juice samples were determined by measuring the peak current from the calibration curves in which the Background current was subtracted and calculated using the volume measured and expressed in milligrams per 100 mL of sample.

2.4 Preparation of Carbon paste electrodes

Graphite powder and paraffin oil were used to prepare the bare carbon paste electrode. Carbon paste electrodes were prepared according as follows: Different ratios (10% -40%) of paraffin oil with respect to graphite powder were prepared by hand mixing and made the mixture homogenous. The prepared mixture was packed in to the syringe. Electrical contact was made by pushing a copper wire down the syringe into the back of the mixture. Percent of paraffin oil (with respect to graphite powder) that showed good sensitivity was selected and this ratio was applied for the determination of ascorbic acid in fruit juices as well as to study the effect of other voltammetric parameters on the developed method.

2.5 Working procedure

0.1M ascorbic acid stock solution was prepared by dissolving 1.7613g of ascorbic acid in 100 mL phosphate buffer solution. Appropriate amounts of the phosphate buffer solution (pH = 2-9) and ascorbic acid standards or sample solutions were pipetted into the voltammetric cell. All solutions were prepared with distilled water and the measurements were made at a 25 ± 0.2 ^{o}C. For each voltammetric measurement, the potential was scanned within the range *0* and *9*00mV, with a 100 mVs^{-1} scan rate, and the value of the background current obtained for the supporting electrolyte solution was subtracted from the current corresponding to the solution/analyzed sample. For investigating the potential scan rate influence, this parameter varied from 20 to 500 mVs^{-1}. The voltammetric working procedure employed for standard ascorbic acid solutions was also applied to analyzed juices. The electrode surface layer of the carbon paste electrode was regularly being removed using a wet paper for surface renewal of the CPE. Data were analyzed using Microcal Origin version 6.0 software.

2.6 Iodometric Titration

Iodometric titration was used to compare the content of ascorbic acid in freshly prepared and bottled fruit juices obtained by cyclic voltammetry. The concentration of the prepared iodine solution was determined by titration with a standard solution of ascorbic acid using a 0.5% starch indicator. Titrations were carried out for freshly prepared orange juice and pineapple bottled fruit juice samples and titrated with 0.005 $molL^{-1}$ iodine solution. The number of moles of ascorbic acid reacted was determined according to the following equation of titration:

Ascorbic acid + $I_2 \rightarrow 2\,I^-$ + dehydroascorbic acid

The endpoint is indicated by the reaction of iodine with starch suspension, which produces a blue-black product. As long as ascorbic is present, the triiodide is quickly converted to iodide ion, and no blue-black iodine-starch product is observed. However, when all the vitamin C has been oxidized, the excess triodide (in equilibrium with iodine) reacts with starch to form the expected blue-black color. The titration was repeated and the average volume iodine solution was taken and the moles of iodine reacting was calculated. The molar concentration of ascorbic acid obtained for the analyzed fruit juices were expressed in milligrams per 100 mL.

RESULTS AND DISCUSSION

3.1 The Effect of Electrode Composition

Different ratios of carbon paste ranging between 60% and 90% with respect of paraffin oil which was used as binder using 5 mM ascorbic acid was tested and the optimum value was obtained at the ratio 70% of carbon paste with respect to paraffin oil and the CPE prepared in this ratio was used for all the voltammetric measurements.

3.2 The Effect of pH of the supporting electrolyte

The electrochemical behavior of ascorbic acid (pKa1 =4.17 and pKa2 =11.5) is dependent on the pH value of the aqueous solution [22]. As a result, pH optimization of the solution seems to be necessary in order to obtain the electrocatalytic oxidation of AA [8]. The effect of pH for 0.1M phosphate buffer solution was investigated in the pH range between 2 and 9 using cyclic voltammetry. The current increases up to 5 and then decreases in which the optimum current was obtained at pH= 5

