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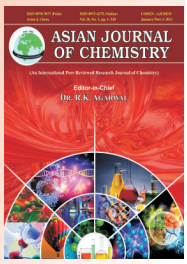

Electrochemical Analysis of Ascorbic Acid in Commercial Fruit Juices and Drinks

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Simple, reliable and inexpensive methods based on oxidation of ascorbic acid, differential pulse polarography and potentiometric titration, were validated for determination of ascorbic acid in commercial fruit juices and fruit drinks consumed in Thailand. For differential pulse polarography, the 0.1 M citrate buffer pH 4.6 was used as supporting electrolyte. The linearity was found in the range of 2.5-100 mg/L ascorbic acid with limit of detection and quantitation at 0.6 and 1.9 mg/L, respectively. The repeatability and reproducibility at 50 mg/L were 0.043 and 0.96 %, respectively. The potentiometric titration was performed using combined Pt-ring electrode with 2,6-dichloroindophenol as titrant. The method showed excellent linearity over the tested concentration ranges (100-350 % of the amount found in the juice samples), good precision (RSD < 2 %) and recovery (> 94 %). The limit of detection and limit of quantitation were 0.05 mg and 0.165 mg, respectively. The quantitation of vitamin C content in commercial fruit juices and drinks determined by both methods were in good agreement at 95 % confidence level with high percentage recoveries in the range of 89-105 %. Found levels of ascorbic acid in most samples by both methods were not in good agreement with those stated on the nutritional label.

Keywords: Ascorbic acid, Differential pulse polarography, Potentiometric titration, Nutritional label, Fruit juice.

INTRODUCTION

Ascorbic acid is the main biologically active form of vitamin C, the most important vitamin for human nutrition. More than 90 % of vitamin C in human diets is supplied by fruits and vegetables[1]. Nowadays, there is a rapidly growing market of commercial fruit juices and fruit drinks and their consumption is increasing quickly. Ascorbic acid as vitamin C is allowed by the European commission to use as an additive in fruit juices, jams, daily products, *etc*[2]. Ascorbic acid is well known for its important role in biochemical processes, such as collagen formation[2], resistance to infections and cellular respiration[3], reduction of plasma cholesterol level[1], enhancement of the immune system and reaction with singlet oxygen and other free radicals[1]. However, high levels of ascorbic acid in the human body could cause adverse effects. A high recommendation of 100-200 mg/day has been suggested, since stress in modern life is known to increase the requirement for vitamin C[1]. It is also known that the actual content of ascorbic acid is important for the infirmed and other specific groups (*e.g.* child, pregnant women and the elderly). Therefore, an accurate determination of its content in different foods is of great importance.

Ascorbic acid is reversibly oxidized to form dehydroascorbic acid and it can be easily converted into ascorbic acid in the human body which also exhibits biological activity[4]. It has been noted that when reporting vitamin C levels, many workers have not taken into consideration dehydroascorbic acid[1]. In many horticultural crops dehydroascorbic acid represents less than 10 % of total vitamin C[1]. In addition, the normal value of dehydroascorbic acid in commercial orange juice ranges from 0 to 0.2 % relative to ascorbic acid level, therefore, the error that is introduced in assessing vitamin C activity is negligible[5].

Various methods have been employed for the analysis of ascorbic acid in foodstuffs including titrimetry[6,7], spectrophotometric[8], spectrofluorometric[9], chromatographic[4,10-13] and electrochemical methods such as potentiometric[3,14], polarographic[15-16], voltammetric[7,16-19] and amperometric[19,20].

Electrochemical methods have some advantages over colorimetric spectrophotometric ones. In this method, the preparation of the sample is relatively simple and requires fewer steps. Because it can be rapidly compared, oxidation of the vitamin is prevented during the sample preparation and the measurement stages[15]. In addition, analysis can be carried out with a few milliliters of the sample. The deeply colored, viscous and turbid fruit juice samples do not necessarily interfere for determination of ascorbic acid[14].

