

# Monitoring of Anthocyanins and Colour in Electrochemically Processed *Hibiscus sabdariffa* Juice

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## Abstract

The vital role of anthocyanins in *Hibiscus sabdariffa* L. is now known to most consumers. The richness of anthocyanins in antioxidants, vitamin C, minerals, etc., provides *Hibiscus* juice with proven nutritional qualities. The health requirements of recent years have made food products with added preservatives or processed at high temperatures less popular, thus explaining the new orientations towards innovative and interdisciplinary technologies. Anthocyanins from *Hibiscus sabdariffa* L. are, however, sensitive to degradation factors such as temperature, light, enzymes and also oxygen. The instability of anthocyanins has long been a subject of research using classic techniques such as heat treatment, the results of which are often limited by the rapid degradation and above all the destruction of the nutritional and organoleptic qualities of the product. Oxygen dissolved in juices is so far treated by bubbling with an inert gas or by adding other molecules such as preservatives which can cause a lot of health damage. The electrochemical approach is a new stabilisation technique that reduces the dissolved oxygen in the juice, cold and without the addition of other molecules. The electrolysis of *Hibiscus* juice was carried out by noble electrodes (Platinum and Stainless Steel) with a well-characterised Time/Potential or Time/Intensity couple. The electroreduced samples and the control were then stored at 4°C, 25°C and 37°C for more than 6 months. Monitoring of anthocyanins in the first month, of the samples and the control, showed a significant difference of 10% between the electroreduced extract and the untreated control at 37°C, which had previously been problematic for the heat treatment and even for the other membrane techniques. Oxygen reduction on the platinum electrode/ECS allowed the retention of more than 10% of anthocyanins after 4 weeks of storage at 25°C and 37°C. At 4°C, a significant difference of 5% between the electro-reduced *Hi-*

*biscus* juice and the control was maintained until the fifth month of storage with the 1/5 ratio (calyx/water). Non-significant losses in anthocyanin (10 mg/l), for the juice with reduced dissolved oxygen, were noted for the 1/5 and 1/15 ratios during the first month of storage at 4°C against 24 mg/l of significant losses for the 1/20 ratio. However, the untreated control showed significant losses for the ratios 1/20, 1/15 and 1/5. Oxygen dissolved in the juice therefore considerably degrades the anthocyanins of *Hibiscus sabdariffa* L. from the first month of storage at 4°C.

## Keywords

Monitoring, Anthocyanin, Colour, Oxygen, Stabilisation, Electrochemical Nafion Membrane, Ratio

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## 1. Introduction

The richness of anthocyanins as an antioxidant [1] [2] and their numerous health benefits [1]-[7] explain the multiple research themes that exist around the subject [1] [8] [9] [10].

Anthocyanins are the most important group of water-soluble natural pigments. They are polyphenols of the flavonoid family, which are secondary metabolites synthesised by plants. Anthocyanins are a heterogeneous group of molecules ranging from pink, red, purple to dark blue in a wide variety of vegetables and fruits [11].

However, the instability of anthocyanins to temperature, light, pH, oxygen [12] and other factors remains problematic.

Food preservation processes usually involve the use of high temperature heat treatment (60°C to 150°C), in order to effectively preserve the food. However, heat treatment has a negative influence on the stability of anthocyanins [13].

The requirements of the joint programme of the World Health Organisation (WHO) and the Food and Agriculture Organisation (FAO) in the new food code [14] [15] for quality foods respect the nutritional properties of the product direct research towards new stabilisation techniques.

The negative impact of oxygen on anthocyanins has been shown by several authors.

The degrading effect of oxygen on the chemical and sensory evolution of red wine was characterised by Laurent PECHAMAT [16]. In his results, he showed a decrease in total anthocyanin content as a function of time, as well as a degradation of sensory qualities.

The effect of the stopper on the evolution of the sensory quality of the wine was also characterised by Maria Adelaine Monteiro de Araujo Silva [17] in her thesis work. These studies revealed an evolution in the composition of wines with a significant decrease in the concentrations of certain compounds such as anthocyanins such as malvidin and cyanidin, which decreased by 60%, as well as

20% of flavanols.

The elimination of dissolved oxygen in the food was achieved by the joint use of enzymes. Prieels Jean Paul *et al.* [18] described in their patent a procedure to prevent the auto-oxidation of food substances that can be degraded by oxidation with molecular and/or radical oxygen by adding an enzymatic composition comprising an oxidase: catalase and super oxide dismutase, chemical stabilisers but also by bubbling with an inert gas.

