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Electrochemical Characteristics and Quantification of Ascorbic Acid by Voltammetric Techniques Using Glassy Carbon Working Electrode in Eight Fruit Juice Samples

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Abstract

Ascorbic acid (AA) is a very potent and widely used reducing agent found primarily in plant foods and fruits. Several classical analytical techniques have become available to quantify ascorbic acid (vitamin C). There is a growing interest in replacing the outdated methods of analysis with easy, fast, sensitive, and efficient methods for quantifying AA in nutritional analysis. The purpose of this study was thus to investigate the voltammetric characteristics and quantification of AA in eight different fruit juice samples using CV and DPV techniques with a glassy carbon working electrode (GCE). The effect of solution pH and scan rates was examined to gain insights into its redox properties and determine the optimal analysis conditions. The content of AA in the studied fruit samples (mg/100 g) ranged from 10.5 to 69.8 in the CV method and 11.0 to 73.5 in the DPV method, with a limit of detection (LOD) of 1.760×10^{-4} and 1.065×10^{-4}, and the limit of quantification (LoQ) 5.868×10^{-4} and 3.550×10^{-4}, respectively. A spike recovery of 104.3% in the CV and 103.8% in the DPV confirmed the suitability of the methods in directly quantifying AA in clear juice samples, with good linearity and sensitivity at bare GCE. Overall, both methods, CV and DPV, offer a quick, cost-effective, and reliable approach for determining the AA content of fruit juice samples compared to other common traditional techniques. These methods can be valuable in quality control and nutritional assessment of vitamin C within the food industry.

Keywords: Ascorbic Acid, Cyclic Voltammetry, Differential Pulse Voltammetry, Fruit Juice, Glassy Carbon Electrode

1. Introduction

Ascorbic acid, also known as vitamin C, is an essential nutrient present in sufficient quantities in vegetables and fruits. Ascorbic acid acts as a potent antioxidant, playing a significant role in human health (Gęgotek and Skrzydlewska, 2022). The presence and concentration of this compound serve as indicators of the nutritional value and

freshness of fruit juices (Ağagündüz D, 2020). Thus, efficient quantification of AA in fruit juices is of utmost interest to guarantee their nutritional quality.

For the quantification of AA, traditional analytical techniques such as titration and spectrophotometry have been commonly employed (Raman et al., 2022). However, these traditional methods have certain limitations. Titration involves a volumetric approach that utilizes oxidizing solutions such as dichlorophenol indophenol (DCPIP), potassium bromate, or iodate. However, these analytical techniques have the drawback of lacking specificity, thereby limiting their applicability to samples without any other reducing agents present, apart from AA (Mohapatra et al., 2024). On the other hand, spectrophotometric methods often encounter interference from other compounds found in juices, and both methods are time-consuming, requiring tedious sample preparation processes (Đurić and Deletic, 2020). Moreover, their sensitivity is not always satisfactory. Consequently, there is an increasing demand for developing alternative methods that are easy, fast, sensitive, and efficient for quantifying AA in nutritional analysis. Currently, electrochemical approaches are preferred over others due to their rapidity, sensitivity, and accuracy (Demir et al., 2021). Among these methods, CV (Wang et al., 2021) and DPV (Bilenler Koc et al., 2023) have shown reliability in quantifying micronutrients and antioxidants in a food matrix.

Determination of the content of AA is essential in many fields, including nutritional composition assessments, drug analysis, and quality assurance in the food and beverage manufacturing industries for both raw ingredients and processed commercial products. There is a scarcity of studies in which CV and DPV methods were used for direct quantification of AA from clear fruit juices. Therefore, this study aims to quantify the amount of AA directly from fruit juice samples using the CV and DPV methods, while avoiding the drawbacks commonly associated with traditional methods. Additionally, this research examines the effects of pH, scan rate, and AA concentration on the electrochemical characteristics using a glassy carbon working electrode. The results of this research could provide significant insights into the food and nutrition sectors in accurately quantifying the nutritional composition in fruit juice products.

2 Materials and methods

2.1 Sample collection

Eight samples of fruits were obtained from the Adet Agricultural Research Center at Woramit Horticultural Site, Ethiopia. The fruit samples analyzed were lemon (*Citrus limon*), strawberry (*Arbutus unedo*), lime (*Citrus aurantifolia*), guava (*Psidium guajava*), banana (*Musa sapientury*), mango (*Mangifera indica*), orange (*Citrus sinensis),* and tomato (*Lycopersicon esculentum*).

2.2 Preparation of supporting electrolyte

Supporting electrolytes containing phosphate buffers (NaH_2PO_4–Na_2HPO_4) and acetate buffers (CH_3COOH–CH_3COONa) within the pH range of 0.5-12 were prepared. To adjust the pH of the solution, drops of HNO_3 and NaOH were added. Additionally, different pH media were utilized to prepare the

required concentrations of vitamin C in both phosphate and ABSs.

2.3 Preparation of a standard solution

For cyclic voltammetric studies, a stock solution of 5 mM AA was prepared in 250 mL of 0.1 M phosphate buffer at pH 2, containing 0.1 mM $Na_2EDTA{\cdot}2H_2O$. The use of $Na_2EDTA{\cdot}2H_2O$ in voltammetric analysis of AA serves to bind metal cations that can interfere, thereby producing more accurate and consistent results. Serial dilutions of the 5 mM AA solution were prepared using 0.1 M phosphate buffer (pH 2.0) to obtain concentrations (mM) of 4, 3, 2, 1, 0.8, 0.7, 0.5, 0.4, 0.3, 0.2, and 0.1, after which the calibration curve was plotted. A similar procedure was repeated for DPV analysis, using a 0.2 M acetate buffer (pH 3.5). Each 16 mL portion of the standard AA solutions, prepared as described above, was transferred into the electrochemical cell and purged with nitrogen for 10 minutes before measurement.

2. 4 Preparation of fruit juice samples

A fruit mixer was utilized to extract juice from each sample fruit for the measurements. An adequate amount of juice was then transferred to a flask. The contents of the flask were subjected to centrifugation at 400 rpm for 5 min, followed by multiple filtrations using a 110 mm filter paper, until a clear juice was obtained. For the CV study, 2 mL of the aliquot was mixed at pH 2 with a solution containing phosphate buffer (0.1 M) and $Na_2EDTA.2H_2O$ (0.1 M), resulting in a final volume of 16 mL. Similarly, for DPV studies, 2 mL of the clear juice was added to a 0.2 M acetate buffer (pH = 3.5), again resulting in a final volume of 16 mL. The potentials of all solutions were then scanned following the procedure described in Section 2.3.

