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Voltammetric Analysis of Ascorbic Acid and Its Application in Commercial Roselle Juices

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ABSTRACT

Ascorbic acid is a water-soluble vitamin and commonly known as Vitamin C. Human cannot synthesize the ascorbic acid. It is naturally rich in citrus fruits and most of the vegetables. Hence, these fruits and vegetables become main source of ascorbic acid to meet the requirement of a dietary intake. The differential pulse anodic stripping voltammetric (DPASV) technique has been proposed for ascorbic acid analysis in commercial Roselle juices based on the electrochemical oxidation of the ascorbic acid at glassy carbon electrode (GCE). Phosphate buffer solution (PBS) at pH 5.0 was used as a supporting electrolyte. The optimum parameters used were E_i = +0.00 V, E_f = 0.80 V, t_{acc} = 60 s, ν = 0.05 V/s, E_{acc}= 0.00 V and pulse amplitude = 0.15 V. The calibration graph obtained shows a linear region between peak height and ascorbic acid concentration. The equation of the calibration graph was $y = 83.168x + 284.03$ with regression coefficient (R^2) of 0.9995. The limit of detection (LOD) was found to be 0.25 mg L^{-1}. The precision in term of relative standard deviation (RSD) were 0.69 %, 0.18 % and 0.17 % for three consecutive days. The recovery of ascorbic acid content in the commercial Roselle juices were 86.53 ± 4.27 % for added 25 mg L^{-1}, 92.53 ± 4.71 % for added 50 mg L^{-1} and 96.73 ± 4.12 % for added 100 mg L^{-1} AA, respectively. It can be concluded that the proposed technique is precise, accurate, rugged, cheap, fast and has potential to be an alternative method for further analysis of ascorbic acid in the commercial Roselle juices.

Keywords: *ascorbic acid; commercial fruit juice; voltammetry*

1. INTRODUCTION

Hibiscus sabdariffa Linn. is known as Roselle or Sorelle, which belongs to the family of Malvaceae [1]. Roselle is a herbaceous plant that can sprout until 8 feet or 2.4 meter tall, with smooth or nearly smooth, cylindrical and red stems [2]. It has been widely used in food and pharmaceutical industries. In food industries, roselle is consumed as herbal drinks in hot and cold beverages, fermented drinks, wine, jam, jellied confectionaries, ice cream, chocolates, puddings and cakes [3]. It also acts as flavouring and colouring agent in food industries [4].

The ascorbic acid (AA) is a water-soluble vitamin. The AA is also known as vitamin C, L-ascorbic and 2,3-didehydro-L-threohexono-1,4-lactone as well as 3-keto-L-gulofuranolactone.

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It has a chemical formula of $C_6H_8O_6$ and molecular weight of 176 $gmol^{-1}$ while its chemical structural is shown in Figure 1.

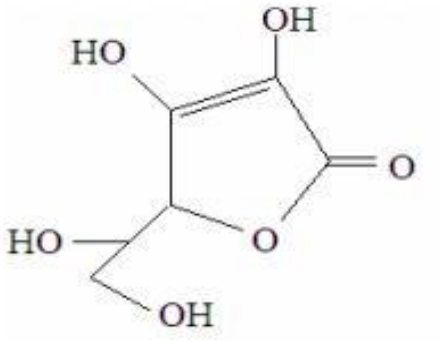

Figure 1: Chemical structure of ascorbic acid [5]

The AA was discovered in the twentieth century in 1907 by Holst and Frolich, for scurvy disease remedies. During that year, an experiment was carried out on guinea pigs by feeding them with fresh apples, potatoes, cabbage and lemon juices which is rich in the AA. The guinea pigs were also fed with a simple diet like oat, barley and wheat. The findings indicated that lack of AA in the diet intake had caused the scurvy disease [6]. The main natural sources of AA are various fruits and vegetables especially kiwi, mangoes, papayas, lettuce, tomatoes, peppers, strawberries, cantaloupe and broccolis [7].

