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Comparative Effect of Adding Synthetic Citric Acid to Natural Lemon Juice (*Citrus aurantiifolia*) on the Stability of *Hibiscus* Drinks Stored at 4˚C and 37˚C

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Abstract

The general objective of the work is to compare the effect of the addition of synthetic citric acid compared to the addition of natural fruit juice of *Citrus aurantiifolia* on the conservation of drink based on red chalice *H. sabdariffa*. The tests were carried out over a period of 5 weeks at 4˚C and 37˚C with seven batches of beverage samples prepared at the rate of a calyx/water ratio of 1/40 ($kg \cdot kg^{-1}$) and added respectively citric acid at 1, 2 and 4 $g \cdot L^{-1}$ and lemon juice at 10, 20 and 40 $mL \cdot L^{-1}$. The characterization of the different batches of beverages was carried out from day one. A follow-up of the residual anthocyanin content and the intensity of the red coloring were carried out over five weeks. The anthocyanin concentration was determined by the pH-differential method. The red color degradation index is determined based on the *CIELAB* color system (L*, a*, b* and L*). R and Minitab 18 software was used for data processing. The results of the monitoring of the parameters showed that the concentration of 1 g AC L^{-1} retains 2.7 mg more of the anthocyanins than adding 10 mL JC L^{-1} and longer maintains red color when stored at 4˚C and 37˚C/5 weeks. The 2 g AC L^{-1} and 4 g AC L^{-1} ratios have the same effects as the addition of 20 and 40 mL of lemon juice, all accelerating the degradation of anthocyanins and the red color. After 5 weeks of storage at 37˚C, the effect of the temperature combined with the increase in the acidity of the samples (2 to 4 g AC L^{-1} and 20 to 40 ml JC L^{-1}), have accelerated the total disappearance of the red color on all samples.

Keywords

Stabilization, Drink, Citric Acid, *Citrus aurantiifolia*, *Hibiscus sabdariffa*, Coloring

1. Introduction

Polyphenols of plant origin have been a major scientific field for many years with many fields of application. As such, *Hibiscus* polyphenols are very widely studied because they have many biological activities that are beneficial to human health [1]. It is unanimously recognized that color is a fundamental dimension for the drink made from the red chalices of *Hibiscus sabdariffa*. Color is an important attribute to increase the acceptability of food products [2]. Commonly called Bissap in Senegal, *Hibiscus sabdariffa* has been cultivated since the 19th century [3]. The drink made from its chalices is widely consumed in Senegal and West Africa. This allowed the emergence of the *Hibiscus sabdariffa* industry. This growth is facilitated by several factors such as the production potential and the presence of several support structures for the sector (State technical services, projects and programs, NGOs, etc.) [4]. Business opportunities are good, especially during Ramadan and hot weather. However, the Bissap-based product line faces a stability issue. The latter results in a degradation of the red color due to anthocyanins giving rise to brown polymers and residues of splitting reactions (aldehydes, gallic acid, etc.) [5]. Anthocyanins are pigments of plant origin expressing bright colors ranging from red to blue [6]. The stability of anthocyanins and red coloration is determined by the degree of oxidation, temperature, ionic strength, acidity, and interaction with other radicals and complex molecules [7]. When formulating this drink, the manufacturers add 4 g of citric acid or 40 ml of *lemon juice* per liter of drink. Pouget announces that the stability of anthocyanins in solution is improved by adding sulphite or metabisulphite [8]. Anti-oxygens are compounds capable of retarding oxidation by indirect mechanisms such as the complexation of metal ions or the reduction of oxygen [9]. Citric acid, which has properties of being strongly reducing, would it contribute to better preservation of Bissap-based products?

In this work the addition of synthetic citric acid powder will be compared with the natural fruit juice of *Citrus aurantiifolia* that has various proportions added during the formulation of drinks. The main objective of this work is to compare the effects of synthetic citric acid, and the citric acid of the natural juice of the fruit of *Citrus aurantiifolia* on the preservation of beverages based on aqueous extract of *Hibiscus sabdariffa*-L stored at 4˚C and 37˚C. The tests will be carried out with seven batches of samples of drinks prepared at the rate of a calyx/water ratio of 1/40 ($kg \cdot kg^{-1}$) and respectively added citric acid 1g L^{-1}, 2 g L^{-1} and 4 g L^{-1} and lemon juice 10 mL L^{-1}, 20 mL $L^{-1}$and 40 mL L^{-1}. Color parameters and residual anthocyanin concentration will be monitored weekly over a period

of five weeks.