The aim of this work was to evaluate the feasibility of using different electrochemical methods, polarographic and

potentiometric titration, for determining the amount of ascorbic acid in commercial fruit juices and drinks produced in Thailand. The reliability of the methods was evaluated in terms of linearity, sensitivity, precision and accuracy. A comparative study of ascorbic acid content was carried out between the nutritional labels and the analysis results from both methods.

EXPERIMENTAL

A voltammetric analyzer (VA 693, Metrohm, Switzerland) including a voltammetric cell with a DME as a working electrode (WE), a platinum rod as a auxiliary electrode (AE) and a Ag/AgCl electrode (3 M KCl) as a reference electrode (RE) was employed for polarographic analysis. Combined Pt-ring electrode (Metrohm, Switzerland) with an electrode cable was employed for potentiometric titration. All pH and potential measurements were made using pH meter (Model 713, Metrohm, Switzerland).

All chemicals used were of analytical reagent grade unless otherwise stated. 2,6-Dichloroindophenol and L-ascorbic acid (standard grade) were purchased from Merck (Germany). Citric acid, sodium citrate, sodium acetate, acetic acid, oxalic acid and sodium hydrogen carbonate were purchased from Carlo Erba (Italy). Deionized water (obtained from a system of Milli-Q, Millipore, Sweden) was used for the preparation of all solutions and at all other stages of analysis. The standard solution of ascorbic acid was prepared daily. The 2,6-dichloroindophenol solution was prepared and stored in an amber glass-stoppered bottle and kept in a refrigerator. This indophenol solution was standardized daily with freshly prepared ascorbic acid standard solution. Other reagents were prepared and stored in bottles. These solutions were stable for 2 weeks.

Sample preparation: Long-life commercial samples of fruit juices and fruit drinks without preservatives were analyzed. The details of each sample are summarized in Table-1. Most of the samples were packed in laminated paperboard packages (Tetrabrik), except sample 8A and 9A which were packed in plastic bottles. The samples were selected and bought directly from local superstores in Maha Sarakham Province, Thailand. All of the samples were produced in Thailand. Once samples were in the laboratory, they were kept under refrigerated conditions. Each sample consisted of 6 bottles or boxes chosen from the same batch which were homogenized immediately before direct analysis. No sample pretreatment was made except filtering of the sample through absorbent cotton or rapid paper just before the analysis.

Determination of ascorbic acid by differential pulse polarography: An aliquot of homogenized sample (200-400 µL) was transferred to the polarographic cell, which contained 0.05 mol/L citrate buffer (10 mL, pH 4.6) and the solution was de-aerated with nitrogen for 10 min. Then anodic scanning was applied from -100 to -225 mV, employing a differential pulse waveform with pulse amplitude of 40 mV, step potential of 6 mV and voltage step time 0.7 s. A polarogram was recorded. Peak potential and peak current corresponding to ascorbic acid was evaluated from the polarogram. Standard addition procedure was carried out by adding a standard solution of ascorbic acid (25 µL, 2.5 mg/mL) to the sample solution and then the measurement step was performed. Standard addition was repeated for 3 times. The concentration of ascorbic acid in the sample was evaluated from the standard addition graph, with subtraction of the concentration of ascorbic acid in the blank solution.

Determination of ascorbic acid by potentiometric titration: The ascorbic acid content in fruit juices and drinks was determined by direct titration with 2,6-dichloroindophenol. The variable potential during potentiometric titration was determined using combined Pt-ring electrode. 10 mL of deionized water, 15 mL of oxalic acid and 1 mL of sodium acetate were transferred into a titration vessel (50 mL beaker with magnetic stirrer). Each 5 mL of the sample or standard solution (containing 0.05 to 0.5 mg) was added and then the solution was directly titrated with 0.001 mol/L 2,6-dichloroindophenol previously standardized with 0.5 g/L ascorbic acid standard. Each mL of 0.001 mol/L 2,6-dichloroindophenol is equivalent to 0.176 mg ascorbic acid. A blank titration was performed prior titration of each sample. All analyses were done in triplicate. The ascorbic acid contents in the samples were obtained from the first derivative titration curves.