The edible portion of the non-juicy fruits, weighing 10 g, was chopped, combined with 50 mL of phosphate buffer (0.1 M), $Na_2EDTA{\cdot}2H_2O$ (0.1 mM), and adjusted to pH 2 before being used in CV experiments. Similarly, 10 g of the minced fruit sample was blended with 0.2 M acetate buffer (pH = 3.5) containing 0.1 mM $Na_2EDTA{\cdot}2H_2O$ for the DPV study. The sample was filtered using a 0.45 µm filter paper and then centrifuged at 400 rpm for 5 min. The resulting solution (16 mL) was pipetted into the cell and purged with nitrogen for 10 min. Finally, the potential was scanned in the range of 0 mV to 800 mV.

2.5 Electrochemical measurement

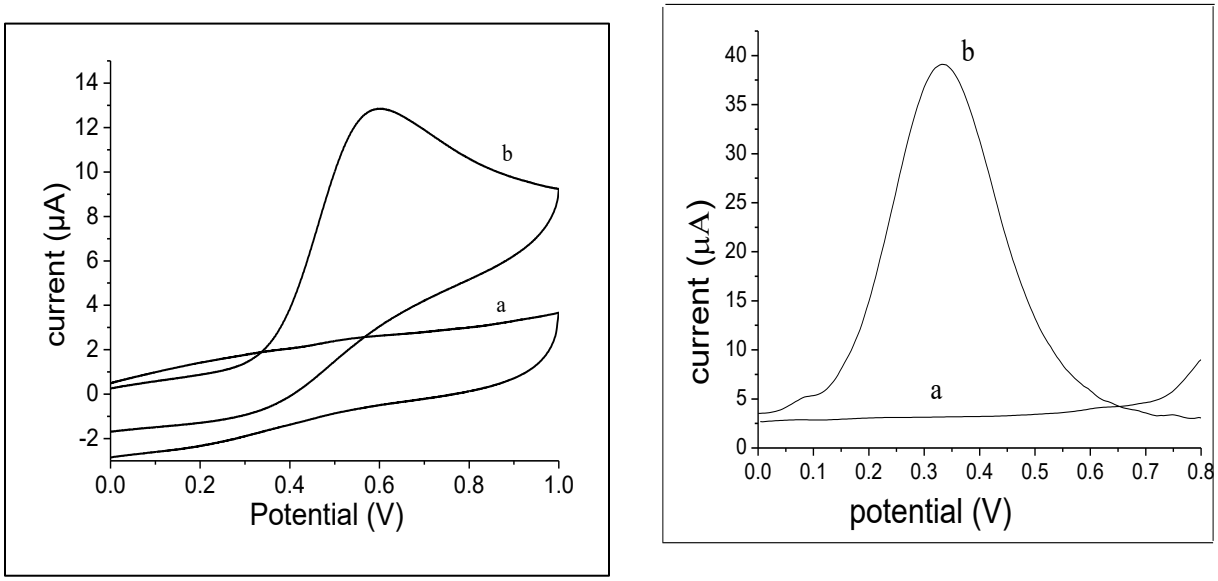
The experiments were performed using the BAS 100 B potentiostat analyzer interfaced to a Dell computer. Experiments were done using a three-electrode cell system with platinum wire, Ag/AgCl, and glassy carbon as the counter, reference, and working electrodes, respectively. Quantitative analysis of AA in samples of fruit juice was conducted by CV and DPV. The CV was measured at a scan rate of 20 mV/s and a potential range of 0 mV to 1000 mV. For DPV, the pulse width was 50 mV/s, the pulse amplitude was 50 mV, and the scan rate was 20 mV/s. Then, a current of AA was measured as a result of the anodic oxidation. They were all measured at room temperature.

3. Results and discussion

3.1 Cyclic and differential pulse voltammetric oxidation behavior of AA

To study the oxidation of AA on the GCE, CV, and DPV techniques were employed

using a solution of 5 mM AA. Figure 1 exhibits the CV and DPV voltammograms for the oxidation of AA. The CV of the 0.1 M PBS alone was performed by scanning the potential between 0 mV and 1000 mV, which resulted in no electrode reaction (Figure 1). However, an oxidation peak at 585 mV was observed for 5 mM AA in the 0.1 M PBS. Similarly, no recorded peaks were observed after scanning the potential for 0.2 M ABS alone in DPV. Nevertheless, an oxidation peak was observed at 320 mV for a 5 mM AA in the 0.2 M ABS. Both studies failed to show a cathodic peak current, which is an indicator of irreversible oxidation.



(A) (B)

Figure 1 (A) Cyclic voltammograms at a scan rate of 20 mV/s with pH = 2 a) background 0.1 M PBS b) 5 mM AA in 0.1 M PBS. (B) Differential pulse voltammograms at a scan rate of 20 mV/s with pH = 3.5 a) background 0.2 M ABS b) 5 mM AA in 0.2 M ABS.

A study conducted by Huang (2021) examined the electrochemical oxidation of AA using a GCE in different electrolyte buffer solutions (Britton-Robinson, acetate, and phosphate) with pH levels ranging from 0.64 to 10.15. Consequently, they obtained a well-resolved peak at 0.342 V in a 0.2 M acetate buffer with a pH of 3.5 for the DPV method (Mohammadi and Mousazadeh, 2022, Huang, 2021). Other research studies also indicated a 0.1 M phosphate buffer at pH 2.0 in CV was a suitable electrolyte for supporting the electrochemical analysis (Erdurak-Kiliç et al., 2006). Consequently, this particular study utilized the same buffer solutions: 0.1 M phosphate buffer at pH 2 for the CV analysis and 0.2 M acetate buffer at pH 3.5 for the DPV analysis throughout the study. Other factors influencing AA oxidation behavior were investigated in the subsequent Results and Discussion Sections.

Effect of solution pH

Ascorbic Acid is a bioactive compound that exhibits electroactive properties that make it prone to oxidation. These characteristics form the foundation for its electrochemical detection and analysis. However, the oxidation property and activity of AA are pH dependent (Hsieh et al., 2025). Figure 2 (A)

shows the effects of pH on the oxidation I_p of AA in 0.1 M PBS. As can be seen in Figure 2 (A), the peak current increases from pH = 0.5 to pH = 2 and then decreases with increasing pH. Therefore, pH = 2 was chosen as the optimum pH. Various factors could be attributed to the variation of peak current with pH, including protonation, deprotonation, and pH-induced reactions (Yenealem et al., 2024, Karastogianni et al., 2021, Feng et al., 2023). Several other studies have shown that the anodic peaks observed in CV do indeed change with pH (Handayani et al., 2020).