Sugawara *et al.* [19] also in their patented invention improved the taste, flavour and physicochemical properties of milk by removing ions from the milk and reducing the concentration of dissolved oxygen in the milk followed by heat treatment.

The electrochemical approach has already been used for the reduction of dissolved oxygen in seawater [20] but not for the preservation of juice to our knowledge.

Our objective is to characterise juices electrochemically and stabilise them, without adding food or using inert gas, while preserving the nutritional and organoleptic qualities of the product.

The monitoring of anthocyanins and colour, after storage for several months at different temperatures, showed the effect of the treatment on the juice of *Hibiscus sabdariffa* L.

The reduction of oxygen in juices by electrochemical means could thus be an alternative to bubbling and other classic techniques for stabilising food products.

## 2. Material and Methods

The plant material is the extract of *Hibiscus sabdariffa* L. (Figure 1).

The electrochemical measurements carried out in this work use a potentiostatic set-up with three electrodes. It comprises a platinum working electrode, the site of the electrochemical reactions studied, a stainless steel auxiliary electrode which closes the electrical circuit and a saturated calomel reference electrode, which makes it possible to control and measure the potential of the working electrode at each moment. The system used is a potentiostat connected to a computer equipped with cyclic voltammetry software (Figure 2).

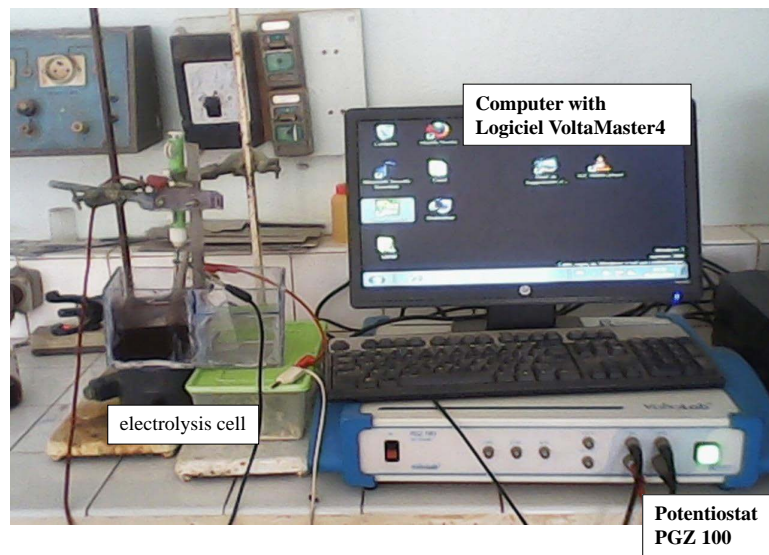
The electrochemical cell is a Plexiglas electrolysis cell with two compartments separated by a cationic nafion membrane (Figure 3). On one side of the membrane, *Hibiscus* juice is in contact with a platinum cathode and on the other side, 0.1M hydrochloric acid (HCl) is in contact with a stainless steel anode.

### 2.1. Electroreduction of Bissap

The potential difference applied between the two electrodes, anode and cathode, is produced by an electric current generator, in potentiostatic mode, connected to a PGZ 100 potentiostat which fixes the potential between the cathode and the reference electrode. The applied electrolysis potentials are fixed in relation to this reference (Figure 4).