The exploitation of the results will allow industrialists to apply the best ratios or optimal doses allowing the best preservation of the red color and anthocyanins in drinks made from *Hibiscus sadariffa*, which is very popular with the populations.

2. Material and Methods

2.1. Material

The stabilization tests were carried out with the powder of red calyxes of the horticultural variety known as Vimto (Figure 1 and Figure 2). Citric acid is supplied by PROLABO as the laboratory reagent (Figure 3). The fruit of *Citrus aurantiifolia* (Figure 4) is bought on the market (***Castor***) and the juice is obtained by pressing followed by double filtration.

Figure 1. Red calyx *H. sabdariffa.*

Figure 2. Red calyx powder.

Figure 3. Synthetic citric acid.

Figure 4. Fruit of *Citrus aurantiifolia.*

2.2. Methods

2.2.1. Beverage Production

Calyxpowder of *H. sabdariffa* is obtained using an electric grinder (Thermomix Vorwerk, France). The dried chalices are crushed and sieved then macerated in demineralized water at the rate of a chalices/water ratio of 1/40 ($kg \cdot kg^{-1}$). Manual stirring is carried out every 10 minutes for 1 hour. The final extract is filtered twice and then divided into 7 batches: 3 batches to which lemon juice is added respectively at the rate of 10, 20 and 40 $ml \cdot L^{-1}$, 3 batches containing citric acid at the rate of 1, 2 and 4 $g \cdot L^{-1}$ and a control batch (**Figure 5**). Drinks at 15˚ Brix are prepared with the addition of cold sucrose sugar until completely dissolved.

2.2.2. Pasteurization and Storage

The beverages are characterized (**Table 1**) for each batch, filled in bottles disinfected with bleach diluted at 100 mg kg^{-1} for thirty minutes, drained and dried in an oven. After filling and cold capping, pasteurization is carried out for each

Figure 5. Beverage batches with varying matrix concentrations.

Figure 6. Pasteurization of samples.

Table 1. Physico-chemical characterization of beverages on the first day before storage at 4˚C and 37˚C over 5 weeks.

Settings	Anthocyanin concentration mg/L	Conductivities in µs/cm 25˚C	pH at 25˚C	Brix at 25˚C g/100g
Control Drink	$113.86^{a} \pm 1.67$	$2255.33^{f} \pm 0.57$	$2.79^{a} \pm 0.01$	$14.97^{cd} \pm 0.05$
Beverage + 1 g AC/L	$115.49^{a} \pm 1.38$	$2307.33^{e} \pm 2.08$	$2.53^{c} \pm 0.00$	$15.10^{bc} \pm 0.07$
Beverage + 2 g AC/L	$113.42^{a} \pm 0.56$	$2351.33^{c} \pm 1.15$	$2.15^{e} \pm 0.01$	$15.22^{ab} \pm 0.02$
Beverage + 4 g AC/L	$114.17^{a} \pm 1.07$	$2414.00^{a} \pm 1.00$	$1.98^{g} \pm 0.01$	$15.29^{a} \pm 0.01$
Drink + 10 ml Juice C/L	$114.36^{a} \pm 0.74$	$2202.33^{g} \pm 0.057$	$2.62^{b} \pm 0.00$	$15.87^{de} \pm 0.07$
Drink + 20 ml Juice C/L	$114.26^{a} \pm 0.41$	$2317.33^{d} \pm 0.057$	$2.33^{d} \pm 0.01$	$15.86^{de} \pm 0.01$
Beverage + 40 ml Juice C/L	$114.17^{a} \pm 0.19$	$2364.66^{b} \pm 0.057$	$2.09^{f} \pm 0.01$	$14.81^{e} \pm 0.01$

AC = Citric acid powder, Juice C = Lemon juice (*Citrus aurantiifolia*).

batch in a water bath at the pasteurizing value (VP 50) of 75˚C/30min (Figure 6), cooling is carried out with cold water. The samples are thus ambered with aluminum foil (Figure 7) and stored at 4˚C and 37˚C.

Analyzes are carried out every week over a period of two months in order to monitor the physicochemical parameters and the evolution of the red coloring of the different batches of the drink.

