Determination of Ascorbic Acid Level in Orange Juice using an Open-source Poteniostat & Screen Printed Electrodes

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Abstract: In this research we describe the use of cyclic voltammetry concept in order to determine the level of Ascorbic Acid in orange juice. The proposed method consists of an open-source poteniostat and screen printed electrodes. The Current result from the chemical reaction is proportional to the concentration of the Ascorbic Acid. This method was applied to different commercial samples of orange juice and the results were used to determine which one has the most preservation of Ascorbic Acid.

1 INTRODUCTION

Ascorbic Acid (Vitamin C) is a water-soluble vitamin that is highly needed to the human body. The human body needs Ascorbic Acid (**AA**) to produce collagen to make connective tissue. (**AA**) is also important the body to absorb iron, heal wounds, build red blood cells and to fight infections. Since orange is a great source of (**AA**) our study is conducted on different samples of orange juice.

There are several factors that affect the amount of (**AA**) in orange juice such as heat, light, exposure to oxygen and how the juice is made during the manufacturing process. In this research we focused the study on the effect of storage temperatures on, both, fresh and packed orange juice.(Royston and Angela, 2003).

According to the present life style, people prefer depending on the packed juices as they are versatile, accessible and just ready to drink. Consequently, these products must be periodically checked to ensure that they contain the right amount of each nutritional element needed by the human body.

Considerately, the proposed method in this research is to determine the level of (**AA**) in orange juice which reflects the quality of the juice. By exposing a variety of orange juice samples to different storage temperatures and checking the preservation of (**AA**).

There are some classical techniques for the determination and assessment of (**AA**) such as: titration with an oxidant solution, Chromatographic methods and Fluorimetric methods. (A.M. Pisoschi et al., 2008)

The need for another method arises from the complexity of the previous methods, the expensive components used and the use of large equipment. On the other hand, the proposed method consists of a hand-held poteniostat in the size of cell phone, screen printed electrodes and a laptop to save the readings. Fig.1

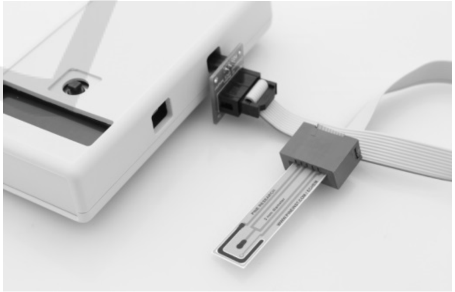

Figure 1: The poteniostat used in the research and the screen printed electrodes.

2 BACKGROUND

Several researches have studied the determination of ascorbic acid in orange juice or in other fruits. Each study and research utilizes different equipment to perform the cyclic voltammetry technique.

Fundamentally, Cyclic Voltammetry (CV) is a

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technique to study the behavior of electrochemical reactions. In the CV, information about the samples undergoing the test is obtained by measuring the output anodic current as the input voltage varied (J. Randles and Trans.Far, 1948).

To preform cyclic voltammetry on a certain chemical element three electrodes are needed (working, reference and counter) electrodes, a poteniostat to vary the potential of the working electrode and a recording device to measure the output current.

It is worth mentioning that a research to determine **(AA)** level in commercial fruit juices was done using a potentiostat-galvanostat KSP, laboratory made by SlawomirKalinowski and Pt disc electrode as a working electrode, saturated calomel electrode as the reference electrode and the counter electrode was Pt strip. This setup proved its accuracy in the determination and recovery of **(AA)** level in fruit juices (Pisoschi et al., 2008).

The complication facing this method is that the test must be done in a chemistry lab, as the equipment used is static.

Another research was done on the determination of **(AA)** level in orange juice using a poteniostat and a lead pencil electrode. This method proved a satisfactory determination of **(AA)** but with a minimum concentration of 0.0326 mg/mL to produce a linear determination curve (D. King et al., 2010).

3 EXPERIMENTAL WORK

3.1 Samples

Three samples were taken from a freshly squeezed orange juice and stored at 25 ºC, 18 ºC and 12 ºC for the first experiment.