**Figure 1.** Extracts of *Hibiscus sabdariffa* L.

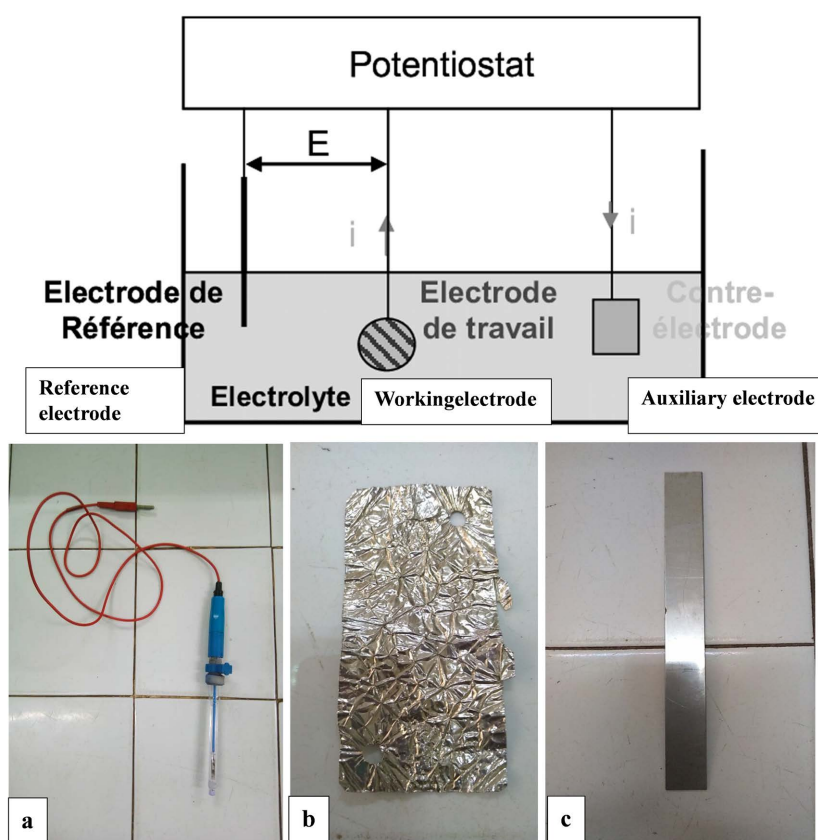


**Figure 2.** Electrochemical device for the electroreduction of *Hibiscus sabdariffa* juice.





**Figure 3.** Electrolysis cell with two compartments separated by (a) a dark nafion cation membrane (b) a transparent nafion cation membrane.



**Figure 4.** Three-electrode set-up. (a) Saturated Calomel reference electrode, (b) Platinum working electrode, (c) Stainless steel.

The treatment was carried out on bissap juice placed in the cathode compartment using an equivalent volume of hydrochloric acid in the anode compartment. The current intensities applied and the duration of the treatment were determined following the voltammetric study in our first article (Ndiaye Khady *et al.*) [21].

The potential scan was performed between  $-0.5$  V and  $1.5$  V/ECS at a rate of  $100$  mV/s.

At the cathode, oxygen is reduced on the platinum electrode according to the acid reaction:



The reduction of one oxygen molecule into two water molecules on the platinum electrode is done in particular according to a 4-electron process (Nathalie BOZEC) [20]. Platinum is not affected by oxygen or water and is only soluble in aqua regia or molten alkali. It is satin-like, ductile and malleable.

At the end of each electrolysis, the treated product is placed in small bottles wrapped in aluminium foil (Figure 1) for storage at 4°C, 25°C and 37°C. Anthocyanins and colour were monitored every month.

## 2.2. Determination of Anthocyanins

The determination was carried out on *Hibiscus sabdariffa* extract.

The principle is based on the modification of anthocyanin coloration as a function of pH (pH-differential method) [22].

After dilution of the calyx extract in two buffer solutions at pH = 1.0 and pH = 4.5, the absorbance is measured at 510 and 700 nm, the values read at the two wavelengths for each solution are used to calculate the anthocyanin concentration by the following formula

$$Ca = \frac{P_m * F_d * A * 1000}{\varepsilon} \quad (2)$$

*Ca*: anthocyanin concentration in mg/L.

*P<sub>m</sub>*: molecular weight of the anthocyanin. In this case,

*Ca* is expressed in relation to Delphinidin Sambubioside, which is the majority anthocyanin in the calyxes of *Hibiscus sabdariffa* L. Its molecular weight is 597 g/mol.

$\varepsilon$ : molecular extinction coefficient and is equal to 26,000 L·mol<sup>-1</sup>·Cm<sup>-1</sup>.

*F<sub>d</sub>*: dilution factor

*A*: absorbance, calculated using the formula:

$$A = (A_1 - A_2) - (A_3 - A_4)$$

*A*<sub>1</sub> = absorbance measured at pH 1 at 510 nm.

*A*<sub>2</sub> = absorbance measured at pH 1 at 700 nm.

*A*<sub>3</sub> = absorbance measured at pH 4.5 at 510 nm.

*A*<sub>4</sub> = absorbance measured at pH 4.5 at 700 nm.

## 2.3. Colour Indices

The evaluation of the colour of *Hibiscus sabdariffa* L. extract is carried out by measuring colour indices of the 1% extract solution at pH = 3 in a 1 cm, at wavelengths of 430, 520, 580 nm. The extracts are diluted with a buffer solution pH = 3.