2.2.3. Dosage of Anthocyanins

The principle is based on the modification of the coloration of anthocyanins as a function of pH (pH-differential method) according to the prescriptions of Wrolstad [10]. After dilution in two buffer solutions at pH 1.0 and pH 4.5, the absorbance is measured with a UV spectrophotometer (Speccord 200 plus, Germany) at 510 and at 700 nm.

2.2.4. Color Determination

The color of the nectar samples was measured using a colorimeter (type: KONICA MINOLTA. Japan) based on the CIELAB color system (L*, a*, b* and L*, C*, h, YI). The color parameters (L*, a*, b* and L*, C*, h, YI) were measured 3 times for each sample. L*, a*, b* describe the colors Black-White, Green-Red and Blue-Yellow respectively: L* (0 = Black, 100 = White); a* (− a = Green, + a = Red); b* (− b = Blue, + b = Yellow) Figure 8. The yellowness index (YI) indicates the degree of yellowness [11].

Figure 7. Amber samples of aluminum foil.

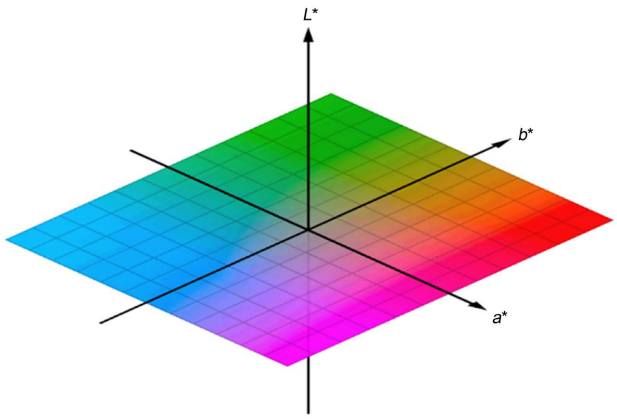

Figure 8. Illustration of color parameters (a, b, L) [11].

2.2.5. Conductivity Measurement

The conductivity is measured with a conductivity meter (Hanna instruments, Germany) at 25˚C incorporating pH measurement. Conductivity measurement is a quick, simple and inexpensive way to determine the ionic strength of a solution. It also reflects the stability of anthocyanins by the note of the flavylium cation. It will be all the higher as the quantity of ions will be important.

2.2.6. Determination of Brix (Total Soluble Solids)

Brix is defined as the concentration of soluble solids in an aqueous solution. This concentration measured at 25˚C. By the refractive index is then expressed by the percentage by mass (g/100g), is measured according to a standardized method (NA 5669) using a universal refractometer. Abbe ATAGO type refractometer with digital reader and temperature correction.

2.2.7. Statistical Analyzes

Statistical analyzes were performed using one-way ANOVA with R software version 3.2.4 Revised (2018-03-16, R-70336) and Minitab software18. The X value of each sample is assigned a superscript letter ($X^{(i)}$ where i = a, b, c,). Samples with the same letter are not statistically different at the 5% level.

3. Results and Discussion

3.1. Characterization of Beverage Batches

The results of analyzes of the different batches of beverage samples on the first day are recorded in Table 1.

The results reveal, compared to the control batch, significant differences in the pH, the Brix and the conductivity from the addition of the matrices on the first day. ANOVA does not show significant differences in anthocyanin concentration. The Brix increases proportionally with the addition of citric acid powder in beverage batches. The latter increases the number of soluble substances of the products obtained and increases the Brix from 15.10 ± 0.07 g/100g for the drink with 1 g AC L^{-1} to 15.29 ± 0.01 g/100g for the drink with the addition of 4 g of citric acid. The conductivity values on the beverage batches increase proportionally with the addition of citric acid. The conductivity of the drink batches changes from 2255.33 ± 0.57 µs/cm to 2414.00 ± 1.00 µs/cm at 25˚C. The addition of citric acid and lemon juice lowers the pH in all beverage batches. Indeed, the concentration of citric acid in lemon is very high and can reach 8% of the dry mass, or about 47 g L^{-1} [12]. This addition on day one contributes significantly to lowering the pH of beverage samples. The pH drops from 2.79 ± 0.01 to 2.09 ± 0.01 as soon as 40 mL of Jus C L^{-1}, and 1.98 ± 0.01 with the addition of 4 g AC L^{-1}.