The AA is important for collagen production, a protein which gives structure to bones, cartilages, muscles and blood vessels [5]. The AA also helps in iron absorption [8] as well as an antioxidant and free radical scavenger [9]. Free radicals? mostly come from environmental pollutants, radiation, chemical and toxins. They can cause several health problems to human such as atherosclerosis, arthritis, cancer and acquired immune deficiency syndrome (AIDS) [10]. These free radicals alter the gene arrangement and induces abnormal proteins. Decide if it is to be in plural or not-> free radicals- they- these

The AA is sensitive to light, heat and oxygen [11]. Due to its instability, the AA tends to degrade during manufacturing processes such as its packaging and in the storage of the foodstuff. Storage of commercial fruit juices in closed containers at ambient temperature for 4 months showed 29 % to 41 % degradation of the AA. On the other hand, for the commercial fruit juices placed in the open containers and in the refrigerator for 31 days, the loss of AA was about 60 % to 67 %. About 12.5 % of AA content has been degraded for the commercial fruit juices placed in open containers , stored outside the refrigerator for 10 days. In addition, about 9 % of the AA has been degraded for refrigerated commercial fruit juices, for the same period of time [12].

Various analytical techniques have been applied for the AA determination in fruit juices such as chromatographic method, particularly a high-performance liquid chromatography (HPLC) [13, 11], titrimetric method [14-16] and spectrometric method [17-19]. However, HPLC method uses expensive equipment, requires a long time of analysis includes sample pre–treatment, requires skilled labours, uses some toxic organic solvents, which depend on the applications as mobile phase and various harmful reagents [20]. In the titrimetric method, the

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difficulties are encountered with titrants and interferences often occur with coloured samples which lead to lack of specificity [9]. Meanwhile, in direct spectrophotometry, there is a matrix effect in ultraviolet (UV) region since many organic compounds in samples may also exhibit UV absorbance during measurement [21]. In addition, the spectrophotometry is beyond the specific limit and the intensity of absorption is not directly proportional to the concentration assuming that the molecules absorbing radiation do not interact with each other [22].

Square wave voltammetric method has been applied to AA in fruit samples by using a modified glassy carbon electrode. The modified electrode best performance was in 0.1 M phosphate buffer at pH 5. The oxidation potential for AA was at 395.24 mV. The inear response range was between 50 μM to 90 mM [23]. The AA content of some freshly prepared and bottled fruit juice was determined by using cyclic voltammetry with carbon paste electrode. Calibration graph equation was I_p (μA) = 5.034 (mM) +1.919 with R^2 of 0.998. The LOD was 0.0221 mM while the LOQ was 0.0735 mM. The recovery for freshly prepared and bottled fruit juices was between 93.35 % to 105.29 % [24].

In addition, glassy carbon electrode (GCE) modified with a perforated film produced by reduction of diazonium generated in situ from p-phenylenediamine (PD) has been proposed for AA determination in orange fruit samples. The bare GCE showed a linear response in the concentration ranged from 5 mM to 45 mM of AA. The limit of detection (LOD) was 1.656 mM. Meanwhile, the modified GCE showed a linear response in the concentration ranged from 5 μM to 45 μM of ascorbic acid with the LOD of 0.123 μM. The RSD obtained was 5.18 % (n=5). The concentration of ascorbic acid obtained in orange fruit was 23.33±0.01 mg per 100 mL [25].

The voltammetric method seems to have a potential as the promising alternative analytical method for determining ascorbic acid in the Roselle juices. Differential pulse anodic stripping voltammetric (DPASV) technique has a good discrimination against capacitive current which resulted in an improved resolution, a capability to give higher sensitivity, low detection limit and effectiveness to be applied in the analysis of various electrochemical active compounds offers simple and fast analysis [8]. The usage of glassy carbon electrode (CGE) in this study instead of the other working electrodes offers excellent electrical and mechanical properties, permeability to gases and low porosity [26]. The purpose of this study is to validate the proposed DPASV technique using bare glassy carbon electrode as a working electrode and phosphate buffer solution (pH 5.0) as a supporting electrolyte for quantitative analysis of AA in the several commercial Roselle juices.