An increase in the colour indices leads to a browning of the extract and thus destroys the visual quality of the product.

The colour indices allow the quality of the dye to be assessed as well as its shade or hue. The tonality of a dye can be quantified by two indices: the brown

index (BI) and the violet index (IV).

The brown index represents the proportion of the yellow colour to the red colour. It is calculated by:  $IB = A_{430 \text{ nm}}/A_{520 \text{ nm}}$ .

The violet index represents the proportion of the blue colour to the red colour. It is calculated by:  $IV = A_{580 \text{ nm}}/A_{520 \text{ nm}}$ .

## 2.4. Reading the Colour

The measurements were carried out with a Minolta CR-5 colorimeter which gives the 3 coordinates  $L^*$ ,  $a^*$  and  $b^*$  directly. The colour of a juice can be defined by three specific attributes or qualities of visual sensation: its hue, its brightness and its chromaticity.

The most characteristic hue is the colour itself: red, yellow, green or blue.

Luminosity is the attribute of visual sensation by which a juice appears more or less brightness. The chromatic characteristics of a juice are defined by the colorimetric coordinates corresponding to lightness ( $L^*$ ), red/green colour component ( $a^*$ ) yellow/blue colour component ( $b^*$ ), and by the derived quantities, namely chroma ( $C^*$ ), tone ( $H^*$ ) and chromaticity ( $a^*$ ,  $b^*$ ) or ( $C^*$ ,  $H^*$ ). In other words, this colour system or CIELab space is based on a Cartesian sequential or continuous representation on 3 orthogonal axes  $L^*$ ,  $a^*$  and  $b^*$ . The  $L^*$  coordinate designates the lightness ( $L^* = 0$  black and  $L^* = 100$  colourless),  $a^*$  designates the red/green colour component ( $a^*$ ) and  $b^*$  designates the red/green colour component ( $b^*$ ). ( $a^* > 0$  red,  $a^* < 0$  green) and  $b^*$  the yellow/blue colour component ( $b^* > 0$  yellow,  $b^* < 0$  blue).

## 3. Results and Discussion

The experimental results are presented in this section with significance tests.

The analyses of variance (ANOVA) were performed with STATISTICA 7.1 software and significance is represented by superscript and lower case letters. Identical superscripts on a result mean that there is no significant difference and when the superscripts are different, the difference is significant.

This is a one-factor analysis of variance (ANOVA), Fisher LSD test, homogeneous groups  $\alpha = 0.05$  inter error term, significance level 0.05.

The monitoring of anthocyanins and colour was carried out on three ratios (Calyxes/Water): Ratio 1/20; Ratio 1/15; Ratio 1/5.

### 1) Chronoamperometry of *Hibiscus sabdariffa* juice: electrolysis cell of dimensions (L = 19 cm; w = 10 cm; h = 11 cm), dark membrane

The electroreduction of 500 ml of *Hibiscus sabdariffa* juice with a potential/treatment time of  $-350\text{mV}/30\text{mn}$ , the calyx/water ratio was 1/20.

#### a) Monitoring of anthocyanins

After electroreduction of the *Hibiscus sabdariffa* juice, the sample was placed in small flasks and stored at  $4^\circ\text{C}$ ,  $25^\circ\text{C}$  and  $37^\circ\text{C}$ .

Every month, the anthocyanins of the treated extract are monitored with the potential of the reduction peak ( $-350 \text{ mV}$ ) for 30 minutes and the control (un-

treated extract) at the three temperatures.

The results obtained are presented in **Figure 5** below.

**Figure 5** shows the monitoring of anthocyanins in electro-reduced (Sample) and untreated (Control) extracts after 1, 3 and 8 months of storage at 4°C, 25°C and 37°C.

The results show a significant difference between the sample and the control at the first month at 25°C and 37°C. However, there is no significant difference between the sample and the control at the third and eighth month for the *Hibiscus* extract of ratio 1/20.

The largest difference between the sample and the control was noted in the first month at 25°C (14%) and at 37°C (12%).

This result can be explained by the fact that most of the dissolved oxygen in the non-electroreduced extract reacts to form colourless products and that these reactions are catalysed by a certain temperature (25°C, 37°C).

However, at 4°C the difference between the sample and the control remained almost constant (2%) throughout the monitoring. It seems that dissolved oxygen in *Hibiscus* juice of ratio 1/20 has no effect on anthocyanins at this temperature.