3.1.1. Monitoring of Anthocyanin Concentration during Storage at 4˚C and 37˚C

Figures 9-11 show the evolution of the anthocyanin concentration during the storage of beverage batches for 5 weeks at 4˚C and 37˚C.

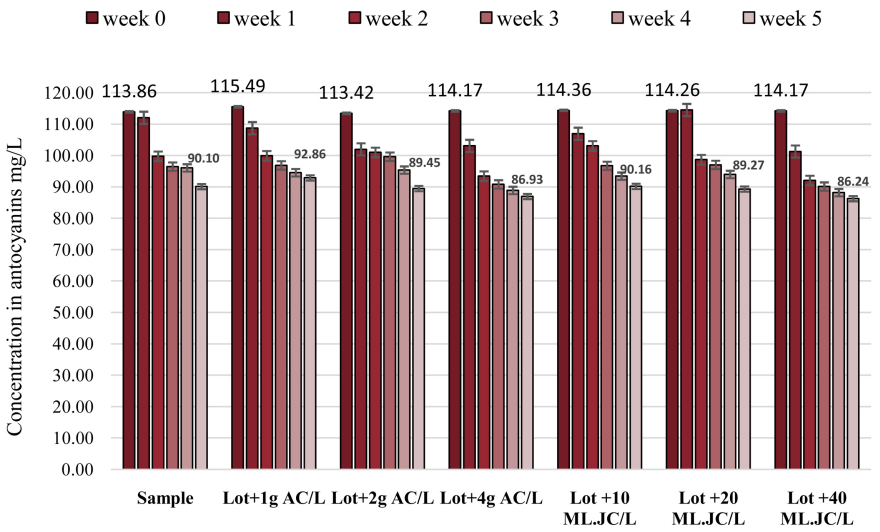

Figure 9. Evolution of anthocyanin concentration during storage at 4˚C/5 weeks.

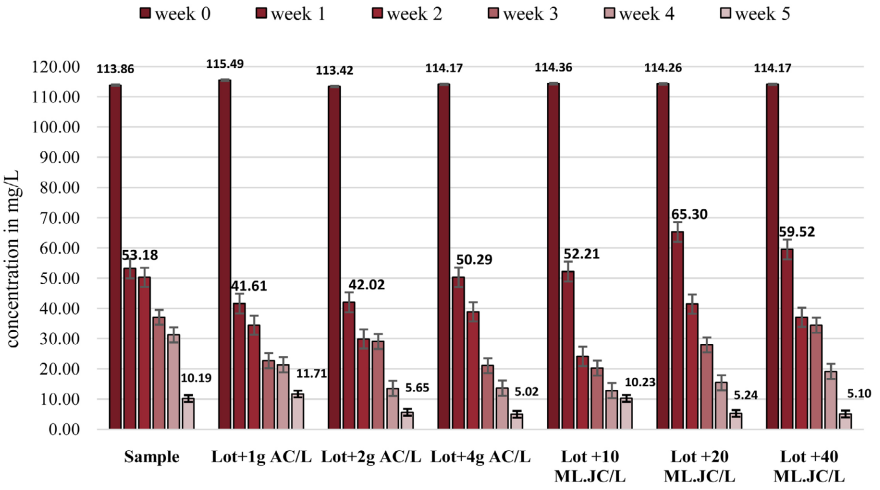

Figure 10. Evolution of anthocyanin concentration during storage at 37˚C/5 weeks.

The results show a very rapid degradation of anthocyanins from the first week of storage at 37˚C (**Figure 10**) compared to batches stored at 4˚C (**Figure 9**). Batches with 1 g AC L^{-1} retain 92.86 mg·L^{-1} of anthocyanins compared to batches with 10 ml JC L^{-1} which retain 90.26 mg·L^{-1}. The samples with the addition of 2 g AC L^{-1} corresponding to 20 mL JC L^{-1} have the same effects and retain 89.27 ± 0.44 mg·L^{-1} of anthocyanins at 4˚C. The respective addition of 4 g AC L^{-1} and 40 mL JC L^{-1} have the same effects and allow only 86.45 ± 0.34 mg·L^{-1} of anthocyanins to be preserved in beverages after five weeks of storage at 4˚C. During storage at 37˚C, the batches with the addition of 1 g AC L^{-1} retained 11.71 mg·L^{-1} of the anthocyanin concentration against 10.23 mg·L^{-1} for the batches with the addition of 10 ml JC L^{-1}. The residual concentration of anthocyanins after five weeks of storage at 37˚C drops to 5.34 ± 0.45 mg·L^{-1} onaverage for the samples with the addition of 2 and 4 g AC L^{-1} and 20 and 40 ml JC L^{-1}. The temperature of 37˚C accelerated all the anthocyanin degradation reactions, confirming the