The effects of pH on the peak current for DPV analysis were significant (Figure 2 B). Ascorbic Acid is an acidic compound that can exist in different forms (AA, ascorbate ion, and dehydroAA) depending on the pH of the solution (Tanikami et al., 2021). Figure 2 B shows the increase in anodic peak current with increasing solution pH up to a pH of 3.5. After this point, the anodic peak current decreases with further increases in pH. The highest peak current was observed in acidic media at a pH of 3.5. Hence, a 0.2 M acetate buffer at pH 3.5 was selected as the optimal pH for the analytical determination of AA in the DPV method. In acidic conditions (lower pH values), the peak current of AA tends to increase due to enhanced electroactivity and increased availability of the AA form. However, in alkaline conditions (higher pH values), the oxidation or reduction potential of AA shifts, leading to a decrease in peak current (Kairy et al., 2022). Additionally, the optimal pH may also vary depending on the specific electrode material and experimental conditions.

Effect of scan rate

The effect of potential scan rates varied from 10 to 200 mV/s on the Ip of AA was assessed (Figure 3). The potential was scanned at various rates under identical experimental conditions, revealing a direct relationship within Ip and υ½. To calculate the effective area, the Randles-Sevick equation ($I_p = 2.69 \times 10^5\ n^{3/2}\ A\ D^{½}\ υ^{½}\ C$) were used; where I_p is the peak current, n is the number of electron (s) transferred in the redox process, A is the area of the electrode surface, D is the diffusion coefficient of the analyte, υ is the potential scan rate, and C is the concentration of the electroactive species. Concidering the obtained plot of peak current vs. $υ^{½}$, (Ipa (μA) = $-37.0 + 7.96\ v^{1/2}$) and the value of n = 2, D = 2.17×10^{-5} cm^2/s (Ang et al., 2020), and C= 1.0 mM for the AA, the active surface area of bare GCE were calculated to be 0.002 cm^2), smaller than expected (for a clean, fully active GCE for a 3.00 mm GCE the geometric area was 0.0707 cm^2). The reduced active area may suggest non-ideal contributions to the current (capacitive background) or adsorption/kinetic limitations, or fouling, or incomplete polishing (Guzmán et al., 2018).

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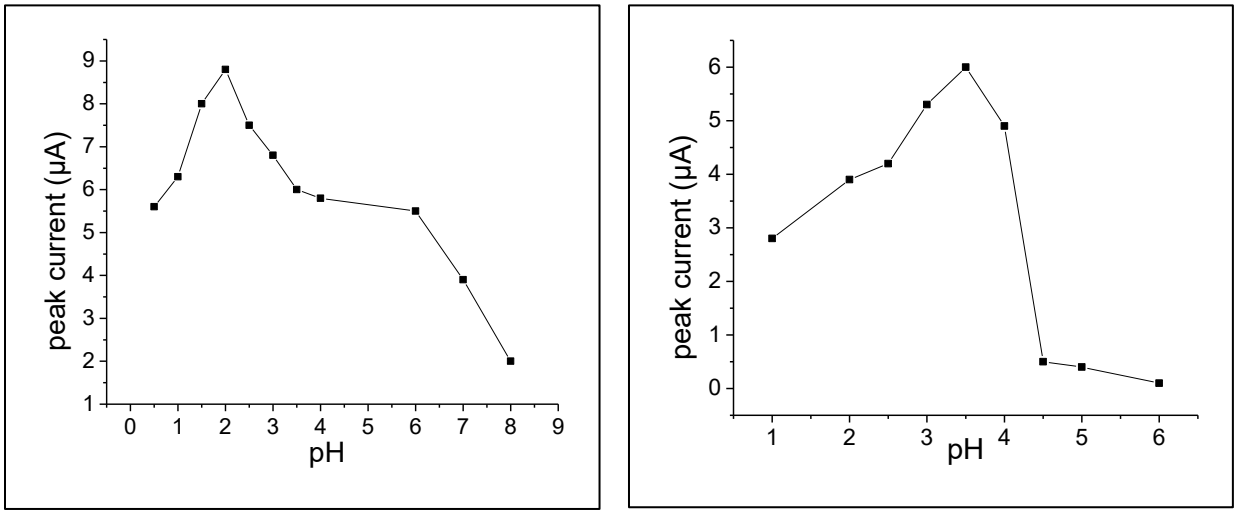


(A) (B)

Figure 2 (A) Plot of the cyclic voltammetry anodic peak current as a function of pH for a 5 mM AA in 0.1 M PBS. (B) Plot of the differential pulse anodic peak current as a function of pH for a 5 mM AA in 0.2 M ABS.

To study the reversibility and the oxidation reaction kinetics of AA at GCE, the effect of the scan rate (ν) on the oxidation peak current (Ipa) of AA was explored. Fig. 3 shows the cyclic voltammograms of AA at GCE in pH 2.0 PBS with 1.0 mM of AA at different scan rates. The Ipa increased with the increase in scan rate within the scan rate range of 40-200 mV/s. As is clear from Fig. 3, all the scan rates give irreversible cyclic voltammograms (i.e., no reverse peak and I_{pa} is proportional to $v^{1/2}$). Fig. 3A shows the increase in the peak current when the scan rate is increased. The anodic-current peak linearly correlates to the scan rates (υ) with a regression equation Ipa (µA) = -0.02 + 0.39v (R^2 = 0.995), indicating that the oxidative process of AA on the GCE is electrochemically surface-controlled. The plot of Ip vs. $v^{1/2}$, the square root of scan rate(υ), with regression equation I_{pa} (µA) = -37.0 + 7.96$v^{1/2}$ (R^2 = 0.979) also shows a good linearity, which is characteristic of a diffusion-controlled process. However, since the correlation with scan rate itself is stronger (R^2 = 0.995) and thus, adsorption control is dominant in the system. The plot of Ep (peak potential) vs. ln(scan rate) with regression equation Ep(V) = 260.8.0 + 40.96 lnv (R^2 = 0.733) indicates a moderate correlation. The less-perfect linearity here further supports the irreversibility of the process. In the case of an irreversible process, the value of αn was determined by the difference between the peak potential (Ep) and the half-wave potential ($E_{p1/2}$).