For the second experiment two samples from commercial orange juice and one sample from a freshly squeezed juice were stored at 18 ºC.

KCl solution with a concentration of 0.1 mole/L was added to all samples as the KCL ions chemically interact with (**AA**) ions and enhance the electric conductivity of the **(AA)** molecules so that the anodic current can be measured be the poteniostat.

3.2 Poteniostat and Screen Printed Electrodes

3.2.1 Poteniostat

Poteniostats are amplifiers used to control the voltage between working and reference electrodes. A simple design of poteniostat is shown in Fig.2 (A.V. Gopinath and D.Russel, 2005).

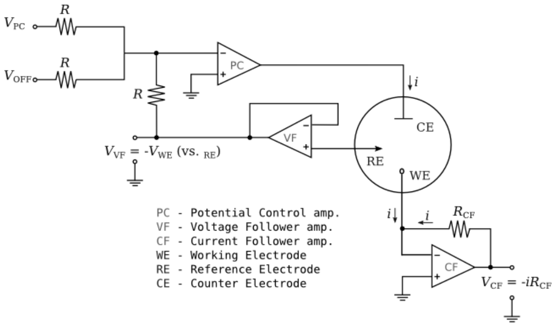

Figure 2: Circuit Diagram of a simple poteniostat.

Several models and designs of poteniostats are available in the market but due to their uneconomical cost (range of thousands dollars) and their huge equipment that is required to be fixed in a certain place as they are not portable (A.V. Gopinath and D.Russel, 2005).

The poteniostat used in this research is an open-source device designed by a team in the biochemistry department, University of California Santa Barbra (Rowe AA et al., 2011).

The total cost of the used poteniostat is in the range of (100$) which is inexpensive compared to any model of a commercially available poteniostat.

Moreover, the poteniostat used is considered a hand held device with a USB power supply. It can be used anywhere due to its portability.

3.2.2 Screen Printed Electrodes

The elimination of bulky materials and instruments is a major concern in the electrochemistry. The printed electrodes offer high accuracy, low cost and more portability.

A major advantage in using Screen Printed Electrodes (SPE) is that it needs small sample volume (~ µL) when compared to the traditional solid electrodes (W. Wonsawat, 2014).

The SPE used in this research is manufactured by Bio-Logic Science Instruments.

SPE consists of a Graphite working and counter electrodes, and Ag/AgCl reference electrode with an alumina substrate. Additionally, the SPE used allows a low sample volume (25 µL-100 µL) which is a great advantage in the on-site detection of the sample under test.

4 PROCEDURES

Two experiments were done to determine the level of (AA) in orange juice. Both were done using the cyclic voltammetry with the following parameters: start voltage 200 mV, end voltage 900 mV, slope 50 mV/S, sample rate 5 mV/sample and number of cycles equal to 1. The current has been evaluated at 550 mV because (AA) is highly chemically active at this voltage so best current reading is taken at 550 mV (Rowe AA et al., 2011).

Both experiments were run for 7 days, with a daily reading for each sample.

The SPE used was mechanically cleaned with distilled water after each reading, also a potential is applied on the SPE (-200 mV to 200 mV) after each reading to ensure there is no interaction between the sample under test and the electrode material (W. Wonsawat, 2014).

For each reading 10 µL of KCl was added to 20 µL of orange juice sample and the mixture was tested by the SPE and the poteniostat and the result of each reading is stored in the PC.