2. METHODOLOGY

2.1 Instrumentation

An Autolab Potentiostat (Metrohm, Switzerland) consisting of a three-electrode- system was used for the overall voltametric measurements. A bare glassy carbon electrode (GCE) acts as the working electrode (WE), a platinum wire acts as the auxiliary electrode (AE) and a silver-silver chloride (Ag/AgCl, 3M KCl) acts as a reference electrode (RE). Before any voltammetric measurements proceeded, the GCE was polished with alumina powder on an alumina pad in order to obtain a mirror-like image and then being rinsed with deionized water. All potentials were referred against the RE. The Autolab Potentiostat was connected to an installed NOVA

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1.1 software computer for data collecting. The pH meter (Hanna Instruments, United Kingdom) was used for pH measurement of phosphate buffer solution (PBS) which acts as a supporting electrolyte [27].

2.2 Chemicals

The AA standard powder (MW = 176 g mol^{-1}) with 99 % purity was brought from Sigma Aldrich, United Kingdom. All solutions were prepared in deionized water. A stock solution of 500 mg L^{-1} AA is being freshly prepared daily by dissolving 0.05 g of AA powder with deionized water in 100 mL volumetric flask until the calibration mark is reached. Dilution of the AA stock solution was carried out in preparing the standard working solution series.

All solutions were protected from light and used within 24 hours to avoid decomposition and affecting the voltammetric measurements. The PBS was prepared by adding and dissolving 2.70 mL of ortho phosphoric acid, 27.218 g of potassium dihydrogen phosphate, 71.630 g disodium hydrogen phosphate with the deionized water in the 1000 mL of volumetric flask. A 0.1 M sodium hydroxide (NaOH) solution was used for pH adjustment of the PBS solution to the 5.0.

2.3 Collection and Preservation of Commercial Roselle Juices

Commercial Roselle juices with three different brands were purchased from markets located in Terengganu, Penang and Johor. All juices were kept in refrigerator at 4°C until ready to be used. The commercial juices containing fruit pulp were centrifuged (1000 rpm, 25°C, 10 minutes) before the analysis and the obtained clear samples were then analyzed [8] within 1 to 3 hours to avoid degradation.

2.4 Validation of Proposed DPASV Technique

An appropriate volume of AA standard solution and PBS solution was pipetted into the measuring vessel. The measuring vessel was covered with aluminium foil. A small size stirrer bar was put into the measuring vessel and the solution was then stirred well. After a period of time, the stirrer was switched off and voltammetric measurements were noted down. The scanning of the solution was initiated by applying voltammetric parameters of E_i = +0.00 V, E_f = 0.80 V, t_{acc} = 60 s, ν = 0.05 Vs^{-1}, E_{acc}= 0.00 V and pulse amplitude = 0.15 V. Peak height (I_p) and peak potential (E_p) were observed from the recorded voltammograms.

The validation method is a procedure employed to confirm whether the proposed DPASV technique is acceptable for the extended use [28]. This method validation ? ensures quality, reliability and consistency in order to verify if the proposed DPASV technique was suitable for analysis of AA content in the commercial roselle juices. A linearity range, limit of detection (LOD), limit of quantification (LOQ), precision, accuracy, ruggedness and robustness were validated. The recovery of the known amount added AA standard in the commercial Roselle juices was carried out in order to investigate the reproducibility of the proposed DPASV technique [29].

The linearity was investigated in the range of 5 mg L^{-1} to 200 mg L^{-1} AA standard solution in the electrochemical vessel. The LOD was estimated by additional lower concentration of the

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AA standard solution until a response that was significantly different from the response of PBS solution at pH 5.0 was obtained. The LOQ were calculated by the equations; LOD = 3 SD/m and the value of the LOQ was 10 SD/m, which means the LOQ was 3.3333 times of the obtained LOQ. A 100 mg L^{-1} AA standard solution was applied for intra-day and inter-day precision with three replicate measurements (n=3). The precision in terms of relative standard deviation (RSD) was then calculated.

