Int. J. Electrochem. Sci., 5 **(2010)** 1464 - 1474

International Journal of
ELECTROCHEMICAL
SCIENCE
www.electrochemsci.org

Determination of the Vitamin C Content of Conventionally and Organically Grown Fruits by Cyclic Voltammetry

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Received: 16 August 2010 / *Accepted:* 19 August 2010 / *Published:* 1 October 2010

Organic fruits are perceived to have a higher nutritional content than conventionally grown fruits, but currently literature is rather ambiguous on the accuracy of this claim. Using vitamin C concentration as a nutritional marker, we found no clear or significant difference in the nutritional content between organically grown and conventionally grown fruit. The fruits analyzed were: oranges, mangoes, kiwi, lemons, gala apples, and red delicious apples. Of the six types of fruits analyzed by cyclic voltammetry only one, lemon, demonstrated a significantly higher vitamin C concentration for organically grown versus conventionally grown fruit. Our results suggest that other factors are more influential on vitamin C levels than whether fruits are organically or conventionally grown.

Keywords: Ascorbic acid, vitamin C, cyclic voltammetry, organic, fruit

1. INTRODUCTION

L-Ascorbic acid (Figure 1), more commonly known as vitamin C, is an essential nutrient. The amount of vitamin C required in a healthy diet varies with age and gender. According to Health Canada, children ages 1-3 require 15 mg/day, adult females 75 mg/day, and adult males need 90 mg/day [1]. The human body is unable to synthesize vitamin C [2]. A vitamin C deficiency can have a number of detrimental health effects with scurvy being the most serious and well known. Other animals such as primates, guinea pigs, Indian fruit bats, bulbuls, and some fish are also unable to synthesize vitamin C naturally and can also develop scurvy [2]. Since vitamin C is water-soluble, excess levels are not a major health concern because it is readily excreted from the body. Extremely high levels can however result in severe diarrhea [3]. Vitamin C functions as a vital electron donor and is an important antioxidant [2]. Antioxidants are key components in the prevention of oxidative damage to proteins and DNA [4]. Oxidative damage is associated with the development of both mild and severe health conditions including cancer, diabetes, cardiovascular disease, arthritis, and cataracts [5].

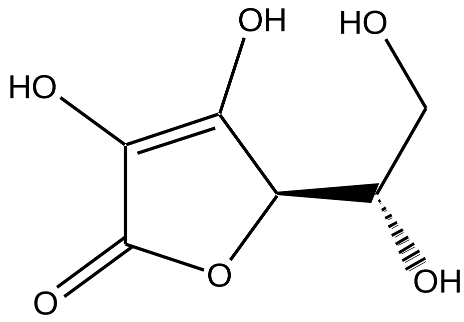


Figure 1. Structural formula for L-Ascorbic Acid

Organic foods have become increasingly popular as they are believed to be more flavourful, nutritious, and environmentally friendly [6]. This rise in consumer demand has led to a wide variety of new certified organic products. Organic foods were once found only in specialty stores, but are now readily available in most grocery stores.

Certification of organic foods varies depending on the regulations set forth by the governing body in which the products are sold. Our study, being carried out under Canadian regulations, requires organic foods to be produced without synthetic: pesticides, fertilizers, growth regulators, processing aids, fungicide packaging, and/or allopathic drugs [7]. Genetic engineering, ionizing radiation, sewage sludge for fertilizer, cloned animals, and the deliberate use of nanotechnology to attain new properties are also prohibited in the production of organic food products within Canada [7]. Any products imported to Canada must also meet these required government specifications in order to be labelled organic [8].

The results from a range of studies reveal uncertainty whether organic foods have higher vitamin C content than conventionally produced foods. We selected six conventionally grown fruits, along with their organic counterparts, and analyzed their vitamin C content, using cyclic voltammetry. Ascorbic acid can be oxidized to dehydroascorbic acid, thus enabling the use of cyclic voltammetry to measure vitamin C content [9-13]. Vitamin C levels were used as a determinant for which fruit group was nutritionally superior.