$$E_p - E_{p1/2} = 48/\alpha n.$$

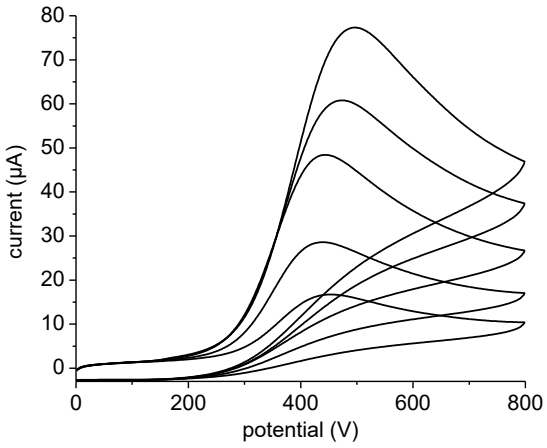
Where α is the charge transfer coefficient and n is the number of electrons transferred. Taking E_p and $E_{p1/2}$ for the cyclic voltammogram at a scan rate of 100 mV s^{-1} (Fig. 3) to be 447 and 400 mV, respectively,

the value of αn was calculated as 1.0. Considering α for an irreversible electrode process to be 0.5, the number of electrons (n) transferred in the electro-oxidation of AA at the surface of GCE was 2.0, which is in agreement with previously reported literature.

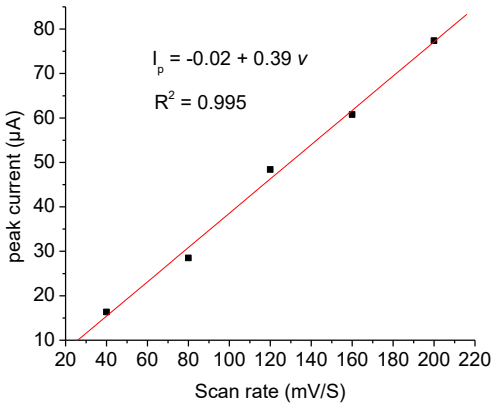
These results have been supported by studies that the oxidation of AA at different electrodes (such as modified carbon and platinum electrodes) is mainly irreversible with increasing potential and scan rates, with stronger dependence on scan rates rather than on the square root of scan rates (Alkhawaldeh, 2020, Álvarez, 2020). In this way, the results are of great indication that the oxidation of AA in these circumstances is irreversible and mainly controlled by adsorption (Kairy et al., 2022). Kinetic investigations of the AA oxidation in different systems, such as metal-catalyzed and electrochemical, all indicate a first-order dependence of AA concentration and irreversibility of the oxidation process (Lungaka et al., 2021). In parallel to our study, Motsaathebe and Fayemi (2022) demonstrated a linear relationship between the anodic peak currents and the square root of scan rates of AA oxidation in a nanocomposite-modified electrode (MWCNT-AONP). Similarly, Wu et al. (2020) Li and Lin (2006) noticed the same relationship in certain while studying AA with polyvinylpyrrolidone (PVP, structurally similar to PVA). The oxidation peak position, resolution of the peak, peak current, and sensitivity of the method are affected by the type of electrode, mediators, and modifiers used (Motsaathebe and Fayemi, 2022, Paul and Kim, 2024, Wu et al., 2020). Mohammadnezhad et al. (2024) reported that modification of GCE with cadmium pentacyanonitrosylferrate (CdPCNF) significantly enhances the electrocatalytic oxidation of AA, an improvement over bare GC electrodes. According to Wu et al. (2020), poly(BCP) film of GCEs greatly enhances the electrocatalytic oxidation of AA, lowering the required overpotential by about 240 mV and significantly increasing the current response, thus improving analytical performance.

3.2 Calibration

The effect of increasing the concentration of AA on the voltammogram is depicted in Figure 4A. The oxidation properties of AA were observed to depend directly on its concentration. This showed that the peaks observed for the samples were only for AA oxidation with controlled diffusion. The linearity range for the peak current versus AA concentration is clearly shown in Figure 4A to be between 0.1 and 5 mM. Similar to this study, several authors have demonstrated the linear dependence of AA peak current with concentration within certain concentration intervals (Mohammadi and Mousazadeh, 2022, Paul and Kim, 2024, Amayreh et al., 2021).



(A)



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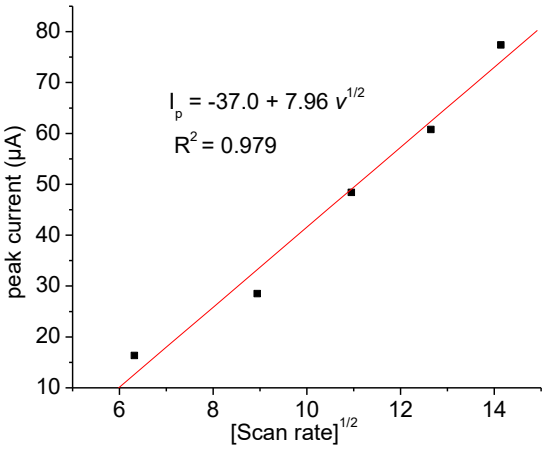
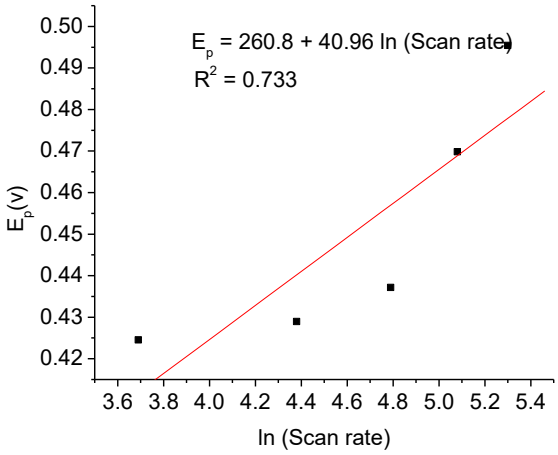


(C)



(D)

Figure 3 (A) CVs of bare GCE in pH 2.0 PBS containing 1.0 mM AA and $Na_2EDTA•2H_2O$ at various scan rates (20, 60, 100, 140, and 180 mV/S) and PBS only (a), (B) plot of I_p vs. v, (C) plot of I_p vs. (v)1/2 , and (D) plot of E_p vs. lnv.