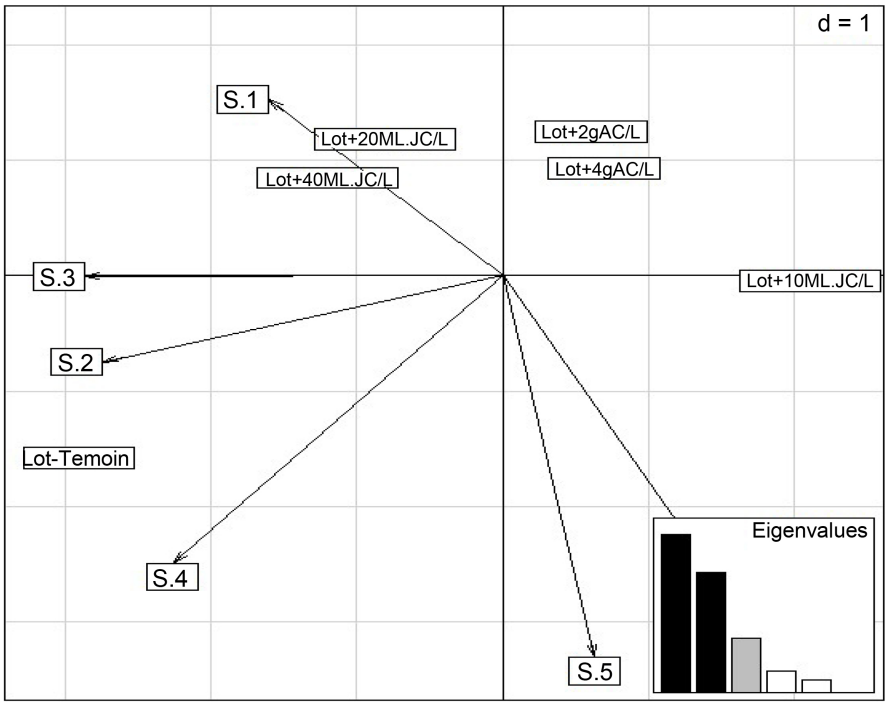

Figure 11. Weeks of retention correlated with added ratios.

work of several authors [13] [14]. The effect of temperature masked the effect of citric acid and lemon juice on the different samples stored at 37˚C. Indeed, raising the storage temperature from 4 to 37˚C combined with a pH of 2 favors the loss by hydrolysis of the glycosyl groups of the anthocyanins. This leads to their degradation since the aglycone forms are less stable than their glycosylated forms [15]. After 5 weeks of storage at 37˚C, only the 1 g AC L^{-1} batches show a degradation rate of less than 90% (**Figure 8**). This degradation increases with the addition of lemon juice. Lemon juice at high concentration contributes to the degradation of anthocyanins in batches of beverages stored at 37˚C. This same tendency is observed during storage at 4˚C with the addition of citric acid powder and addition of lemon juice. During storage at 4˚C, the batches with the addition of 1 g AC L^{-1} show the lowest anthocyanin degradation rates after 5 weeks of storage (18.80%) compared to the control batches (20.86%). Cisse *et al*., (2012) studied anthocyanin losses in extracts of *Hibiscus sabdariffa* during heat treatment and during storage [16]. During pasteurization at 90˚C for 5 min, they observed anthocyanin losses of 4%, which confirms the effect of high temperature on the degradation of anthocyanins. Sugar breakdown products are known to decrease the stability of anthocyanins [17]. In a study by Daravingas and Cain (1968), all sugars tested (sucrose, fructose, glucose, and xylose) increased the rate of anthocyanin degradation in a model medium at pH 3 after one month of storage at 50˚C [18]. After one week (W1) storage at 37˚C, the respective addition of 20 and 40 ml JC L^{-1} retained more anthocyanins (**Figure 11**). Weeks two and three (W_2, W_3 and W_4) remain favorable to the control which better preserves the anthocyanins and the red color in the batches of the control drink. Week five

(W_5) remains favorable to the batch with the addition of 1 g AC L^{-1} (Figure 11).