The accuracy of the proposed DPASV technique was examined by spiking the three-known volume of AA standard solution which gave final concentration of 25 mg L^{-1}, 50 mg L^{-1} and 100 mg L^{-1} AA in the electrochemical vessel. The actual concentrations of AA standard solution found in the electrochemical cell by the proposed DPASV technique were then calculated using the regression equation obtained in linearity range study. Ruggedness of the proposed DPASV technique was investigated using the same instrument (Autolab Potentiostat, Metrohm) operated by two different analysts under the same optimum parameters with three replicating (n=3) measurements. Statistical *F*-test was carried out for the ruggedness. Last but not least, voltammetric measurements were carried out under small variation of ν (48 and 52 mV s^{-1}), t_{acc} (58 and 62 s) and pulse amplitude (148 and 152 mV) for the robustness investigation of the proposed DPASV technique.

2.5 Recovery Studies and Determination of AA in the Commercial Roselle Juices

The recovery of spiked AA in commercial Roselle juices was determined by spiking three different known amounts of AA standard into commercial Roselle juices, which gave the final concentration of 25 mg L^{-1}, 50 mg L^{-1} and 100 mg L^{-1} in the measuring vessel. Found concentrations of spiked AA standard solution into the Roselle juices samples were calculated using the regression equation from calibration curve and the following formula;

$$\text{Recovery (\%)} = \frac{(Q_{DET} - Q_P)}{Q_{ADD}} \times 100 \quad (1)$$

Q_{DET} represents concentration of AA determined in the juices, Q_P represents concentration of AA previously present in the juices and Q_{ADD} represents the concentration of AA added into the juices.

3. RESULTS AND DISCUSSION

3.1 Linearity, Limit of Detection (LOD) and Limit of Quantification (LOQ)

The capability of the proposed DPASV technique as an analytical method for determination of AA was determined by evaluating the obtained I_p as the function of AA concentrations. A linear region between the I_p and concentrations of AA was observed. The linear equation obtained was y = 83.168x + 284.03 with 0.9995 of correlation coefficient (R^2), as shown in Figure 2. The R^2 obtained closest to 1.0 is considered good as it is acceptable fit of the I_p against AA concentrations to the regression line. The LOD was found to be 0.25 mg L^{-1} and the LOQ was 0.83 mg L^{-1}.

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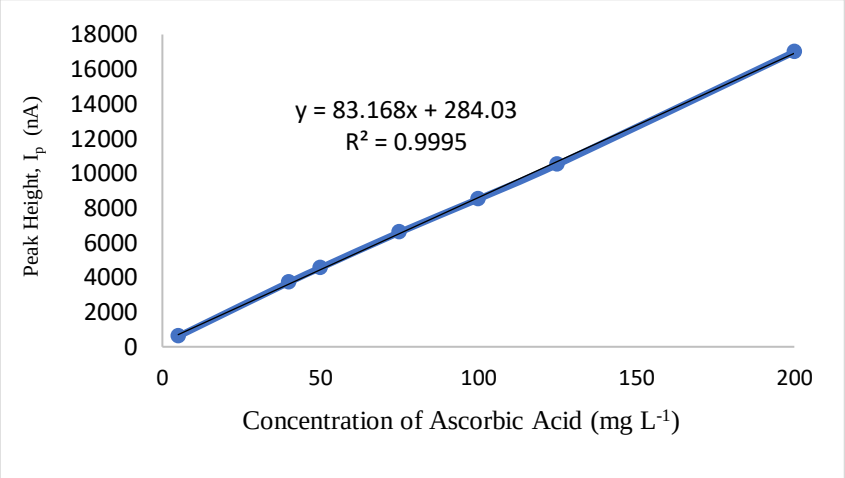

Figure 2: Linear curve of I_p against concentration of AA in PBS solution at pH 5.0

3.2 Precision

The precision of the proposed DPASV technique was determined with three replicating measurements (n=3) of 100 mg L^{-1} AA solution. The RSD obtained for the intra-day precision was 0.69 %. Meanwhile, the obtained RSDs for day 1, day 2, and day 3 were 0.69 %, 0.18 %, and 0.17 %, respectively. The RSD values found to be less 5 % indicating that the proposed DPASV technique was precise and of confidence [31]. On the other hand, the RSD values less than 2 % indicating that the measurements were more precise [32].