RESULTS AND DISCUSSION

Determination of ascorbic acid by differential pulse polarography: The polarographic method of ascorbic acid based on electrochemical oxidation of ascorbic acid at DME was proposed. Electrochemical conditions for oxidation of ascorbic acid were investigated. A differential pulse waveform was employed as it provided fast scanning and good sensitivity. A differential pulse waveform with pulse amplitude of 30, 40 and 50 mV at various voltage step time from 0.4-1.4 s was applied for analysis of standard ascorbic acid by scanning the potential in the range of -100 to -225 mV. The optimum condition that provided high sensitivity and fast analysis time (41 s. per run) was obtained from pulse amplitude of 40 mV at voltage step time of 0.7 s. The results show in Fig. 1.

The type of supporting electrolyte, acetate buffer (pH 4.6) and citrate buffer (pH 4.6), was studied on the sensitivity of the method. Comparable results were obtained. However, citrate buffer (pH 4.6) was chosen as the supporting electrolyte due to the similarity in the composition of fruits and vegetables, anticipating that they would resist the pH change upon the addition of fruit and vegetable extract.

Under the selected condition: 0.1 M citrate buffer as an electrolyte solution, potential range -100 to -225 mV, pulse amplitude of 40 mV at voltage step time of 0.7 s., measurement time 20 ms., pulse time 40 ms., sweep rate 8.571 mV/E and voltage step 6 mV, a polarogram was obtained as shown in Fig. 2.

A linear calibration graph in the concentration range of 2.5-100 mg/L could be obtained with the calibration equation $y = 18.014x - 15.784$, $R^2 = 0.9999$. The limits of detection (3s/s) and quantification (10σ/s) (where σ is standard deviation of reagent blank (n=11) and s is the slope of calibration curve) for ascorbic acid were obtained at 0.6 and 1.9 mg L^{-1}, respectively. The repeatability and reproducibility for eleven replicate determinations of 50 mg L^{-1} were 0.043 and 0.96 %, respectively.

Determination of ascorbic acid by potentiometric titration: The ascorbic acid content in fruit juice was determined