2. EXPERIMENTAL METHOD

2.1. Reagents and Apparatus

Sodium phosphate monobasic (Fisher Scientific Company), phosphoric acid (Fisher Scientific Company), potassium chloride (J. T. Baker Chemical Company), disodium EDTA dihydrate (J. T. Baker Chemical Company), sodium 2,6-dichloroindophenolate hydrate (DCIP) (Sigma-Aldrich

Chemie), L-ascorbic acid (Aldrich Chemical Company), and anhydrous ethyl alcohol (Commercial Alcohols) were all of analytical grade and used as received.

All fruits tested were purchased from a supermarket during June and July 2010. Comparative testing of conventionally versus organically grown fruits was carried out on: lemons, oranges (navel and Valencia), apples (red delicious and gala), green kiwi, and mangoes. Organic navel oranges and conventional Valencia oranges were commercially unavailable.

Cyclic voltammetry was carried out using Pine Instrument Company bipotentiostat model AFCBP1 and data were displayed using Aftermath™ software. Data were analyzed using t-tests, Mann-Whitney U-test (for non-parametric data), and standard deviations with SigmaPlot™ software.

2.2. Electrode Polishing

A standard three-electrode cell was used with a glassy carbon working electrode, platinum auxiliary electrode, and a silver/silver-chloride reference electrode. Both the working and auxiliary electrodes were polished prior to each run. Polishing involved using a slurry of 1.0 micron Buehler Micropolish II® alumina polishing powder with distilled/deionized water on a Buehler microcloth® polishing pad followed by a thorough rinse with distilled/deionized water. The rinsed electrodes were polished again on a new pad with only distilled/deionized water to remove any film left by the alumina polish and again rinsed thoroughly with distilled/deionized water. The electrodes were sonicated for five minutes in absolute ethanol, followed by rinsing with distilled/deionized water to remove any ethanol residue and loosened particulates. Electrodes were blown dry with nitrogen gas and placed immediately in the electrochemical cell.

2.3. Calibration Curve

A calibration curve of vitamin C was prepared by diluting a stock solution of 3.00 mM L-ascorbic acid in a pH 2.0 phosphate buffer. Phosphate buffer was prepared with 0.1 M KCl, 0.1 mM EDTA, and 0.1 M sodium phosphate monobasic. The pH of the buffer was adjusted by a dropwise addition of phosphoric acid. Calibration solutions were made using 1.00 mL, 2.00 mL, 5.00 mL, 20.00 mL, and 35.00 mL of stock solution diluted to 50.00 mL with buffer solution. Solutions were degassed by bubbling nitrogen for 5 minutes prior to each cyclic voltammetry experiment.

2.4. Vitamin C Extraction from Fruits

Only the traditionally consumed portions of the fruit were used in the extraction procedure. For example, kiwi were peeled while the apples remained unpeeled but cored. Vitamin C was extracted, from the fruit, into solution using pH 2.0 phosphate buffer, which was prepared daily with 0.1 M KCl, 0.1 mM EDTA, 0.1 M sodium phosphate monobasic and adjusted to the proper pH with phosphoric acid as outlined by Ogunlesi et al. [14]. Extraction was performed by combining 50 g of fruit with 50 mL of buffer solution and blending for 1 minute. An additional 50 mL of buffer was used to wash the unblended residue along the inside of the blender, producing 100 mL of solution. This solution was further blended until a uniformly smooth consistency was achieved. The blended mixture was then vacuum filtered to remove pulp, resulting in a transparent solution. The filtrate was degassed with nitrogen for 5 minutes and immediately tested.

2.5. DCIP Titration

DCIP titration was used as a comparative method with cyclic voltammetry in order to compare the vitamin C concentrations obtained. Titrations were performed using only kiwi because an accurate endpoint could easily be determined. Vitamin C was extracted from kiwi, as described above, with the following exceptions: 200 g of kiwi were weighed out and combined with a total of 400 mL of buffer. Three 15.00 mL samples of solution were titrated with 1.00 mM DCIP until the solution had a light pink tinge [15, 16]. This resulted in an endpoint with a light peach hue as a result of the initial light green colour of the kiwi filtrate.

3. RESULTS

The calibration curve of vitamin C in the phosphate buffer is provided in Figure 2. Serial dilutions from a 3.00 mM stock solution of vitamin C were measured to produce five data points at 0.0600 mM, 0.120 mM, 0.300 mM, 1.20 mM, and 2.10 mM.