3.3 Accuracy

The satisfactory recoveries of 99.08 %, 97.14 % and 99.75 %, were respectively achieved for spiked 25 mg L^{-1}, 50 mg L^{-1} and 100 mg L^{-1} AA standard into a voltammetric vessel containing PBS solution at pH 5.0. These results showed that the proposed DPASV technique is considered accurate since acceptable recoveries were successfully obtained [33].

3.4 Ruggedness and Robustness

In the study, ruggedness was carried out by two different analysts but using the same instrument, Autolab (Metrohm) under the same experimental procedures. The RSD obtained for three replicating (n=3) measurement of 100 mg L^{-1} AA standard solution were 0.69 % and 0.42 % for the first and second analyst, respectively. From the two-tailed *F* test, there was no significant difference between the obtained variances for I_p when the measurements were performed by two different analysts (people or process?) with the same instrument (Autolab Potentiostat, Metrohm) at the 5 % significance level. Hence, the results indicated that the proposed DPASV technique was considered rugged. The robustness study was carried out by considering variation of ν (48 and 52 mV s^{-1}), t_{acc} (58 and 62 s) and pulse amplitude (148 and 152 mV) on the I_p of 100 mg L^{-1} AA. There was no significance difference of variances by the small changes of the optimum parameters as proved by two-tailed *F* test with 95 % confidence level [34].

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3.5 Determination of AA in Commercial Roselle Juices

The recovery studies which was carried out using commercial Roselle juices (Juice A) were 86.53 ± 4.27 %, 92.53 ± 4.71 % and 96.73 ± 4.12 % for respective 25 mg L^{-1}, 50 mg L^{-1} and 100 mg L^{-1} of spiked AA standard solution, as shown in Table 1. According to the *t*-test, there was no significant difference between recovery and added value at the 95 % confidence level with degree of freedom (n-1=2) since all the calculated *t* values in the experiment are lower than theoretical *t* value, which is 4.303 [35]. The proposed DPASV technique was tested in three commercial Roselle juices (Juice A, Juice B, Juice C). There were no AA detected in Juice A and B. Meanwhile, there was 135.4 $\pm$ 10.5 mg L^{-1} of AA in the Juice C, as shown in Table 2.

Table 1: Recovery for AA standard solution in the commercial Roselle juice (n=3)

Sample	Spiked Concentration of AA Standard Solution (mg L^{-1})	Found Concentration of AA Standard (mg L^{-1}) n=3	Average Recovery ± SD (RSD)
Juice A	25	21.5	86.53 ± 4.27 (4.93 %)
		20.6	
		22.8	
	50	45.1	92.53 ± 4.71 (5.09 %)
		47.4	
		46.3	
	100	97.8	96.73 ± 4.12 (4.25 %)
		95.9	
		96.5	

Table 2: The content of ascorbic acid in the commercial Roselle juices (n=3)

Sample	Concentration of AA (mg L^{-1})
Juice A	N.D
Juice B	N.D
Juice C	135.4 $\pm$ 10.5

Notes:N.D = not detected

4. CONCLUSION

The proposed DPASV technique has been successfully applied to determine the AA in the commercial Roselle juices. The proposed technique possess advantages on very simple sample pre-treatment, practically rapid, sensitive, accurate, precise, rugged, robusted and low in cost. Ambiguous structure and meanings >> are “practically rapid... to low in cost the advantages”?The degree of accuracy of the proposed DPASV technique is confirmed by the values obtained for the degree of recovery, which ranged between 97.14 % and 99.75 %. The

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limit of detection (LOD) and the limit of quantification (LOQ) obtained by the proposed DPASV technique were 0.25 mg L^{-1} and 0.83 mg L^{-1}, respectively. Hence, it could be an excellent alternative method for the routine determination of AA in the commercial Roselle juices in future.

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