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A higher concentration of AA generates more ascorbate radicals, which then react with the electrode surface, resulting in a higher measured oxidation current (Zhang et al., 2021, Njus et al., 2020). The Randles-Sevick equation was also confirmed, where the current is directly proportional to the concentration of electroactive species. However, factors such as electrode fouling or reaction kinetic saturation may render the linear relationship between oxidation current and AA concentration at extremely high concentrations (Mohammadnezhad et al., 2024, Mohammadi and Mousazadeh, 2022, Xiao et al., 2021)

According to the results obtained from the linear range, the fitting regression equation is as follows: I_{pa} (μA) = - 0.08428 + 13154.4 [AA]. The R^2 value was found to be 0.998. This linear relationship demonstrates the inherent sensitivity of CV for AA detection and the efficacy of the method for accurately quantifying it within a given concentration range. The limit of detection (LOD) and quantification (LOQ) were calculated according to equations LOD = 3 s/m and LOQ = 10 s/m, respectively.Where s is the standard deviation of the peak currents for 12 measurements (n = 12), and m is the slope of the calibration curve**.** Accordingly, the values of LOD and LOQ of AA in the CV method were 1.76×10^{-4} and 5.87×10^{-4}, respectively. A similar study by Aishwarya et al. (2024) reported a bit lower LOD (1.5×10^{-7} M) value. However, a much lower value of LOD (5×10^{-8} M) was obtained by using methylene blue intercalated MnO_2 nanosheets. The same author explained methylene blue facilitated a diffused L-ascorbate at the surface of the GCE and thereby catalyzed the oxidation of L-ascorbate. According to Deng et al. (2021), the sensitivity of the instrument is also influenced by the size of the working electrode. As the working electrode area decreases, only a small current passes through and is sensitive enough to be detected, and hence, the quantification limits improve, as exemplified by the ultra-micro working electrode.

The influence of AA concentration on the oxidation peak current in DPV was investigated. The peak current was proportional to its concentration as it is shown in Figure 4 B. DPV generates voltammograms with more noticeable and higher peaks. This indicates the absence of interfering background current and the suitability of the DPV method over CV. The dependent nature of i_p on concentration enables the construction of calibration curves. Consequently, a straight calibration line for DPV analysis was established within the range of 0.08 mM to 5.5 mM (Figure 4 B).

The LOD and the LOQ were computed, and the values were 1.07×10^{-4} and 3.55×10^{-4}, respectively. The fitting regression equation

was: I_P (μA) = 1.38 + 7599 [AA], with an R^2 value of 0.999, suggesting an excellent relationship with the experimental measures and theoretical values. A similar study in DPV analysis on a graphene–multiwalled carbon nanotube modified GCE reported an LOD of 6.71 μM for AA, while other advanced modifications achieve LODs in the nanomolar or even picomolar range (Hsieh et al., 2025, Zaidi et al., 2023). The use of a modifier, *p*-ABSA, on the GCE resulted in a much lower LOD value (2.0×10^{-8} M) for AA in DPV analysis (Ahmed et al., 2021). In this study, the value of LOD in DPV was lower, indicating DPV is more sensitive. Furthermore, DPV offers a wider linearity range for AA, allowing for the detection of both higher or lower quantities in samples.

3.3. Analysis of AA from fruit juice samples using cyclic voltammetry and differential pulse voltammetry

A cyclic and differential pulse voltammogram of AA in real samples, orange, and tomato juices, is shown in Figure 5. The shape and peak position of the voltammograms (CV and DPV) were found to be similar to that of the standard AA voltammograms (Figures 4A and 4B), revealing the oxidation of AA, which is found in fruit juices, at the GCE. Therefore, the anodic I_p recorded was used to quantify the amount of AA in the real fruit juice samples by comparing it to the calibration. The amount of AA in eight fruit juice samples was assessed similarly. Representative voltammograms of orange and tomato (shown in Figure 5 and chosen for demonstration) were used for this purpose.

Two types of fruits were studied: non-juicy fruits and juicy fruits. For non-juicy fruits, the amount of AA in 100 g of the edible fruit part was computed using equation 1.

$$\frac{\text{Ascorbic acid (mg)}}{\text{100 g of fruit}} = \text{mg of ascorbic acid per aliquot} \times \frac{\text{100}}{\text{Weight of fruit (g)}} \quad (1)$$

Milligrams (mg) of AA per aliquot were obtained by comparing the peak current to the calibration curve. Similarly, for juicy fruits, the AA concentration in the aliquot was determined using a similar method, and the degree of dilution was also taken into account.

Degree of recovery

In addition to the LOD, LOQ, and linear dynamic range, spike recovery was used to validate the methods for the quantification of AA in real samples. The degree of recovery determines whether the methods can detect and quantify the analyte of interest. A high degree of recovery (nearly 100%) indicates that the measurement method accurately measures the analyte from the sample.