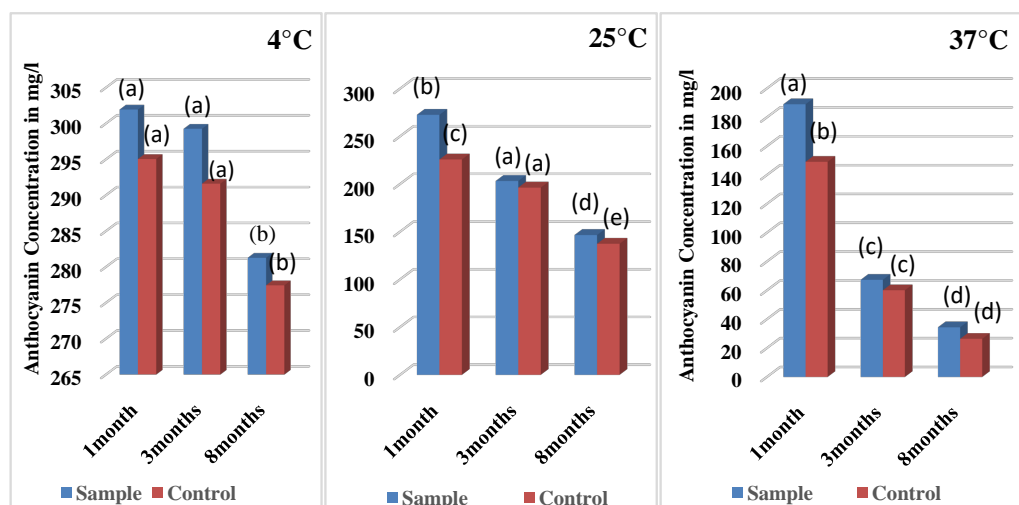
#### b) Colour monitoring

The colour of the electrochemically treated extract with a peak reduction potential/treatment time of -350 mV/30min and the untreated extract (control) was also monitored after storage at 4°C, 25°C and 37°C.

The colorimetric coordinate corresponding to the red/green component (a\*) only is presented.

The results obtained are shown in the following **Table 1**.

**Table 1** shows the results of monitoring the red colour (a\*) of *Hibiscus* juice with dissolved oxygen reduced on a platinum/ECS electrode (Sample) and non-electro-reduced *Hibiscus* extract (Control) after 8 months of storage at 4°C, 25°C and 37°C.



**Figure 5.** Monitoring of anthocyanins in the electrochemically treated extract (Sample) and the untreated extract (Control) after storage at 4°C, 25°C and 37°C.

**Table 1.** Red/green colour component ( $a^*$ ) of electro-reduced (sample) and untreated (control) juice stored at 4°C, 25°C and 37°C.

		Red/green component $a^*$		
		1 month	3 months	8 months
4°C	Sample	56.14 <sup>a</sup>	55.38 <sup>c</sup>	54.47 <sup>b</sup>
	Control	56.11 <sup>a</sup>	55.07 <sup>d</sup>	54.44 <sup>b</sup>
25°C	Sample	56.06 <sup>a</sup>	54.79 <sup>c</sup>	52.72 <sup>e</sup>
	Control	55.52 <sup>b</sup>	53.75 <sup>d</sup>	51.83 <sup>f</sup>
37°C	Sample	55.12 <sup>a</sup>	54.61 <sup>c</sup>	50.51 <sup>e</sup>
	Control	52.6 <sup>b</sup>	51.73 <sup>d</sup>	50.49 <sup>e</sup>

The results corroborate those obtained with the monitoring of anthocyanins. Indeed, after one month of storage at 25°C and 37°C, there were significant losses of red colour in the untreated extract (Control) (55.52 at 25°C and 52.6 at 37°C) compared with the treated extract, which retained its red colour better, as shown by the values of the red coordinate  $a^*$  (56.06 at 25°C and 55.12 at 37°C), whereas at 4°C the difference was not significant.

In the third month at all temperatures, the difference in red colour between the sample and the control is significant, unlike in the eighth month when the sample and the control do not show a significant difference.

These colour results confirm the degrading effect of dissolved oxygen in the *Hibiscus* juice in the first three months at 4°C, 25°C and 37°C.