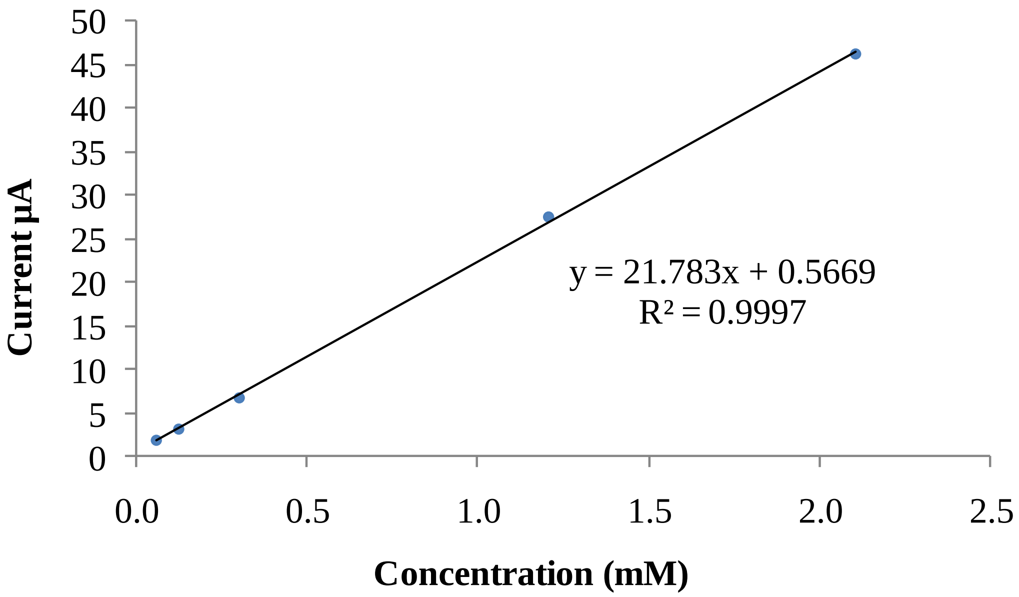


Figure 2. Calibration curve for vitamin C in a pH 2.0 phosphate buffer (0.1 M), with its linear regression and correlation coefficient values. (n = 3).

The calibration curve was generated by voltammetric measurements of the diluted solutions. The maximum anodic current was measured for each voltammogram; a sample voltammogram is shown in Figure 3. Cyclic voltammetry produced a linear calibration curve with a correlation coefficient of 0.9997.

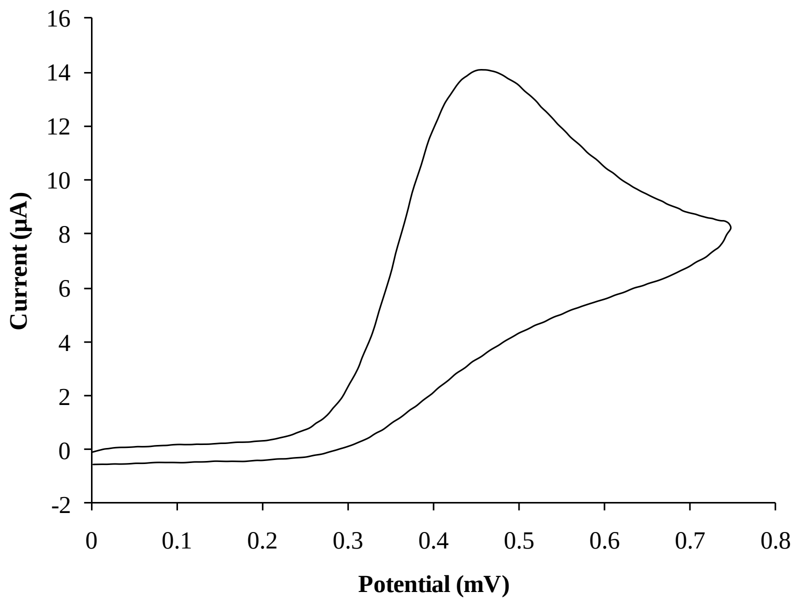


Figure 3. Sample cyclic voltammogram for a 0.300 mM vitamin C solution in a pH 2.0 phosphate buffer (0.1 M). Scan rate = 100 mV/s.