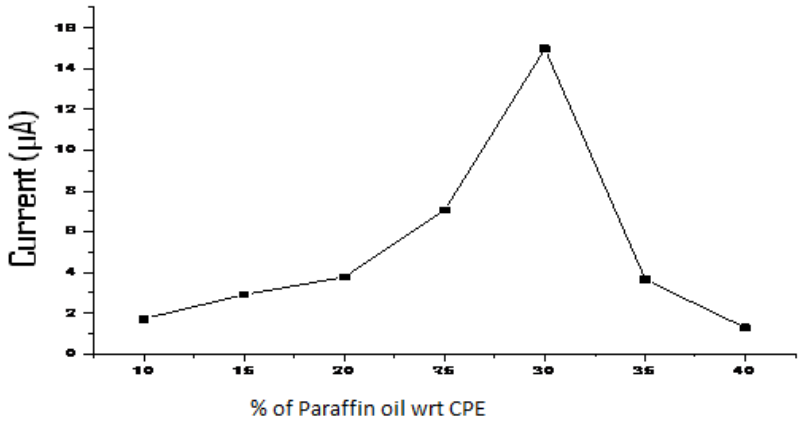

Figure 1: graph showing the amount of paraffin oil with respect to carbon paste and the current obtained

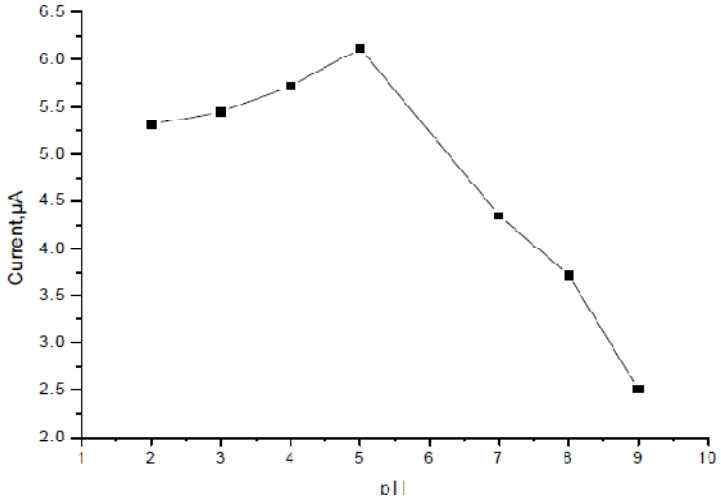

Figure 2: Current-pH curve for electrooxidation of 5.0 mM Ascorbic acid at carbon paste working electrode at various pH values (2, 3, 4, 5, 7, 8 & 9) in 0.1 M phosphate buffered solution at a scan rate of 100 mVs^{-1}

The cyclic voltammograms shape and peak position of analyzed fruit juices is similar with the result obtained for standard ascorbic acid solution and no reduction peak is appeared. This shows the data reported in literature [4] that electrochemical oxidation of ascorbic acid is an irreversible process. Therefore, cyclic voltammetric method can be used to determine the concentration of ascorbic acid in natural fruit juice.

3.3 The effect of scan rate

The effect of the potential scan rate on the electrocatalytic properties of CPE in 0.1 M phosphate buffered solution containing 5 mM ascorbic acid was studied. The measurements were performed at the potential scan rate between the range 20 and 500 m mVs^{-1} in which the background current was subtracted.

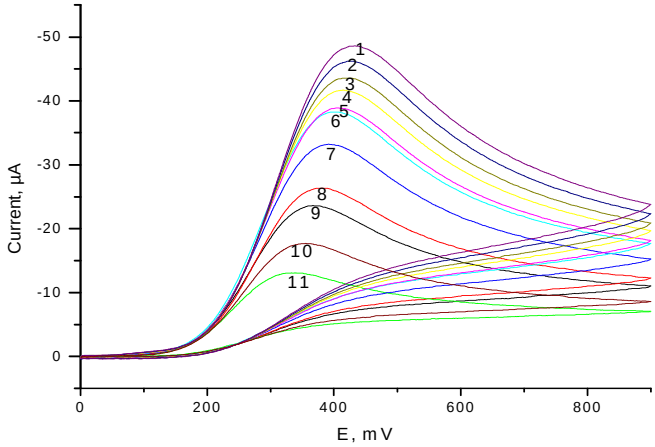

Figure 3: (A) Cyclic voltammograms of the CPE in the presence of 5.0 mM AA at various scan rates: 20 (11), 50 (10), 100 (9), 150 (8), 200 (7), 250 (6), 300 (5), 350 (4), 400 (3), 450 (2) and 500mVs^{-1}(1) in 0.1M phosphate buffer solution (pH=5)