## 2) Chronoamperometry of *Hibiscus sabdariffa* extract: electrolysis cell with dimensions (L = 8; w = 7; h = 8.5 cm), transparent membrane

### a) Monitoring of anthocyanins

The reproducibility of scientific results is one of the requirements of research.

In order to confirm the results on the electrochemical stabilisation of anthocyanins, we used another (transparent) membrane.

The treatment was carried out in a small electrolysis cell with a peak potential/treatment time of -250 mV/15mn. The ratio used was 1/15.

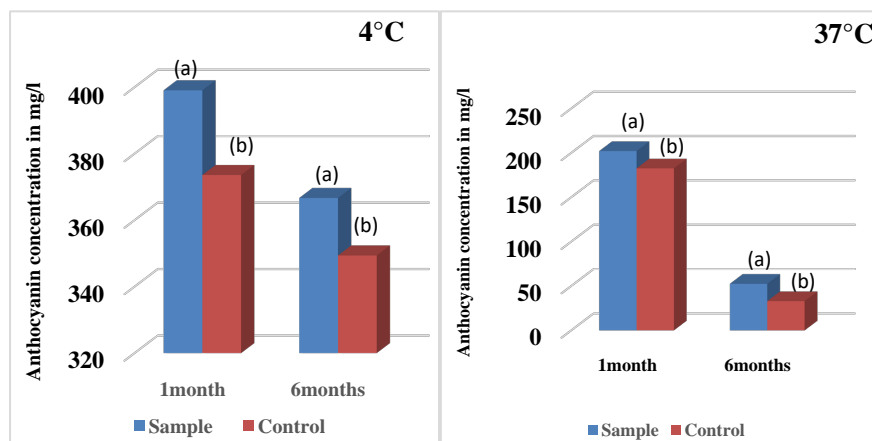
The conservation lasted 6 months at 4°C and 37°C.

The following **Figure 6** shows the results obtained.

**Figure 6** shows the concentration of anthocyanins in *Hibiscus sabdariffa* extract with oxygen reduction on a platinum/ECS electrode (Sample) and the non-electro-reduced extract (Control) after 1 and 6 months of storage at 4°C and 37°C.

The results show a significant difference between the electrochemically treated juice with a potential/time couple of -250 mV/15mn (Sample) and the untreated juice (Control) at the first and sixth month of storage at 4°C and 37°C.

The difference in anthocyanin concentration between the sample and the control is 7% and 4% respectively after 1 month and 6 months of storage at 4°C. At 37°C, the difference between the sample and the control is 5% at the first month and 3% at the sixth month of storage.



**Figure 6.** Monitoring of anthocyanins in the electrochemically treated extract (Sample) and the untreated extract (Control) after 6 months of storage at 4°C and 37°C.

The effect of dissolved oxygen in the *Hibiscus* juice (ratio 1/15) is therefore significant at 4°C. This could be explained by the fact that the electrolysis was performed in a small cell (Figure 3(b)) and that the experiment is an electron transfer.

We also noticed a slower degradation of the extracts after the first month of storage compared to the initial concentration: initial concentration (413.31 mg/l) and sample concentration after 4 weeks of storage at 4°C (399.07), *i.e.* a difference of 13 mg/l for the 1/15 ratio, whereas for the 1/20 ratio there was a difference of 24 mg/l between the initial concentration and the sample for the same storage time at the same temperature, thus confirming Mady Cisse's work [13].

#### b) Monitoring of colour

The following Table 2 presents the monitoring of the red/green component a' of the sample and the control after storage at 4°C and 37°C.

The results on colour corroborate those on anthocyanins.

The red colour component of the electro-reduced juice is significantly different from the control at 4°C and 37°C after 1 month and 6 months of storage.

### 3) Chronoamperometry and Chronopotentiometry of *Hibiscus sabdariffa* juice

Chronoamperometry consists in treating the solution with the potential corresponding to the reduction peak during a well-defined treatment time, the intensity remaining constant. Whereas chronopotentiometry translates a treatment of the juice by the intensity of the reduction peak predefined at a well given time with constant potential.

#### a) Monitoring of anthocyanin concentration

The electrochemical treatment of *Hibiscus* juice was first carried out with a potential of the reduction peak/treatment time of -125 mV/30mn, *i.e.* the chronoamperometry method. Then a treatment by chronopotentiometry with an intensity of the reduction peak/treatment time of -5 mA/30mn. The ratio used is 1/5, based on the work of M. CISSE [13]. The electrolysis of the juice was done with the small cell (Figure 3(b)).