Sample voltammograms for conventional and organic oranges, gala and red delicious apples are provided in Figure 4. Figure 5 displays sample voltammograms for conventional and organic lemons, kiwi, and mangoes. The average calculated vitamin C concentration (Table 1) for both conventional and organic fruits were calculated from their corresponding voltammograms.

A DCIP titration was used to confirm the accuracy of our voltammetric measurements. The average concentration of conventional kiwi by DCIP titration was found to be 1.39 ± 0.06 mM. The same kiwi solution had a concentration of 1.48 ± 0.03 mM using cyclic voltammetry. Cyclic voltammetry detected a 6% higher concentration of vitamin C than DCIP titration.

To test for any significant difference in the vitamin C concentration between conventional and organic kiwi, mango, lemon, gala apple, and red delicious apple, t-tests were used. A t-test was also performed to check for a significant difference between conventional gala and red delicious apples as well as between organic gala and red delicious apples.

A Mann-Whitney U-test was performed on the oranges, as they failed the equal variance test. No significant difference was found in kiwi ($p = 0.273$), mango ($p = 0.500$), orange ($p = 0.690$), gala apple ($p = 0.059$), and red delicious apple ($p = 0.403$). Significance as defined by a p value of less than 5 % ($p < 0.05$) was found only in lemon ($p = 0.010$).