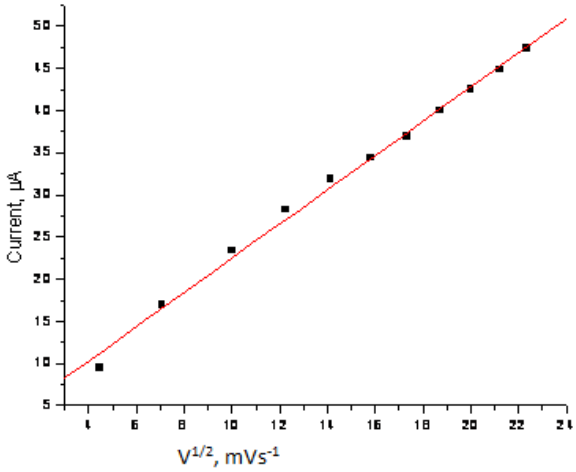

Figure 4: Graph of the anodic peak currents vs square root of scan rate obtained from Figure 3

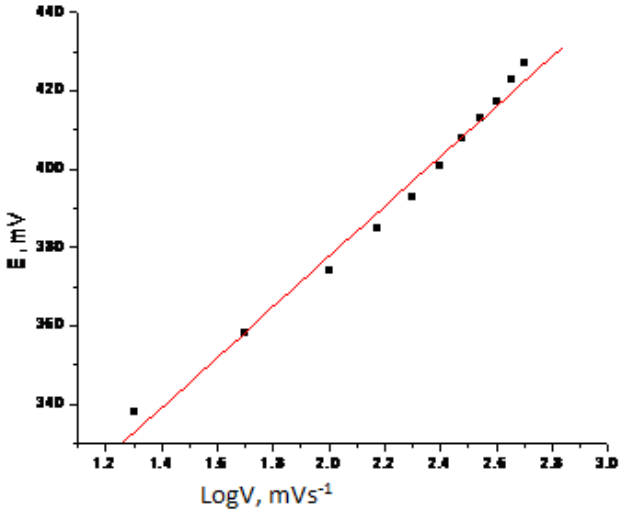

Figure 5: Graph of the anodic peak potential versus log v obtained from Figure 3

As shown in Figure 4, the voltammetric peak current at the carbon paste working electrode was linearly proportional to the square root of scan rate (R^2=0.9975), which indicates that the process of ascorbic acid oxidation in the developed method is diffusion controlled. The diffusion coefficient (D) in the developed voltammetric method was determined to be $2.2x10^{-5}$ cm^2s^{-1} and the geometric surface area of the carbon paste working electrode is $0.053cm^2$. From figure 3, it can be also noted that with an increasing the scan rate, the peak potential for the catalytic oxidation of ascorbic acid shifts to the more positive potentials, which indicates characteristics of totally irreversible systems [20]. Thus, the oxidation ascorbic acid is totally irreversible systems. The electron transfer rate (k_s) on CPE for developed voltammetric technique was calculated based on the plot of E_p vs log v. The slope of the resulted curve of E_p vs. log v for cathodic and anodic peaks are $-\frac{2.3RT}{\alpha nF}$ and $\frac{2.3RT}{(1-\alpha)nF}$ respectively, where E_p is the cathodic or anodic peak potential, v is the scan rate, α is the electron transfer coefficient and n the number of electrons involved in the electrochemical reaction. F, R and T are faraday's constant, universal gas constant and temperature respectively [22]. By calculating α from the slope of E_p vs log v curve, k_s can be obtained from the Equation:

$$Logks = \alpha \log(1-\alpha) + (1-\alpha)log\alpha - \log\left(\frac{RT}{nFv}\right) - \frac{\alpha(1-\alpha)nF\Delta Ep}{2.3RT}$$

From the slope of equation of plot E_p vs logv in Figure 5, the electron transfer coefficient (α) was calculated to be 0.57. By substitution this parameter in equation (i), K_s was calculated as $6.34x10^{-5}$ $M^{-1}s^{-1}$.

3.4 Calibration curve

In Figure 6, several cyclic voltammograms, obtained for different ascorbic acid concentrations (0.07 mM-20 mM were presented. The calibration graph (Figure 7), shows a linear range obtained between 0.07 and 20 mM ascorbic acid (R^2=0.998, Ip (μA) = 5.034 (mM) + 1.919. The value calculated for the relative standard deviation R.S.D. was 2.138 and n = 10)