TABLE-1
ASCORBIC ACID LEVELS OBTAINED IN ANALYSIS OF DIFFERENT FRUIT JUICES AND DRINKS

Sample	Composition	Days before expiration	Ascorbic acid (mg/L)		
			In label	Differential pulse polarography	Potentiometric titration
1A	100 % red grape juice from grape juice concentrate	276	180	21.01 ± 0.88	23.51 ± 1.18
2A	100 % tangerine orange juice from tangerine orange concentrate with orange pulp	283	180	523.06 ± 7.72	509.04 ± 1.01
3A	100 % juice, including juices such as carrot, orange, pineapple, apple, lemon and pumpkin from juice concentrate	310	180	311.35 ± 2.79	323.83 ± 0.26
4A	100 % juice, including juices such as carrot, orange, pineapple, beetroot, strawberry, passion fruit, apple, raspberry, blackcurrant, cherry and tomato from juice concentrate	357	180	293.79 ± 3.32	290.50 ± 4.39
5A	100 % juice, including juices such as blood orange, apple, orange, tomato, carrot, beetroot and red bell pepper from juice concentrate	294	150	107.56 ± 1.73	119.23 ± 0.77
6A	100 % juice, including juices such as carrot, pineapple, orange, apple, tomato, lemon, celery, pumpkin, purple carrot and red grape from juice concentrate	317	240	414.30 ± 5.41	414.90 ± 1.57
7A	100 % juice, including juices such as orange, pineapple, white grape, apple, lemon, celery, spinach, asparagus, cucumber, kiwi, banana, broccoli, cabbage, Chinese cabbage from juice concentrate	315	135	132.97 ± 1.99	137.15 ± 0.53
8A	Nectar, 98 % including juices such as carrot, pineapple, orange, apple, tomato, lemon, celery and pumpkin from juice concentrate	231	200	7.92 ± 1.34	5.98 ± 1.02
9A	Nectar, 96 % including juices such as apple, white grape, orange, pineapple, celery and pumpkin from juice concentrate	296	220	37.92 ± 1.54	35.65 ± 2.32
10A	Nectar, 40 % juice including juices such as pineapple, orange, carrot, lemon, tomato, pumpkin, celery, apple from juice concentrate; and sugar	274	504	179.90 ± 3.06	180.45 ± 0.26
11A	Nectar, 40 % juice including juices such as apple, orange, pineapple, white grape, lemon, celery, spinach, asparagus, cucumber, kiwi, banana, broccoli, cabbage, Chinese cabbage from juice concentrate; fructose syrup and sugar	312	192	146.25 ± 1.08	151.64 ± 0.78
12A	Nectar, 40 % juice including juices such as carrot, orange, pineapple, apple, lemon from juice concentrate; and sugar	275	480	185.92 ± 2.35	178.16 ± 0.51
13A	Nectar, 40% juice including juices such as purple carrot, pineapple, orange, grape, lemon, carrot, apple from juice concentrate; and sugar	342	120	369.01 ± 0.48	391.95 ± 0.10
14B	100 % guava juice	242	120	392.20 ± 8.41	354.86 ± 6.53
15B	100 % shogun orange juice	212	150	258.25 ± 1.50	269.69 ± 0.26
16B	100 % juice, including juices such as kiwi and grape	192	135	334.02 ± 6.37	328.42 ± 1.59
17B	100 % juice, including juices such as mangosteen, orange, grape, beetroot, passion fruit, banana and lychee	231	510	62.17 ± 1.92	59.80 ± 0.13
18B	Nectar, including juices such as 32 mixed vegetable and mixed fruit, orange, apple, cheng orange, mango from juice concentrate; apple cider; fiber; vitamin C; vitamin A; vitamin B2 and vitamin B1	240	270	549.06 ± 5.38	543.24 ± 2.65
19B	100 % juice, including juices such as gojiberry, carrot, orange, grape, golden tomato, pineapple and pumpkin from juice concentrate	149	180	105.94 ± 4.36	124.84 ± 1.06
20B	Nectar, 40 % juice including juices such as purple carrot, pineapple, orange, grape, lemon, carrot, apple from juice concentrate; and sugar	301	480	119.54 ± 1.89	115.32 ± 3.20
21C	100 % tangerine orange juice from tangerine orange juice concentrate	281	150	547.45 ± 2.64	560.24 ± 1.94
22C	100 % orange juice from orange juice concentrate	260	300	211.29 ± 9.62	247.93 ± 1.94
23C	100 % juice, including juices such as carrot, orange, pineapple and apple from juice concentrate	359	300	147.34 ± 7.60	171.52 ± 2.01
24C	Nectar, including juices such as strawberry, grape, apple, yumberry, pomegranate, black currant, raspberry, cranberry from juice concentrate with added garcinia extract, collagen and vitamin E	151	150	25.16 ± 3.76	20.45 ± 0.13
25D	Nectar, 50 % juice including juices such as tomato, orange, passion fruit and sugar	285	-	ND	ND

A-D: the same letter indicates the same manufacturer. ND: Not detected. The experiments were performed in triplicate.

by direct titration with 2,6-dichloroindophenol. The method based on the oxidation of ascorbic acid to dehydroascorbic acid. The variation of potential during titration was determined using a combined Pt-ring electrode. The titration curve was constructed by plotting of first derivative of obtained potential versus volume of titrant as presented in Fig. 3.

The method was validated for linearity, precision, accuracy, ruggedness, limit of detection and limit of quantitation using 100 % orange juice as samples.

The linearity of the method was determined by adding standard ascorbic acid at 100, 150, 200, 250, 300 and 350 % of the amount found in the juice sample into the sample.