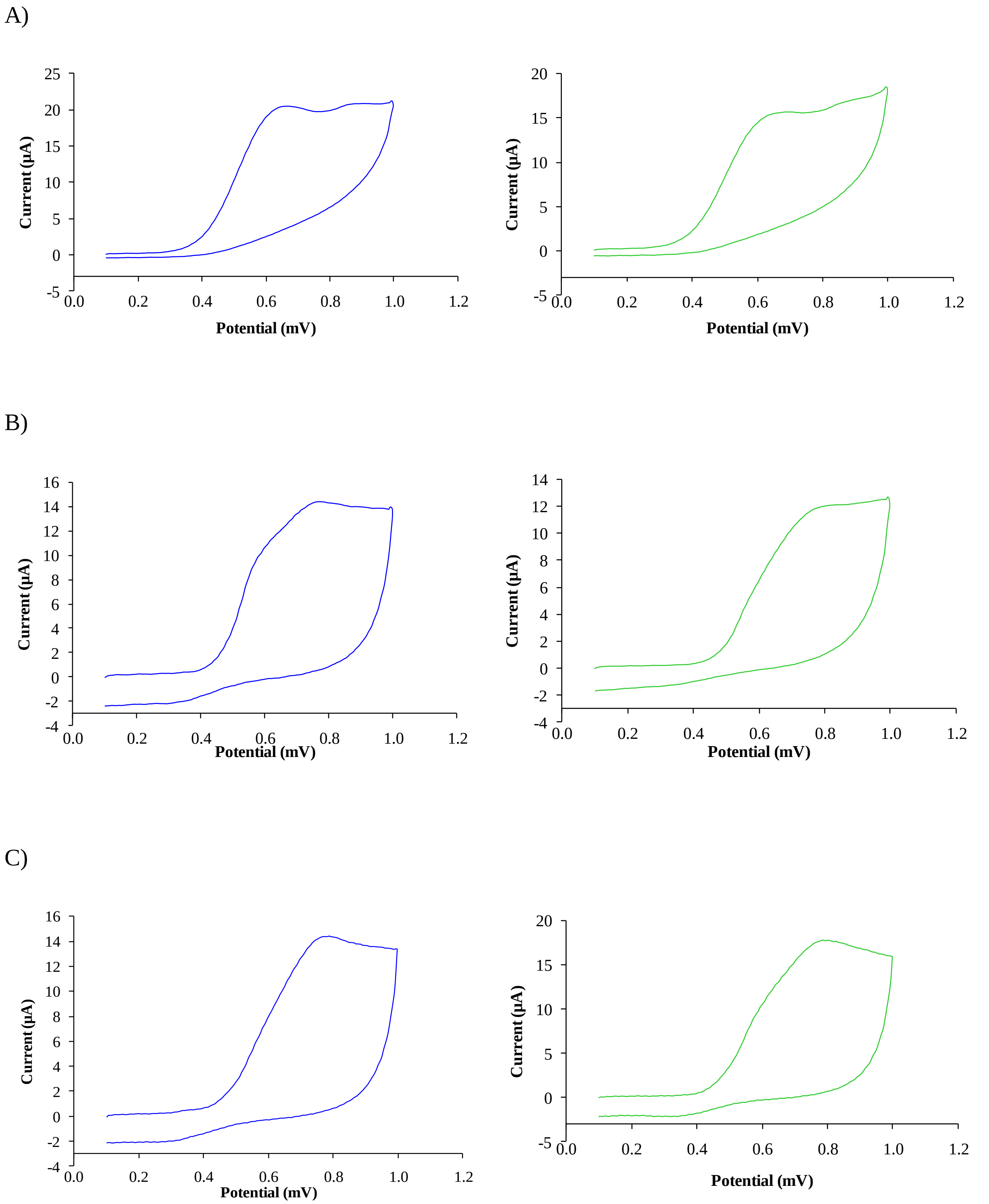


Figure 4. Sample cyclic voltammograms for orange (a), gala apple (b), red delicious apple (c), where blue (left) is conventional fruit and green (right) is organic fruit. Scan rate = 100 mV/s.

Table 1. Vitamin C concentration in mg/100 g of fruit and their respective standard deviations for conventionally and organically grown fruit samples. n = 5.

Fruit		***Conventional***	***Organic***
Common Name	Botanical Name		
Kiwi	*Actinidia chinensis*	46.8 ± 4.2	51.4 ± 7.8
Mango	*Mangifera indica*	11.0 ± 3.6	12.5 ± 2.9
Lemon	*Citrus limon*	26.2 ± 4.8	34.5 ± 4.9
Orange*	*C. sinensis*	27.7 ± 2.7	26.2 ± 1.4
Gala apple	*Malus domestica*	21.5 ± 1.8	19.0 ± 1.9
Red delicious apple	*M. domestica*	19.8 ± 2.2	21.4 ± 3.3
***Conventional = navel, organic = Valencia**			

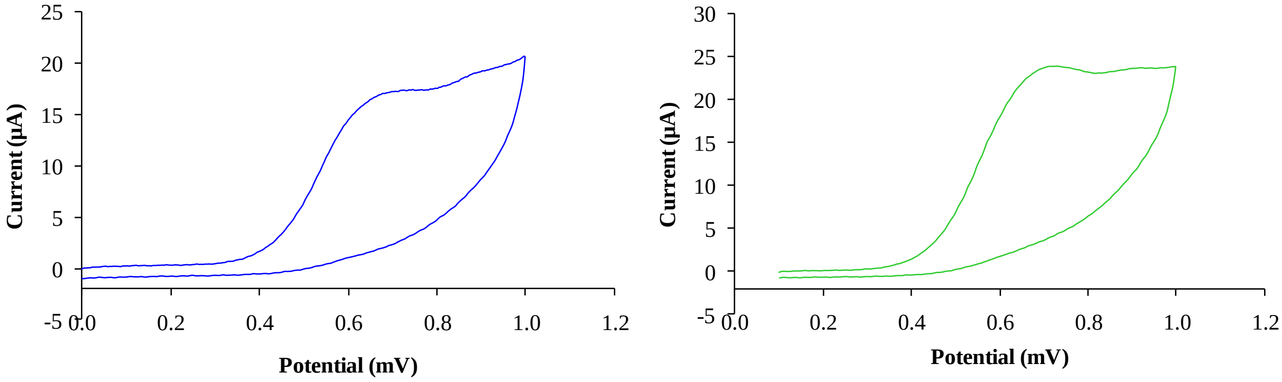
The t-tests used to compare the vitamin C content of the two cultivars of apples resulted in no significant difference in either the conventional apples ($p = 0.224$) or the organic apples ($p = 0.190$). A t-test found no significant difference between the vitamin C concentrations found by DCIP titration and cyclic voltammetry methods ($p = 0.080$).

4. DISCUSSION

Our results illustrate the quantitative measurements of vitamin C concentration by cyclic voltammetry. The order of our measured fruits from highest to lowest vitamin C content are organic kiwi, conventional kiwi, organic lemon, conventional orange (navel), conventional lemon, organic orange (Valencia), conventional gala apple, organic red delicious apple, conventional red delicious apple, organic gala apple, organic mango, and conventional mango.