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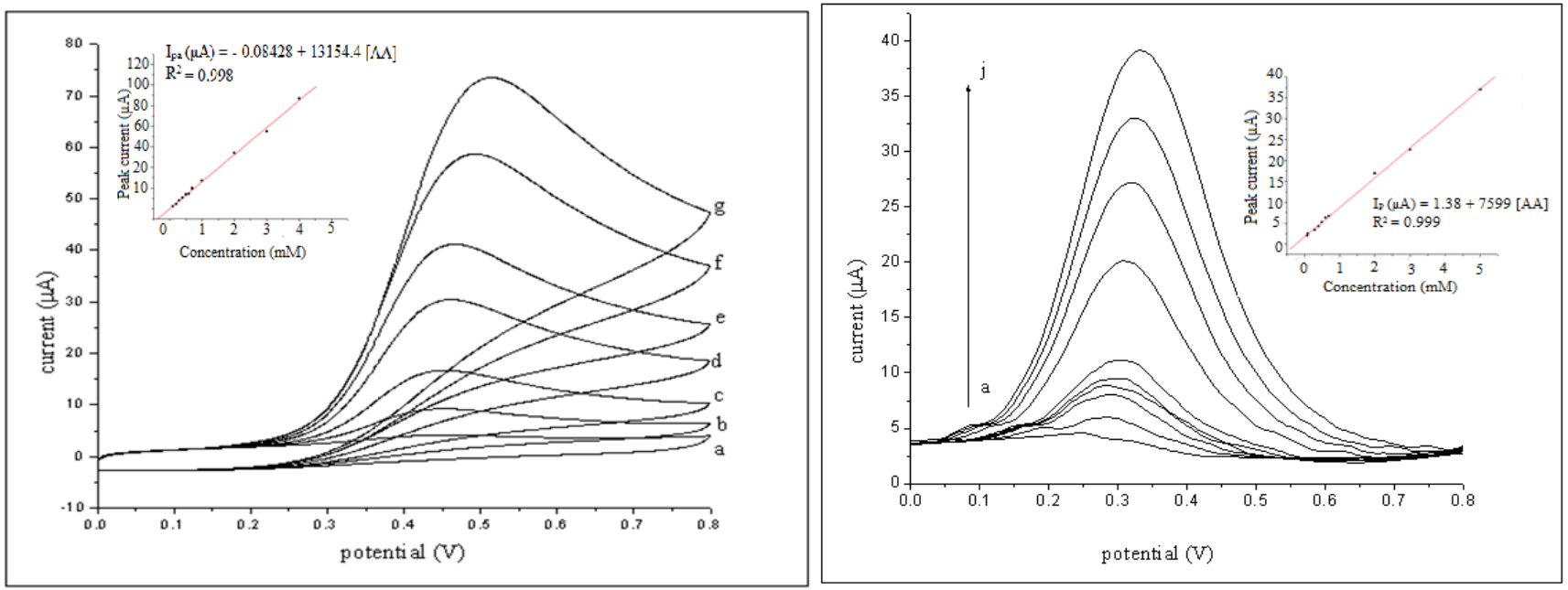
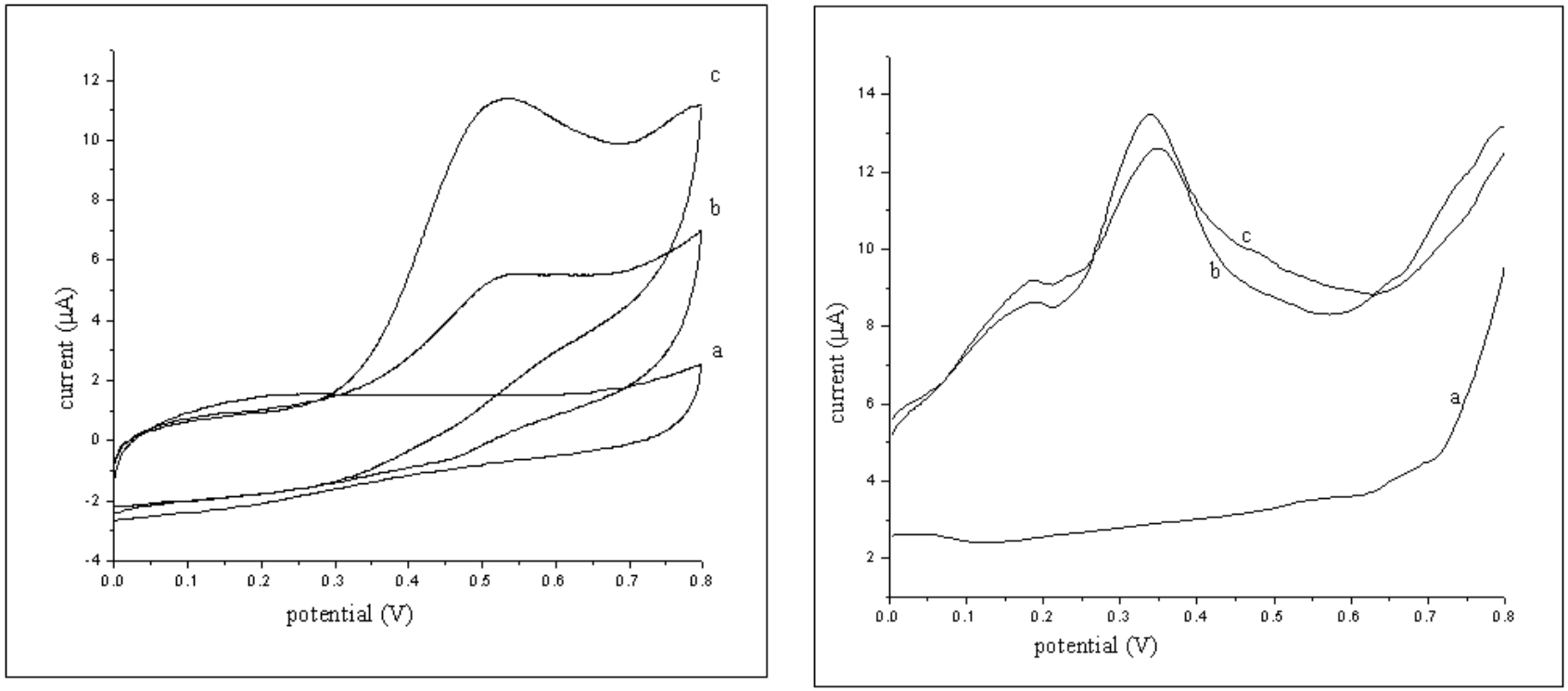


(A) (B)

Figure 4 (A) Cyclic voltammograms obtained with pH = 2, and 0.1 M PBS with a scan rate of 50 mV/s for different concentrations of AA (mM) (a) 0.1, (b) 0.6, (c) 1.0, (d) 2.0, (e) 3.0, (f) 4.0, and (g) 5.0.

(B) Differential pulse voltammograms at a scan rate of 20 mV/s with pH = 3.5 in 0.2 M ABS of different AA concentrations (mM). (a) 0.08, (b) 0.1, (c) 0.3, (d) 0.5, (e) 0.6, (f) 0.7, (g) 2, (h) 3, (i) 4, (j) 5.



(A) (B)

Figure 5 (A) Cyclic voltammograms at a scan rate of 50 mV/s with pH = 2, and 0.1 M PBS a) Background), b) Tomato juice, c) Orange juice. (B) Differential pulse voltammograms at a scan rate of 20 mV/s with pH = 3.5 in 0.2 M ABS a) Background, b) Tomato juice, c) Orange juice.

The analyte-specific degree of recovery, along with the sample matrix and analytical technique employed, enables comparison and evaluation of the method's performance. In this study, the addition of a standard solution of 0.6 mL of 4 mM AA to the orange juice (2 mL) increased the peak current substantially in the CV and DPV (Figure 6).

Table 1 Degree of recovery of AA (AA) in the CV and DPV method for an orange extract.

Analysis methods	**Initial AA (M)**	**Spiked AA (**M)	**Amount of AA (M) after spike**	**% Recovery**
CV	8.365×10^{-4}	2.4×10^{-5}	$(8.616 \times 10^{-4}) \pm 0.04$	$104.4\% \pm 0.03$
DPV	9.0609×10^{-4}	2.4×10^{-5}	$(9.3100 \times 10^{-4}) \pm 0.03$	$103.8\% \pm 0.02$

Data of AA (AA) after spike are presented as mean ± standard error of replicate measurments (n = 3).