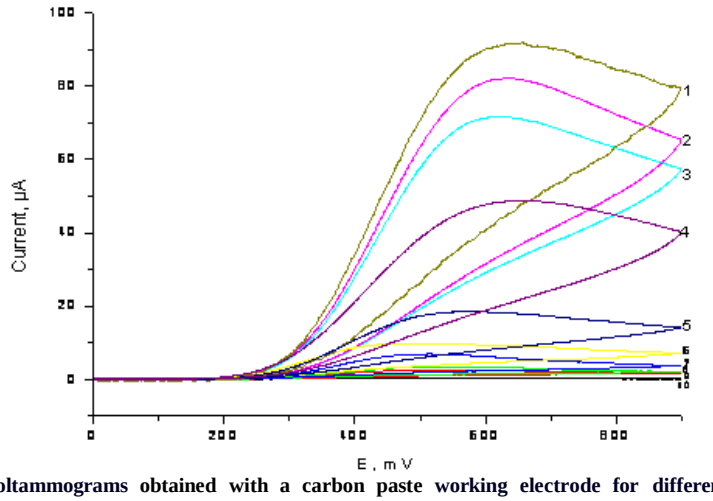

Figure 6: cyclic voltammograms obtained with a carbon paste working electrode for different ascorbic acid concentrations, expressed as mM: 20 (1), 15 (2), 10 (3), 5 (4), 2.5 (5), 1.2 (6), 0.6 (7), 0.3 (8), 0.14 (9) and 0.07 (10) at 100m mVs^{-1} potential scan rate

3.5 Determining the amount of ascorbic acid content in fruit juice samples

The amount of ascorbic acid in some of freshly prepared and bottled fruit juices was determined using cyclic voltammetric technique. Iodometric titration was used to determine ascorbic acid concentration in one freshly prepared and one bottled fruit juice which was used to compare with cyclic voltammetric technique. The average ascorbic content of freshly prepared orange juice and bottled pineapple juice using iodometric titration were obtained to be 43.60 ±1.56 mg/100mL and 12.02 ± 0.87 mg/100mL respectively. On the other hand, in cyclic voltammetric technique the amount of ascorbic acid were determined to be 44.26±1.13 mg/100mL and 11.90±0.95 mg/100mL for freshly prepared orange juice and bottled pineapple juice respectively. Generally the determined amount of ascorbic acid the fruit juices using cyclic voltammetric method and iodometric titration method were in a good agreement which indicated that ascorbic acid content of various fruit juices can be quantified

using cyclic voltammetric technique.

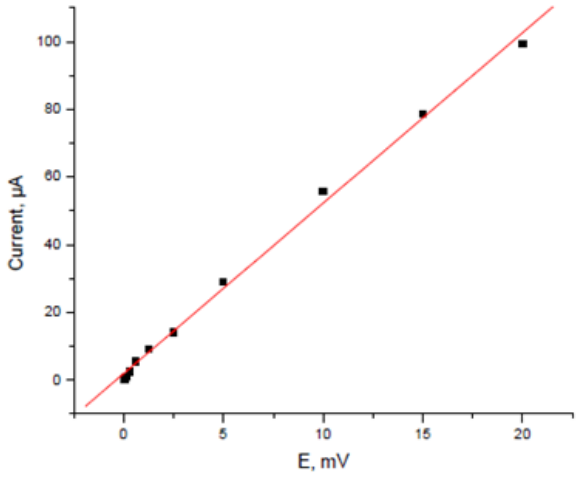

Figure 7: calibration graph for standard ascorbic acid obtained from Figure 6

Table 1: Amount of Ascorbic acid in some freshly prepared and bottled fruit juices using CV

Analyzed fruit sample	Content of AA (mg/100 mL)	
	Freshly prepared fruit juice	**Bottled fruit juice**
Sweet Orange juice	44.26 ± 1.13	30.56±0.89
Mango juice	15.88± 0.41	10.79±0.69
Pineapple juice	14.63±0.52	11.90 ± 0.95
Tomato juice	17.84±0.28	13.32±0.63