3.1.2. Monitoring of Changes in Color Intensity during Storage at 4 and 37˚C

Figure 12 and Figure 13 show the evolution of the intensity of the red color during the storage of the beverage batches for 5 weeks at 4˚C and 37˚C.

The results show that the drop in the intensity of the red color given by the color parameter (a+), remains very low during storage at 4˚C. For the control samples the value of a+ drops from 67.90 ± 0.42 to 65.07 ± 0.33. The samples with the addition of 1 g AC L^{-1} retained more of the red color with a+ = 66.11 in the 5th week. The same effects are observed for batches of samples with the addition of 10 mL of lemon juice stored at 4˚C with a+ values on average of 65.12 ± 0.80 (Figure 12). The drop in storage temperature slowed down the degradation of the red color of the sample batches of drink made from the red calyces of *Hibiscus sabdariffa*, thus confirming the work of Cissé [19].

Figure 13 shows a significant decrease in red color intensity is obtained with storage at 37˚C for 5 weeks on all samples. The red color parameter (a+) drops

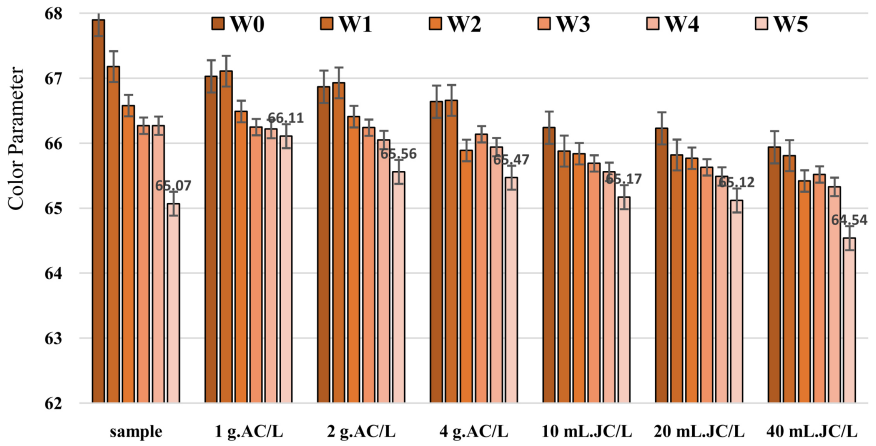

Figure 12. Evolution of the intensity of the red color (a+) during storage at 4˚C/5 weeks.

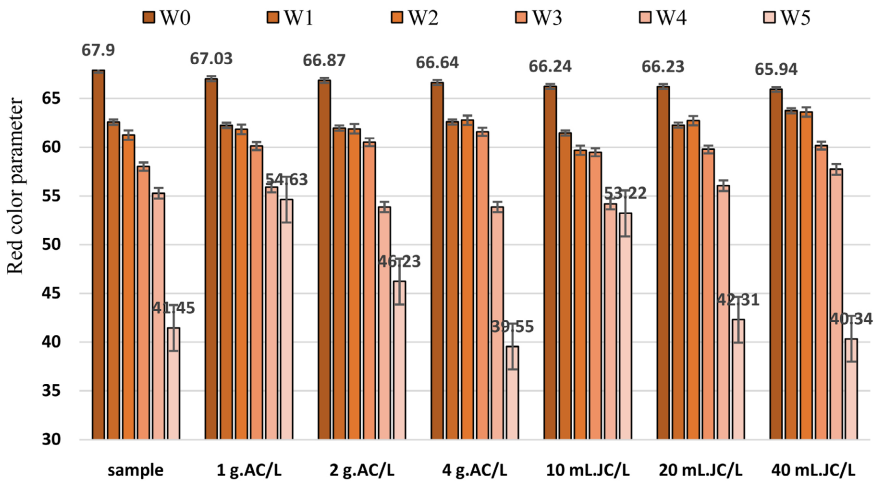

Figure 13. Evolution of the intensity of the red color (a+) during storage at 37˚C/5 weeks.