4.1 Experiment 1

In the first experiment three samples of freshly squeezed orange juice were taken and stored at 25 ºC, 18 ºC and 12 ºC, in order to study the effect of storage temperature on fresh orange juice. Fig.3 shows the cyclic voltammetry of **(AA)** at different storing temperatures**.**

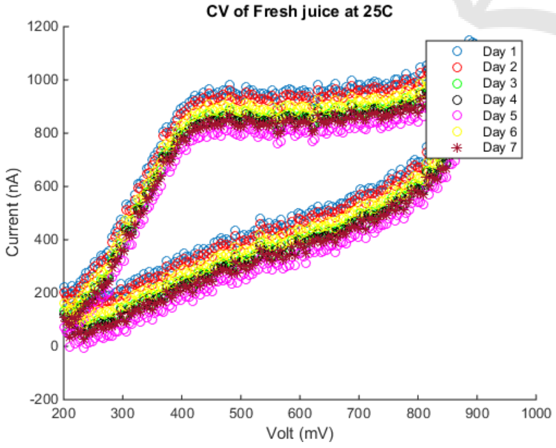

Figure 3a: Cyclic Voltammetry output of Fresh orange juice stored at 25 ºC.

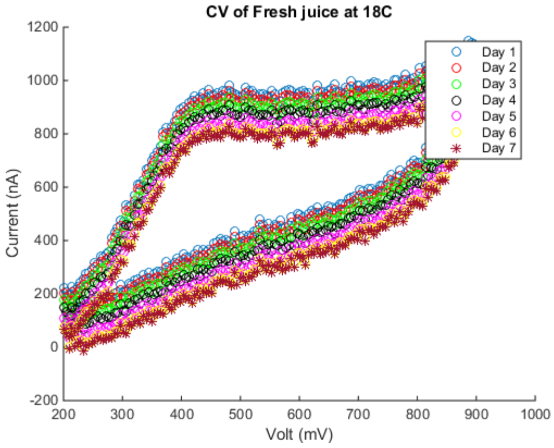

Figure 3b: Cyclic Voltammetry output of Fresh orange juice stored at 18 ºC.

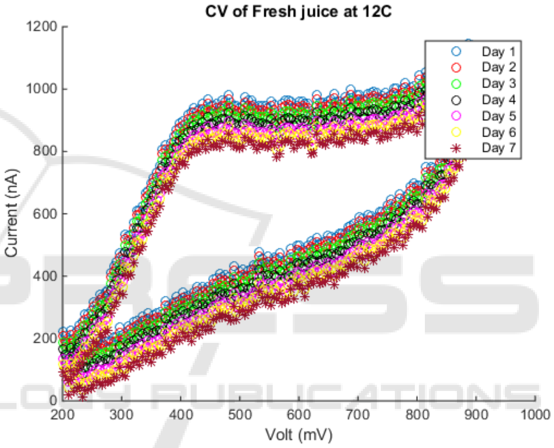

Figure 3c: Cyclic Voltammetry output of Fresh orange juice stored at 12 ºC.

4.2 Experiment 2

In the second experiment three samples were used, two of which were from commercial orange juice , one of them was a Natural Identical product and the other was a Nectar product while the third sample was a freshly squeezed orange juice, in order to study which type will have the ability to preserve **(AA)** for more time keeping the storage temperature (18 ºC.) stable. Fig.4 shows the cyclic voltammetry for the samples.

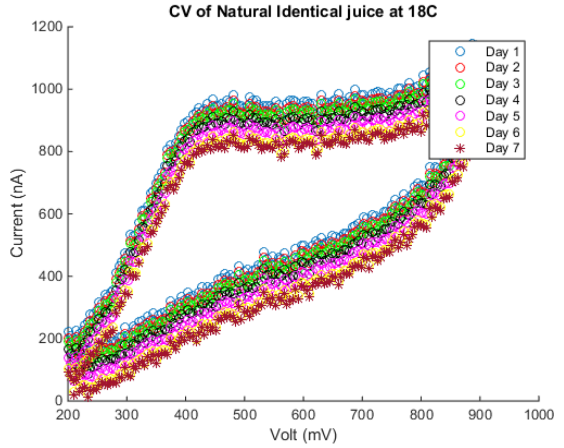

Figure 4.a: Cyclic Voltammetry output of Natural Identical orange juice stored at 18 ºC.