**Table 2.** Red/green colour component (a\*) of electro-reduced (sample) and non-electro-reduced (control) juice stored at 4°C, 25°C and 37°C.

		Red/green component a*	
		1 month	6 months
4°C	Sample	50.09 <sup>a</sup>	48.63 <sup>b</sup>
	Control	49.39 <sup>b</sup>	47.53 <sup>c</sup>
37°C	Sample	48.56 <sup>a</sup>	45.36 <sup>c</sup>
	Control	47.96 <sup>b</sup>	44.18 <sup>d</sup>

The results obtained are shown in the following **Figure 7**.

The results showed that there was no significant difference between the two methods: chronopotentiometry and chronoamperometry.

The significant difference was noted in the first month at all temperatures (4°C, 30°C and 37°C). A significant difference of 6% at 30°C and 10% at 37°C was noted between the sample and the control at the first month.

At 4°C, a significant difference of about 5% was noted until the fifth month, whereas at 30°C and 37°C, there was no significant difference between the sample and the control at the fourth and fifth month of storage: the temperature factor predominates over the oxygen factor.

According to the results of the experiment, the two methods—chronoamperometry illustrated by sample 1 (−125 mV/30mn) and chronopotentiometry corresponding to sample 2 (−5 mA/30mn) are equivalent. In fact, apart from a few differences between the two samples, which are certainly due to the instability of anthocyanins in general, the two methods make it possible to reduce the oxygen dissolved in water, thus allowing a certain stability of anthocyanins during storage.

The experiment confirms the predominance of temperature over the other factors (more than 88% loss at 37°C after 5 months of storage).

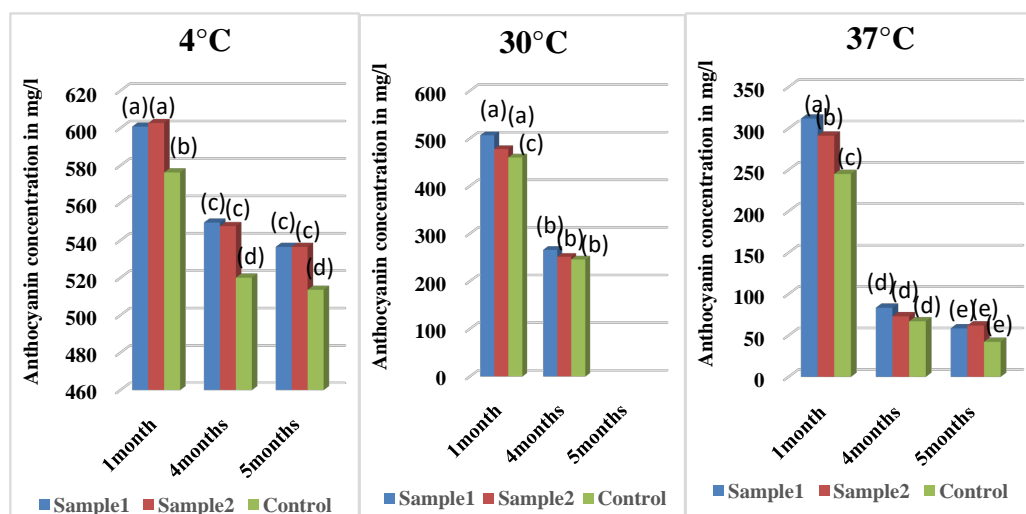
#### **b) Monitoring of colour indices**

Colour indices are one of the techniques used to characterise the colour of a product. Indeed, the higher the index, the more the product deteriorates, especially if it turns brown. We have the brown index (BI) which is the proportion of the yellow colour to the red colour (yellow/red) and the violet index (PI) which translates the ratio between the blue colour and the red colour (blue/red).

The results of the monitoring of the brown index and the violet index are recorded in the following **Table 3** & **Table 4**.

The yellow/red (BI) colour ratio showed no significant difference between the sample and the control at the first month at 4°C. The *Hibiscus* juice did not turn brown after 5 months of storage at 4°C. Thus, it can be said that dissolved oxygen does not brown the *Hibiscus* juice at 4°C during the first five months of storage.

At 25°C, the sample and the control do not show any significant difference but the product browns significantly between the first and fourth month of storage.