The degree of recovery was obtained using the equation below.

$$\text{Recovery}\ (\%) = \frac{(Q_{DET} - Q_P) \times 100}{Q_{ADD}} \quad (2)$$

Where Q_{DET} represents the amount of AA determined after the spike in 100 mL of juice, Q_P denotes the amount of AA earlier remaining or before the spike in 100 mL of juice, and Q_{ADD} signifies the added (spiked) amount of AA in 100 mL of juice. As observed in Table 1, the recovery of AA was very satisfactory, suggesting the validation and reliability of the methods. A similar degree of recovery (92.0% - 107.0%) was obtained by Al-Refai et al. (2021) and Motsaathebe and Fayemi (2022) for AA in an orange fruit juice sample. The calculated degree of recovery in DPV (103.8%) was better than the values obtained in the CV (104.4%), indicating the higher efficiency of the method. In other working electrodes apart from glassy carbon, Zainudin and Azis (2021) achieved a comparable level of recovery (101.9%) for AAs in orange juice. Zhu et al. (2020) found a similar result using a NiO/polyaniline-modified electrode, recovery rates for AA in orange juice between 97.95% and 104.93% using both CV and DPV.

It is important to note that the degree of recovery can also be influenced by interferences caused by various electroactive compounds present in the sample media, potentially impacting the accuracy of the analysis (Zhang et al., 2024, Wang et al., 2022). However, the study conducted by Younis et al. (2024) for DPV analysis confirmed that common interferences (citric acid, glucose, benzoate anion, and tartaric acid) of AA in fruit juices had no significant influence (determination error <5%) on the analytical signal of the CV and DPV. The obtained degree of recovery and the calibration results (values of R^2, LOQ , and LOD), which were discussed in Sections 3.1 and 3.2, encourage the appplicability of the methods in the determination of AA in real food samples.

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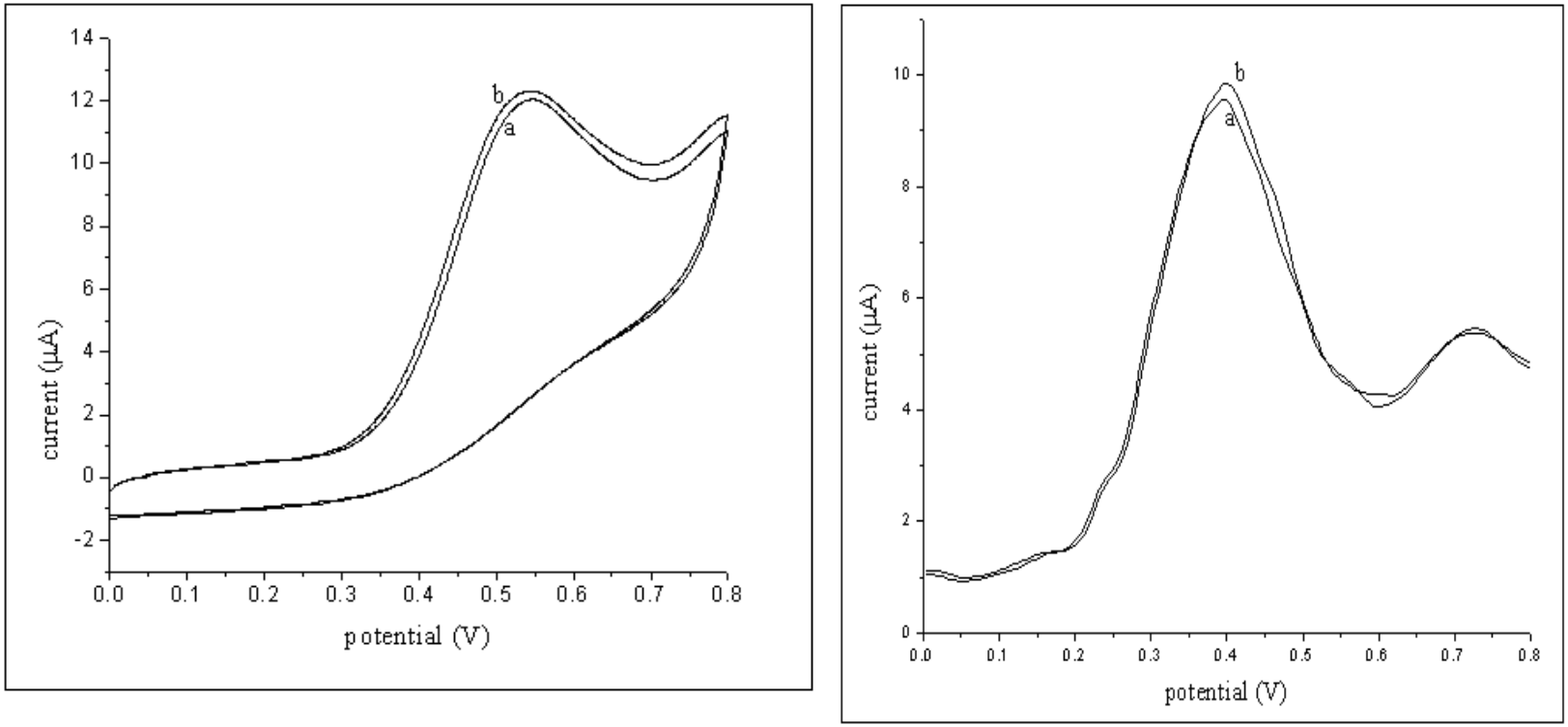

(A) (B)

Figure 6 (A) Cyclic voltammograms a) AA from juice sample (orange), b) AA from an orange juice sample after the addition of a standard solution of 0.6 mL 4 mM AA. (B) Differential pulse voltammograms a) AA from an orange juice sample, b) AA from an orange juice sample after the addition of standard solution of 0.6 mL 4 mM AA.

Generally speaking, the DPV exhibited lower detection limits, improved resolution, and a better degree of recovery than CV, making it a more suitable method for directly quantifying AA in clear and filtrated juice samples using a simple GCE. The calculated amounts of AA in the CV and DPV methods for eight fruit samples are presented in Table 2.

Table 2. The AA amount of fruit juice samples by CV and DPV methods using bare GCE.