As indicate in Table 1 above, freshly prepared sweet orange juice has higher amount of AA than the other analyzed fruit juices. It is reported that the ascorbic acid content of freshly prepared orange juice is 39.25 mg/100mL [11]. Mahdavi *et al*. [21] also indicated that the ascorbic acid contents of freshly prepared orange juice was 42.4 mg/100 ml and the vitamin C contents of commercial pure orange juices immediately after production was mentioned to be in the range of 36.15-40.85 mg/100 mL. In other investigation, the vitamin C content of commercial orange juices was reported in the range of 27.5-73.11 mg/100 mL [21] in which the present study can be included in this range. It was also reported that the ascorbic acid contents of freshly prepared mango juice, commercial mango juice, freshly prepared pineapple and commercial pineapple fruit juices were obtained to be 14.65±0.151, 2.57±0.02, 15.46±0.17 and 13.6±0.21 mg/100 mL respectively [21]. However, there are significant differences in the values of ascorbic acid obtained in this study with those reported by some other studies for some fruit juice samples. It was reported that the ascorbic acid content of freshly prepared orange juice and pineapple juice are 64.00 mg/100 mL and 24.93 mg/100 mL respectively [13] which show some deviations from the present investigation. The observed differences in the contents of vitamin C studied in the same method may be as a result of differences in maturity stage and regional varieties of fruits. The amount of vitamin C could even vary between different samples of the same species. Different techniques of measuring and squeezing process may also affect the vitamin C content of fruit juices [22]. Factors including climate, temperature and amount of nitrogen fertilizers used in growing the plant and climatic conditions such as light can affect the concentration of AA in fruits. For instance, increasing the amount of nitrogen fertilizer from 80 to 120 $kgha^{-1}$ decreased the vitamin C content by 7% in cauliflower [12]. The amount of vitamin C content in fruit juices can also be affected by the type of storage. Fruit juices must be stored at cool temperature. When the fruit juices are stored at cool temperature, the vitamin C content does not loss, however, storing fruit juices at higher temperature result in loss of vitamin C content. This is because vitamin C is more sensitive to temperature and it can easily oxidize [16]. Previously published studies have showed that compounds commonly found in juice and foodstuffs (citric acid, tartaric acid, phenylalanine, glutamic acid, aminoacetic acid, and glucose) do not interfere in the ascorbic acid determination in fresh as well as commercial (bottled) fruit juices by cyclic voltammetry [14].

3.6 Determining Limit of detection (LOD) and Limit of Quantification (LOQ)

In this voltammetric experiment, LOD and LOQ were calculated by measuring ten blank samples, taking their

current, measuring their standard deviation then applying the formula: $LOD = \frac{3sd}{m}$ and $LOQ = \frac{10sd}{m}$, where sd= standard deviation of the blank samples and m= slope of the calibration curve. Thus LOD and LOQ for the developed method were determined to be 0.0221 mM and 0.0735 mM respectively.

3.7 Determination of the degree of recovery of Ascorbic Acid added to the analyzed sample

In order to calculate degree of recovery of the developed voltammetric method, the standard addition method was used to some of the analyzed fruit juices. For each addition, the degree of recovery was calculated and the obtained according to the following formula.

$$\textbf{Recovery } \% = \frac{\textbf{Qdet} - \textbf{Qp}}{\textbf{Qadd}} \textbf{x100}$$

Where *Qdet* represents mg determined ascorbic acid in100mL juice, *Qp* represents mg ascorbic acid previously present in 100 mL juice and Q_{add} represents amount of added (mg) ascorbic acid in 100 mL juice.

Table 2: Results obtained for degree of recovery for some fruit juices

Fruit juice sample	Amount of AA (mg/100 mL)	Amount of AA Added (mg)	Degree of recovery(%)
Orange (freshly prepared)	44.26	35.226	105.29
Mango (bottled)	10.79	35.226	93.35
Tomato juice (freshly prepared)	17.84	35.226	102.4

As can be seen from Table 2, the mass recovery obtained was in the range 93.35% - 105.29% which indicates the developed method can be applied for quantitative determination of ascorbic in fruit juices as well as to study some common cyclic voltammetric parameters.

CONCLUSION

Cyclic voltammetric method was applied for the determination and comparison of ascorbic acid content in some freshly prepared and bottled natural fruit juices. It was found that the vitamin C content of freshly prepared fruit juices which were obtained by fruit pressing is higher than those of bottled fruit juices. In addition to cyclic voltammetric method, the content of ascorbic acid in some fresh and bottled fruit juices was determined using iodometric titration method and approximately close results were obtained. Thus, cyclic voltammetry can be easily used to estimate the ascorbic acid content of various natural fruit juices. Since the method is clean and simple to set up and use, it is advantageous, furthermore it does not require any reagents apart from a simple buffer solution, and is cheaper and more time-efficient. The mass recovery for the developed voltammetric method was calculated and the results were obtained in the range 93.35% - 105.29%. The detection limit and limit of quantification were found to be 0.0221 mM & 0.0735 mM respectively. The reported results agree with most of the results obtained by the literature review regarding to the determination of ascorbic acid in natural fruit juices.

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