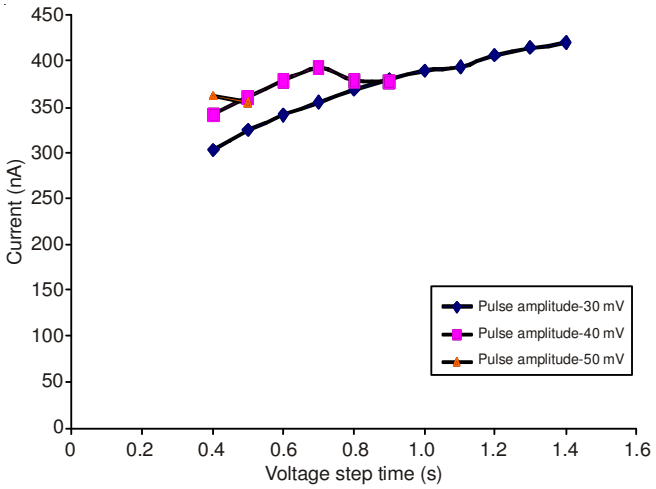

Fig. 1. Effect of pulse amplitude and voltage step time on sensitity

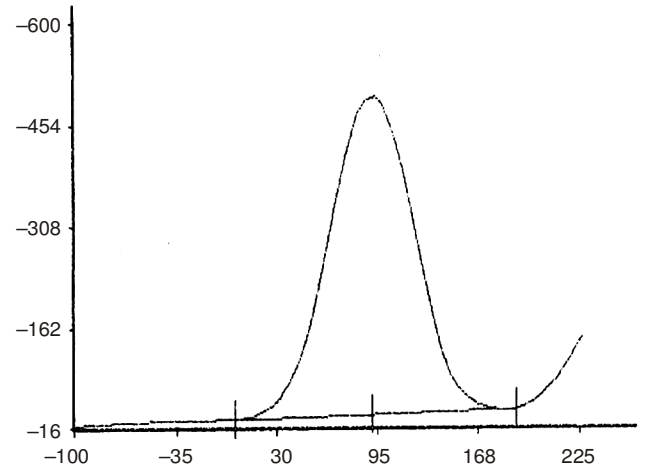

Fig. 2. Polarogram of 25 mg/L ascorbic acid

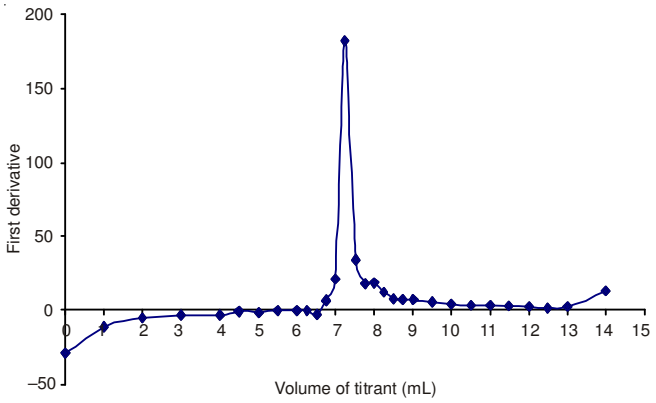

Fig. 3. First derivative titration curve of ascorbic acid in fruit juice

Triplicate titrations were made for each standard added solution. The linear regression line was plotted between the amount of standard ascorbic found and the amount of standard ascorbic added. A linear relationship of the amount of standard ascorbic acid added over concentration of 100-350 % of the amount found in the orange juice was obtained.

Intra-day and inter-day precision were studied by determining ascorbic acid content in orange juice from the same source by the same analyst on the same and different days. Five titrations were made for each sample. The method was precise and the % RSD for intra-day and inter-day assays was 0.31 and 1.87 %, respectively.

The accuracy calculated from percentages of bias and recovery was determined using the standard addition method. Standard ascorbic acid at 100, 150, 200, 250, 300 and 350 % of the amount found in the orange juice was added into the juice samples. Triplicate titrations were made for each standard added solution. The percentage biases of 4.4-6.3 % were obtained. Recoveries of standard ascorbic acid were within 94-96 %.

Ruggedness of the method was performed by analyzing the ascorbic acid content in orange juice from the same source by different analysts. Five replicates were made for each sample. According to t-test at 95 % confident limit, the results obtained from two analysts were in close agreement ($t_{critical}$ = 2.31, $t_{calculate}$ = -0.0153).