No	**Fruits**		**Amount of AA in mg/100 g for non-juicy fruits, and in mg/100 mL for juicy fruits***	
	Common name	**Scientific name**	**CV**	**DPV**
1	Strawberry	*Arbutus unedo*	20.1 ± 0.03	22.6 ± 0.02
2	Lime*	*Citrus aurantifolia*	23.1 ± 0.02	25.5 ± 0.01
3	Lemon*	*Citrus limon*	45.4 ± 0.01	49.9 ± 0.01
4	Orange*	*Citrus sinensis*	55.4 ± 0.02	61.9 ± 0.03
5	Guava	*Psidium guajava*	69.8 ± 0.07	73.5 ± 0.05
6	Banana	*Musa sapientury*	10.5 ± 0.02	11.0 ± 0.02
7	Mango	*Mangifera indica*	15.1 ± 0.05	16.3 ± 0.04
8	Tomato	*Lycopersicon esculentum*	19.0 ± 0.02	19.9 ± 0.01

Data are presented as mean ± standard error of replicate measurments (n = 3). * Represents the juicy fruits.

The results obtained from the CV and DPV methods show strong agreement (Table 2). Moreover, these results line up well with the data described in the previous works. The dissimilarities in our findings and those of other researchers for some of the fruit analyzed studied could be rationalized by environmental factors that influence AA amount in fruits. Among these factors are temperature, climate, and the amount of nitrogen fertilizer used to grow the plants (Okiei et al., 2009).

Comparatively speaking, the current results align more closely with the HPLC results than with the titration and UV-Vis spectrometric methods. While the official technique for measuring AA is a titration with DCIP, it cannot be used for a sample matrix comprising Sn (II), Cu (I), and Fe (II), which are commonly found in normal fruit juices (Umegaki et al., 2024). Additionally, the presence of colored chemicals in the juice hinders precise identification of the titration endpoint, making it challenging to determine the actual amount of AA present. Consequently, there is a risk of either overestimating or underestimating the levels of AA, rendering this method inadequate (Umegaki et al., 2024).

Table 3 Comparison of AA content of fruit samples obtained by other methods (titration, UV- VIS spectroscopic, and HPLC).

Fruits	AA content	Methods	References
Strawberry (*Arbutus unedo*)	52.0 mg/100 g	Titration	(Sahari et al., 2004)
	33.3 mg/100 g	UV-VIS	(Kapur et al., 2012)
	32.6 mg/100 g	HPLC	(Valente et al., 2014)
	20.1 mg/100 g	CV	Present study
	22.6 mg/100 g	DPV	Present study
Lime (*Citrus aurantifolia*) *	64.5 mg/100 mL	Titration	(Al-Musharfi and Al-Wahaibi, 2015)
	21.0 mg/100 mL	HPLC	(Valente et al., 2011)
	23.1 mg/100 mL	CV	Present study
	25.5 mg/100 mL	DPV	Present study
Lemon (*Citrus limon*) *	49.5 mg/100 mL	Titration	(Okiei et al., 2009)
	56.4 mg/100 g	UV- VIS	(Desai and Desa, 2019)
	42.2 mg/100 mL	HPLC	(Scherer et al., 2012)
	45.4 mg/100 mL	CV	Present study
	49.9 mg/100 mL	DPV	Present study
Orange (*Citrus sinensis*) *	37.9 g/100 mL	Titration	(Silva et al., 2018)
	35.6 mg/100 g	UV-VIS	(Da Silva et al., 2017)
	24.9 mg/100 mL	HPLC	(Sánchez-Moreno et al., 2003)
	55.4 mg/100 mL	CV	Present study
	61.9 mg/100 mL	DPV	Present study

Guava (*Psidium guajava*)	181.8 mg/100 g	UV-VIS	(Desai and Desa, 2019)
	65.8 mg/100 g	HPLC	(Valente et al., 2011)
	69.8 mg/100 g	CV	Present study
	73.5 mg/100 g	DPV	Present study
Banana (*Musa sapientury*)	12.7 mg/100 g	Titration	(Okiei et al., 2009)
	13.1 mg/100 g	UV-VIS	(Kapur et al., 2012)
	10.0 mg/100 g	HPLC	(Proteggente et al., 2002)
	10.5 mg/100 g	CV	Present study
	11.0 mg/100 g	DPV	Present study
Mango (*Mangifera indica*)	27.7 mg/100 g	Titration	(Tasie et al., 2020)
	36.4 mg/100 g	UV-VIS	(Desai and Desa, 2019)
	40.9 mg/100 g	HPLC	(Valente et al., 2011)
	15.1 mg/100 g	CV	Present study
	16.3 mg/100 g	DPV	Present study
Tomato (*Lycopersicon esculentum*)	21.0 mg/100 g	Titration	(Okiei et al., 2009)
	15.9 mg/100 g	UV-VIS	(Kapur et al., 2012)
	18.0 mg/100 g	HPLC	(Proteggente et al., 2002)
	19.0 mg/100 g	CV	Present study
	19.9 mg/100 g	DPV	Present study

The UV-Vis spectroscopic method can also be influenced by interference from other organic compounds with similar absorption characteristics, potentially causing an overestimation of AA concentration (Olmo et al., 2020). The typical interferences when quantifying AA in fruit juice samples using spectroscopic methods are phenolic compounds, flavonoids, and carotenoids, which can absorb at similar wavelengths as AA, leading to inaccurate readings (Munteanu and Apetrei, 2021).

Apart from methodological variations, discrepancies between the present study and others could be credited to factors such as the time of measurement. Ascorbic acid is susceptible to oxidation at room temperature, affecting its spectral properties and the accuracy of the measurement (De Faria et al., 2020, Mohammadnezhad et al., 2024). Prolonged exposure to oxygen during sample preparation or analysis can lead to deterioration and reduce the observed amounts. Ascorbic Acid can degrade when exposed to light and high temperatures, making quantification challenging (Pawar et al., 2024). These variables must be closely monitored to ensure accurate quantification of AA in fruit juice samples.

4. Conclusion

The oxidation behaviour of AA at the GCE is irreversible and pH-dependent. The results show that CV and DPV methods can be applied for quantitative study of AA in fruit juice samples. Both techniques, CV and DPV, were rapid to run with no sample preparation other than processes like centrifugation, filtration, dilution, and putting an aliquot into the buffering electrolyte. DPV was highly sensitive and detected low concentrations of AA in samples, making it suitable for accurate quantification. CV and DPV are the best alternatives for quantification of AA compared to spectroscopic and chromatography analysis techniques. Guava (*Psidium guajava*) has been proved to be the richest source of vitamin C among the fruits analyzed.

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