The levels of vitamin C previously reported on similar fruits vary greatly among published studies. For example Vinci et al. [17] reported fresh kiwi, which we assumed to be conventionally grown, to have a vitamin C content of 67.23 mg/100 g. Amodio et al. [18] presented conflicting values of 29 mg/100 g in conventionally grown kiwi and 33 mg/100 g in organically grown kiwi. Our kiwi measurements are intermediate to these published values with 46.8 mg/100 g and 51.4 mg/100 g for conventional and organically grown kiwi respectively. Vinci et al. [17] also reported a value of 25.32 mg/100 g vitamin C in mangoes while we found a lower level of 11.0 mg/100 g for conventionally grown and 12.5 mg/100 g for organically grown mangoes. Lemons were found to have a vitamin C content of 51.30 mg/100 g by Vinci et al. [17], whereas we found values of 26.2 mg/100 g for conventional and 34.5 mg/100 g for organic lemons.

A)



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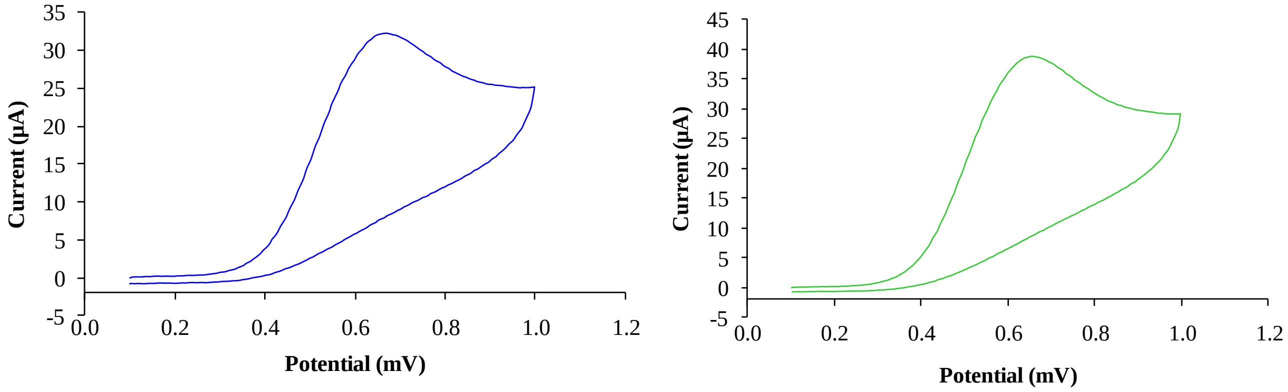

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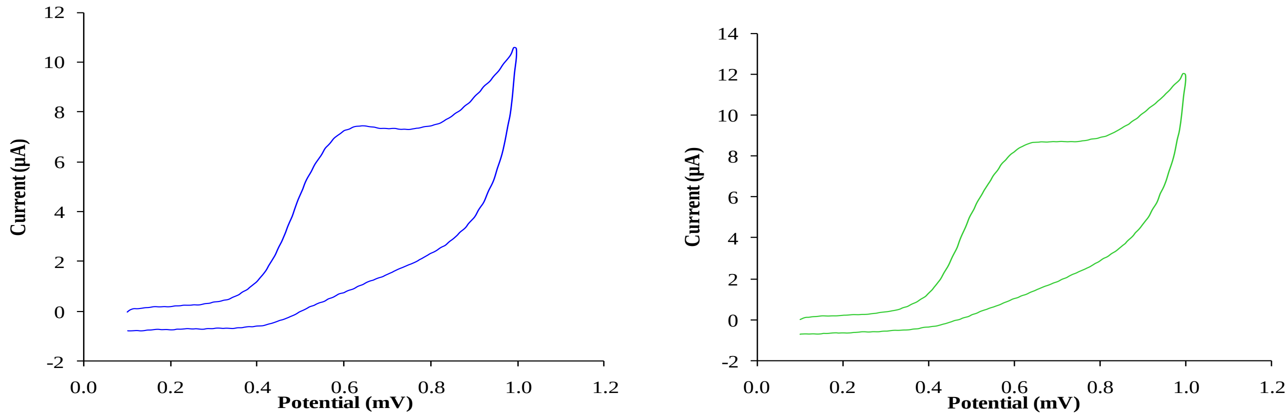

Figure 5. Sample cyclic voltammograms for lemon (a), kiwi (b), mango (c), where blue (left) is conventional fruit and green (right) is organic fruit. Scan rate = 100 mV/s.