The limit of detection (LOD) was determined by decreasing the concentration of standard ascorbic acid 10-fold each time. The amount of standard ascorbic acid, which could be detected by observation of end-point, was considered to be limit of detection. The lowest amount of standard ascorbic acid that could be quantified with reasonable precision and accuracy was considered the limit of quantitation (LOQ). The LOQ was calculated by multiplying the LOD by a factor of 3.3. The LOD and LOQ of ascorbic acid content were 0.05 and 0.165 mg, respectively.

Application to real samples: Twenty five commercial fruit juice and fruit drink samples were analyzed to investigate the degree of correspondence between the label and the ascorbic acid levels found by differential pulse polarographic method and the potentiometric titration method. Table 1 summarizes the results of all samples analyzed. The days before the expiration date are also shown, since the concentration of ascorbic acid in the juice depends on the period of staying[5].

According to *t*-test at 95 % confident limit, the results obtained from both methods were in good agreement ($t_{critical}$ = 2.064, $t_{calculate}$ = -1.036). The results correlated with each other well (Potentiometric titration = 0.9867 Polarography + 6.1659, R^2 = 0.9925). Satisfactory recoveries in the range of 89-105 % for all samples were obtained. However, the contents found were not in agreement with the quantities specified in the label except for sample 7A. The level determined from samples 2A, 3A, 4A, 6A, 13A, 14B, 15B, 16B, 18B and 21C was much higher than the value indicated by the producer. This fact could be due to the label only displaying the amount of ascorbic acid added but not the natural content in vitamin C of the fruits[21].

The level determined from samples 1A, 5A, 8A, 9A, 10A, 11A, 12A, 17B, 19B, 20B, 22C, 23C and 24C was lower than the value indicated by the producer. This could be due to ascorbic acid degradation during the shelf-life of the product. The differences in the degradation process in these samples could be attributed to many variables, such as sample composition, processing conditions and different packaging[2].

The ascorbic acid content in different batches of orange juice product (batch 1:E1; batch 2: F1-F3; batch 3: G1-G6)

was investigated and compared with the nutritional labels. Sample F1, F2 and F3 are samples of the same batch but bought from three different local stores. Samples G1-G6 came from the same batch and were purchased from the same local store. The results are presented in Table-2. It was found that the contents in all three batches studied were not in agreement with the quantities specified in the label. Also, the ascorbic acid content in samples F1-F3 of batch 2 was not equal. This could be due to the different in storage condition of different store which affected to the degradation of ascorbic acid during storage. In addition, the concentration of ascorbic acid trends to increase with time before expiration.

TABLE-2
ASCORBIC ACID CONTENT IN ORANGE JUICE SAMPLES OF DIFFERENT BATCHES

Sample	Days before expiration	Ascorbic acid (mg/L)		% Label
		In label (mg/L)	Differential pulse polarography	
E1	198	300	154.96 ± 6.14	51.65 ± 0.27
F1	260	300	185.40 ± 2.82	61.80 ± 0.94
F2	260	300	219.07 ± 8.03	73.03 ± 2.68
F3	260	300	211.29 ± 9.62	70.50 ± 3.30
G1	295	300	234.02 ± 5.33	78.01 ± 1.78
G2	295	300	237.62 ± 1.76	79.21 ± 0.59
G3	295	300	239.14 ± 0.08	79.71 ± 0.23
G4	295	300	232.57 ± 0.19	77.52 ± 0.06
G5	295	300	244.86 ± 3.22	81.62 ± 1.07
G6	295	300	238.33 ± 2.77	79.44 ± 0.92

E-G: The same letter indicates the same batch. The experiments were performed in triplicate.

Conclusion

The proposed polarographic and potentiometric titration methods are simple, reliable and cost-effective. The advantages of these methods are easy sample preparation steps and applicability to highly colored solutions. Detection and quantification limits were shown to be satisfactory in the analysis of very different fruit beverages available in the market. The method could be useful for food control purpose.

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