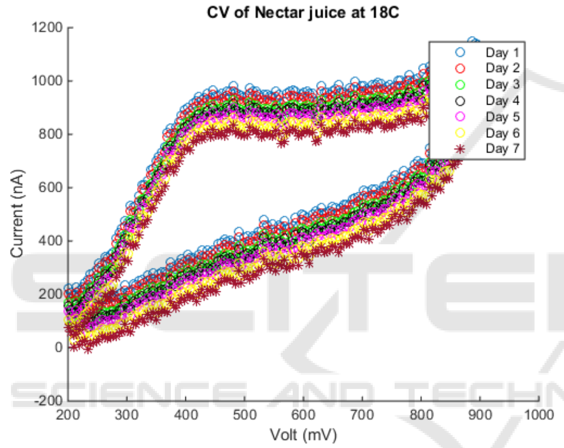

Figure 4.b: Cyclic Voltammetry output of Nectar orange juice stored at 18 ºC.

5 RESULTS AND DISCUSSION

According to Randles-Sevcik equation:

$$I_d = 2.69.10^5\, n^{3/2} A D^{1/2} v^{1/2} c \qquad (1)$$

Where *c* is the concentration, *v* is the voltage scan rate, *A* is the electrode surface, *D* is the diffusion coefficient, *n* is the number of electrons transferred during the process and I_d is the anodic current. (A.M. Pisoschi et al., 2008)

It is obvious that the, concentration is directly proportional to the anodic current. Accordingly, the anodic current can be used as a mirror to the concentration for the study of the **(AA)** behavior at different storage temperatures.

5.1 Experiment 1 Results

Starting with the cyclic voltammetry curves, we can deduce that for a freshly squeezed orange juice, the lower the storage temprature, the more slower losing the **(AA)**.

The anodic current values at 550 mV were taken to plot the relation between the anodic current and measurments time because at 550 mV the ascorbic acid ions are dominant over other ions in the orange juice as shown in Fig5.

By performing a fitting curve to deduce the equation that shows an approximate form of the production time of the juice and hence control techniques can be applied to accomplish different qualities depending on the level of **(AA).** This relationship is given by the following equations.

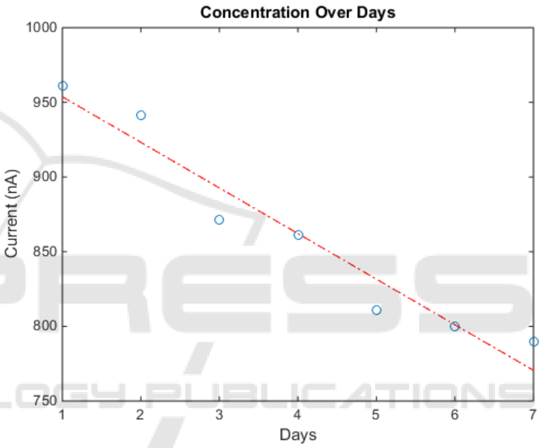

Figure 5.a: The anodic current at 550 mV at daily basis for a fresh orange juice at 25 ºC.

$$y=-30.5357x+ 984.2857 \qquad (2)$$

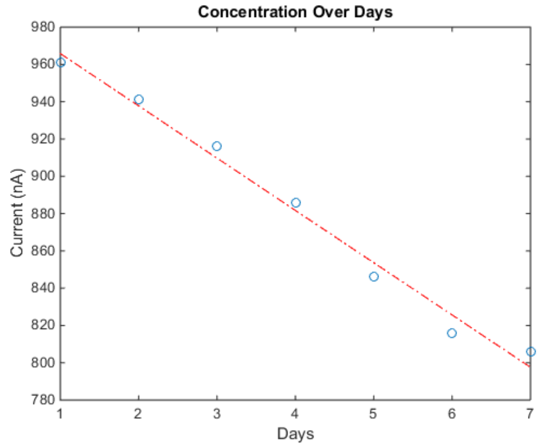

Figure 5.b: The anodic current at 550 mV at daily basis for a fresh orange juice at 18 ºC.