Gazdik et al. [19] reported apples to have a vitamin C concentration range of 11 to 19 mg/100 g. This is comparable to our results which showed the average value of organic gala apples to be 19.0 mg/100 g and 21.5 mg/100 g for conventional gala apples. Our average concentrations of red delicious apples are also comparable, being 19.8 mg/100 g for conventionally grown and 21.4 mg/100 g for organically grown. Gala apples and red delicious apples are the same species and are only different varieties [20] which may explain why we found no significant difference between conventional or organic apple cultivars. Valencia oranges have been reported to have 43.4 mg/100 g and 51.8 mg/100 g of vitamin C for conventionally and organically grown oranges respectively [21]. In our study, we found levels of approximately half that with 27.7 mg/100 g in conventionally grown navel oranges and 26.2 mg/100 g in organically grown Valencia oranges. Valencia and navel oranges are varieties of the same species [22]. Since we found no significant difference between the vitamin C content of the two varieties of apples we can assume a negligible difference between the two varieties of oranges. We thus made a direct comparison between the two orange varieties.

With the exception of lemons, we found no significant difference between the vitamin C content of organically and conventionally grown fruit. A lack of significant difference in vitamin C content of conventionally and organically grown fruits and vegetables has also been previously shown by Masamba and Nguyen [21]. We also found a fairly high variation in the vitamin C content within the same fruit groups, with organic kiwi showing a range of 18.5 mg/100 g in our data set. Other factors such as growing season, storage, and shipping conditions may have a more important influence on vitamin C content than the growing methods employed by organic and conventional farmers. Warman and Harvard [23] found that storage influenced the vitamin C content in cabbage and carrots.

A multiyear study on the antioxidant content of organic and conventionally grown tomatoes and bell peppers carried out by Chassy et al. [24] found that there is variability in vitamin C content from year to year and therefore concluded other factors such as the environment and growing conditions having an important impact on vitamin C levels.

The levels of vitamin C have also been found to vary in the fruit from a single variety [24]. Wunderlich et al. [25] reported that the season in which broccoli are harvested has a significantly greater influence on vitamin C content than whether they are organically or conventionally grown. These studies along with our data further suggests that environmental factors prior to and after harvesting are more influential on vitamin C levels than whether the fruit is conventionally or organically grown.

The comparison of DCIP titration and cyclic voltammetry was used to confirm the accuracy of the voltammetric analysis. This comparison yielded no significant difference; however, the voltammetric analysis did provide a slightly higher vitamin C concentration for the same fruit. This slightly higher detection of vitamin C by cyclic voltammetry than DCIP was also found by Wawrzyniak et al. [26]. A similar increase in detection of vitamin C by cyclic voltammetry has also been noted by Okiei et al. [27] in comparison with N-bromosuccinimide titration, and by Esteve et al. [28], who used high performance liquid chromatography (HPLC). These findings suggest that because of the ease of use, accuracy, minimal sample preparation, and rapid detection, cyclic voltammetry is a superior method for vitamin C detection compared to other methods reported in the literature.

5. CONCLUSION

In this study the nutritional difference, as determined by vitamin C content, between six sets of conventionally and organically grown fruits were analyzed. There was no significant difference found in five of the six fruits considered. Only organic lemons displayed a significantly higher vitamin C level than their conventionally grown counterparts. Our results show that organically grown fruits are not nutritiously superior to conventionally grown fruits. The previously published literature is ambiguous as to whether or not organically grown fruits contain higher vitamin C content. It is more reasonable to assume that other external factors, such as storage and shipping conditions, may have a more considerable influence on the vitamin C content of fruits. Further research to assess the potential nutritional superiority of locally grown foods, over imported foods, is a natural progression of this research.

ACKNOWLEDGMENTS
This work was carried out with financial support from the Vitamin Fund administered by the Faculty of Agriculture, Life & Environmental Sciences (ALES), University of Alberta. We also wish to thank David King for his help with electrochemical measurements. We are grateful to Dr. Michael Mucz and Dr. John Olson for helpful suggestions during the writing process.

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