**Figure 7.** Monitoring of electroreduced anthocyanins and the control, stored at 4°C, 30°C and 37°C for 5 months, of the extract treated by chronoamperometry –250 mV/30mn (sample1), of the extract treated by chronopotentiometry –5 mA/30mn (sample2) and of the untreated extract (control).

**Table 3.** Monitoring of the brown index (Yellow/Red colour ratio) of samples treated with a potential/time pair E1 (–125 mV/30mn) and a current/time pair E2 (–5 mA/30mn) and the untreated control stored at 4°C, 30°C and 37°C for 5 months.

		Colour ratio Yellow/Red (BI)		
		1 month	4 months	5 months
4°C	Sample1	0.62 <sup>a</sup>	0.63 <sup>a</sup>	0.63 <sup>a</sup>
	Sample2	0.59 <sup>a</sup>	0.63 <sup>a</sup>	0.62 <sup>a</sup>
	Control	0.62 <sup>a</sup>	0.63 <sup>a</sup>	0.65 <sup>a</sup>
25°C	Sample1	0.6 <sup>a</sup>	0.79 <sup>b</sup>	
	Sample2	0.63 <sup>a</sup>	0.77 <sup>b</sup>	
	Control	0.64 <sup>a</sup>	0.81 <sup>b</sup>	
37°C	Sample1	0.72 <sup>a</sup>	1.03 <sup>c</sup>	1.05 <sup>c</sup>
	Sample2	0.72 <sup>a</sup>	1.03 <sup>c</sup>	1.04 <sup>c</sup>
	Control	0.77 <sup>b</sup>	1.02 <sup>c</sup>	1.14 <sup>d</sup>

However, at 37°C, there is a significant difference between the sample and the control and the juice also browns significantly between the first and fourth month.

From these results on the brown index, it seems that the degrading effect of oxygen in *Hibiscus sabdariffa* juice is catalysed by a certain temperature (37°C).

The results of **Table 4** on the violet index corroborate those obtained with the monitoring of the brown index. Indeed, the indices increase with time and temperature, which translates into a browning of the product.

The colour indices also show that the two methods, chronoamperometry for sample1 (S1) and chronopotentiometry for sample2 (S2) are equivalent. The juice treated with one of the previous methods (Chronoamperometry or Chronopotentiometry) presents smaller indices significantly different from those of the control at 37°C.

**Table 4.** Monitoring of the violet index (Blue/Red colour ratio) of samples treated with a La **Figure 7** présente les suivis de la concentration des anthocyanes de l'extrait électro réduit et du témoin au bout de 5 mois de stockage à 4 °C, 30 °C et 37 °C.

		Colour ratio Bleu/Red (PI)		
		1 month	4 months	5 months
4 °C	Sample1	0.28 <sup>a</sup>	0.32 <sup>b</sup>	0.33 <sup>b</sup>
	Sample2	0.28 <sup>a</sup>	0.33 <sup>b</sup>	0.32 <sup>b</sup>
	Control	0.29 <sup>a</sup>	0.33 <sup>b</sup>	0.33 <sup>b</sup>
25 °C	Sample1	0.33 <sup>b</sup>	0.45 <sup>c</sup>	
	Sample2	0.36 <sup>a</sup>	0.48 <sup>c</sup>	
	Control	0.35 <sup>ab</sup>	0.47 <sup>c</sup>	
37 °C	Sample1	0.43 <sup>a</sup>	0.61 <sup>c</sup>	0.59 <sup>c</sup>
	Sample2	0.44 <sup>a</sup>	0.61 <sup>c</sup>	0.6 <sup>c</sup>
	Control	0.48 <sup>b</sup>	0.61 <sup>c</sup>	0.64 <sup>d</sup>

#### 4. Conclusions

Generally speaking, the monitoring of anthocyanins and colour clearly showed that the electrochemical approach contributes to better conservation of anthocyanins in *Hibiscus sabdariffa* L.

Furthermore, the imposition of the intensity of the reduction peak for a specific time is equivalent to the application of the peak potential for the same duration. Both methods allow a reduction of dissolved oxygen for a better preservation of the juice with a considerable difference of more than 10% at 25 °C and 37 °C between the sample and the control.

The electrochemical reduction of dissolved oxygen in *Hibiscus sabdariffa* L. juice is significant for the ratios 1/5 and 1/15, with a notable reduction in anthocyanin losses of 5% constant from the first to the fifth month of storage at 4 °C.

The red colour coordinate a\* as well as the brown and violet colour indices corroborated the results on anthocyanin monitoring.

#### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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