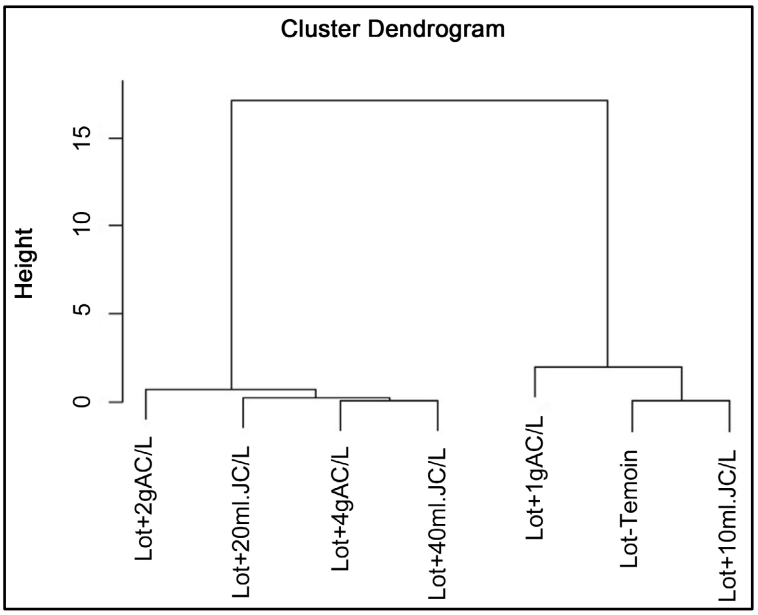

Figure 14. Classification of added matrices according to effects on anthocyanin concentration and final red color.

from 67.90 ± 0.42 to 38.43 ± 0.32 for the control sample lots. The smallest drop is obtained with the sample at 1 g AC L^{-1} (67.0 ± 0.52 to 54.63 ± 0.47).

Raising the storage temperature to 37˚C, combined with the acidity and the presence of sucrose sugar in the drinks, promotes the acceleration of the red color degradation reactions. Nebesky confirms that the presence of oxygen and high temperature is the most favorable combination for the deterioration of the color of various fruit juices and anthocyanins [20]. The oxygen associated with ascorbic acid is detrimental to the anthocyanin stability of cranberry juice [21]. The deleterious effect of oxygen on anthocyanins can take place through the mechanism of oxidation in which the oxidized components of the medium react with the anthocyanins, giving rise to colorless or brown products [22] [23] [24] [25]. Among the typical degradation products of sugar, furfural accelerates the degradation of anthocyanins [17]. The reactions of anthocyanins with these degradation products of sugars and ascorbic acid lead to the formation of polymers and brown pigments [17].

Figure 14 summarizes the classification of the added matrices according to the effects on the concentration of anthocyanins and the final red color of the samples studied.

The 1 g AC L^{-1} ratio has the best effect on color and anthocyanin preservation after five weeks of storage at 37˚C. The concentration of 10 ml JC L^{-1} has the same effects as the control batch. The 2 to 4 g AC L^{-1} and 20 to 40 ml JC L^{-1} ratios have the same effects on the preservation of drinks.

4. Conclusion

Citrus aurantiifolia fruit juice on the stability of drinks based on *Hibiscus sabda-*

riffa-L. The results showed that the concentration of 1 g AC L^{-1} slowed down the degradation of anthocyanins and the reduction in the red color of the samples during storage at 4˚C and 37˚C for 5 weeks followed by the addition of 10 ml JC L^{-1}. The 2 to 4 g AC L^{-1} and 20 to 40 ml JC L^{-1} ratios have the same effects on the preservation of bissap-based drinks. The increase in acid concentration combined with the rise in storage temperature accelerated the disappearance of the red color of the samples adding 2 to 4 g AC L^{-1} and 20 to 40 ml Jus C L^{-1}, which limits the shelf life of samples at 37˚C to five weeks. On the other hand, products stored at 4˚C can exceed 5 weeks of storage given their high anthocyanin concentration. Today, transformation and stabilization processes could use the 1 g AC L^{-1} ratio or the addition of 10 ml JC L^{-1} for the storage at 4˚C of beverages and avoid ratios 2 to 4 g AC L^{-1} and 20 to 40 ml Jus C L^{-1} which accelerate the degradation of red color and anthocyanins in drinks based on *Hibiscus Sabdariffa*.

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Conflicts of Interest

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

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