$$y=-28.0357x+993.8571 \quad (3)$$

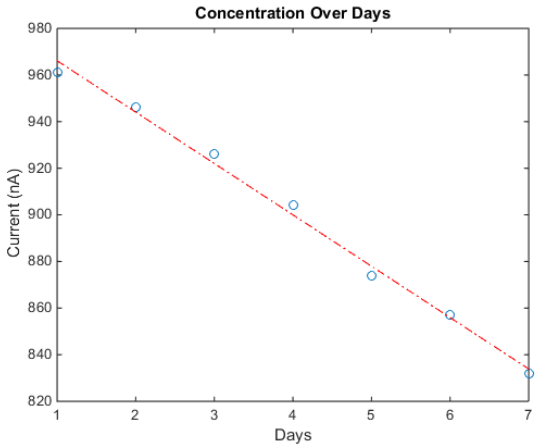

Figure 5.c: The anodic current at 550 mV at daily basis for a fresh orange juice at 12 ºC.

$$y=-22.0357x+988.1429 \quad (4)$$

Equations (2, 3, 4) can be adopted to determine how long has the juice been produced on top of checking the expiry date that gives an indication for the suitable use date.

5.2 Experiment 2 Results

Arising from the cyclic voltammetry curves of the three samples we can deduce that at the same storage temperature, the freshly squeezed orange can preserve **(AA)** for more time than the Natural Identical and the Nectar. Fig.6 shows the degradation rate of **(AA)** of the three juice samples at the same temperature which is 18 ºC.

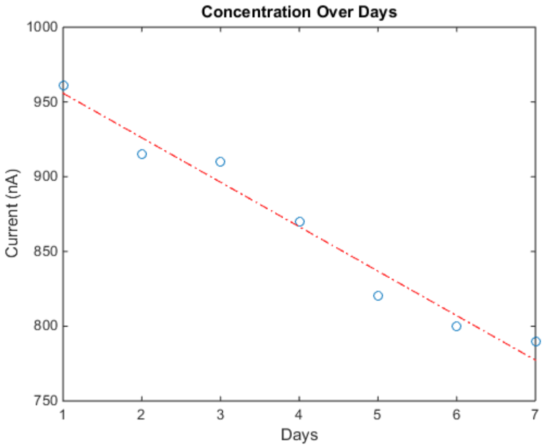

Figure 6a: The anodic current at 550 mV at daily basis for a Natural Identical orange juice at 18 ºC.

$$y=-29.7500x+985.5714 \quad (5)$$

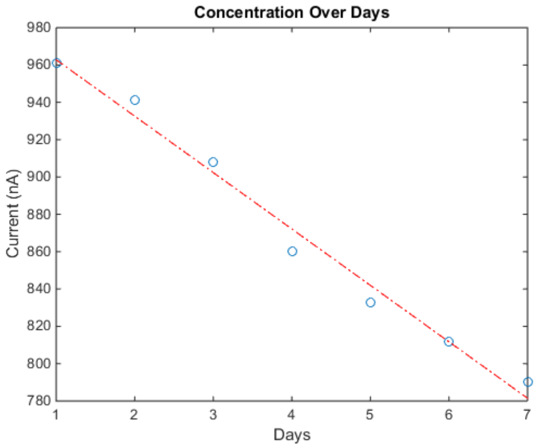

Figure 6.b: The anodic current at 550 mV at daily basis for a Nectar orange juice at 18 ºC.

$$y=-30.2143x+993.0000 \quad (6)$$

We can also use equations (5, 6) to determine how long has the juice been produced and check its expiry date. Ergo, it can give an indication for the best use date.

6 CONCLUSIONS

In this research we show how to employ the cyclic voltammetry concept to determine the **(AA)** level in orange juice. In this work we used an open-source poteniostat which was chosen based upon its inexpensiveness, portability and reliability for **(AA)** measurements. Additionally, we used a low cost screen printed electrodes for the complete process.

The results show that the freshly squeezed orange juice at the lowest possible storage temperature can preserve **(AA)** more than any type of orange juice at the same temperature.

Wherefore, the deduced relation between the anodic current and the time can be used to determine the quality of the juice and an approximation to the production and expiry dates.

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