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Hibiscus sabdariffa anthocyan stabilization tests by co-maceration of crushed calices with powder of Saba senegalensis fruit bark and combretum Micranthum leaf

Papa Guedel Faye[1,2+]
Khadim Niane[1]
Lahat Niang[1]
Samba Balde[1]
Oumar Ibn Khatab Cisse[2,3]
Alioune Sow[2,4]
Nicolas Cyrille Ayessou[1,2]
Mady Cisse[1,2]

[1]*Cheikh Anta Diop University, BP 5080 Dakar Fann, Polytechnic School of Dakar, Chemical Engineering and Applied Biology Department, Water, Energy, Environment and Industrial Processes Laboratory, Senegal.*
[1]*Email: gti.khadim@gmail.com*
[1]*Email: lahatniang5@gmail.com*
[1]*Email: balsamba2013@yahoo.fr*
[2]*Center for Studies on Food Security and Functional Molecules, Dakar, Senegal.*
[1,2]*Email: papaguedel.faye@esp.sn*
[1,2]*Email: nicolas.ayessou@ucad.edu.sn*
[1,2]*Email: mady.cisse@ucad.edu.sn*
[3]*Iba Der THIAM University, National School of Agriculture BP A296 Thiès, Senegal.*
[2,3]*Email: omaribn.cisse@univ-thies.sn*
[4]*Gaston BERGER University, UFR Agronomic Sciences, Aquaculture and Agrifood Technologies, Saint Louis, Senegal.*
[2,+]*Email: alioune.sow@ugb.edu.sn*


(+ Corresponding author)

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ABSTRACT

Tannins can interact with anthocyanins to form complexes that stabilize color and protect anthocyanins from degradation. Thus, this work proposes to study the effect of the tannins of bark of the fruit of *Saba Senegalensis* and *Combretum Micranthum* on the stability of the anthocyanins of drink based on red calyxes of *hibiscus* obtained by co-maceration. The anthocyanin concentration was determined by the pH-differential method. The red color degradation index is determined based on the CIELAB (International Commission of Lighting) color system (L*, a*, b* and L*). R and *Minitab 18* software were used for data processing. Monitoring of the residual anthocyanin content and the intensity of the red color was carried out over eight weeks. The results showed that *Saba Senegalensis* tannins interact better with anthocyanins. After eight weeks of storage at 4°C, 89.36 % of the anthocyanins are preserved with the *Saba S* tannins against 83.62 % for the control batches and 80.38 % for the *Combretum* tannins. Storage at 37°C for eight weeks reveals very high loss rates, 14.86 % of the anthocyanins are preserved (Saba *tannins)* against 3.38 % for the control and 1.84 % with the *Combretum tannins*. Moreover, the intensity of the red color is better preserved at 4 °C and 37 °C with the tannins of *Saba Senegalensis*.

Contribution/Originality: New source of tannins: The use of *Saba senegalensis* and *Comretum microanthum* as sources of tannins could be novel, as these plants may be widely available in some regions and could be effective as natural alternatives to synthetic tannins.

1. INTRODUCTION

Both anthocyanins and tannins are phenolic compounds found in many fruits, vegetables, plants, and beverages, and their interaction can influence the stability, color, taste, and antioxidant activity of food products [1]. *Saba*

Senegalensis, also known as "sabalé", is a tree native to West Africa, particularly Senegal. Different parts of the plant including bark, leaves, fruits and roots are used for medicinal and food purposes. *S aba Senegalensis* is rich in phenolic compounds such as flavonoids and tannins [2]. These compounds have antioxidant and anti-inflammatory properties [2]. Kinkeliba, scientifically known as *Combretum Micranthum*, is a plant native to West Africa, widely used in traditional medicine and as a refreshing drink [3]. Kinkeliba *is* rich in flavonoids such as *quercetin*, kaempferol and luteolein [4]. Flavonoids are powerful antioxidants and may have anti-inflammatory, antiviral, and antidiabetic properties [5]. Tannins are phenolic compounds present in Kinkeliba, which may contribute to its astringent and antioxidant properties [4]. *Hibiscus sabdariffa* is a plant that belongs to the *Malvaceae* family [5]. It is commonly known by different names such as roselle, sorrel of Guinea, or even karkadé. Native to West Africa, it is also cultivated in other parts of the world for its culinary, medicinal and ornamental uses [6].

The most commonly used part of *Hibiscus Sabdariffa* is the flower calyx, which is bright red and fleshy. The calyx is harvested and dried to be used in the preparation of drinks, in particular infusions and herbal teas. It is known for its tart and refreshing taste [7, 8].

Chemically, *Hibiscus Sabdariffa* contains various bioactive compounds, including anthocyanins, organic acids (like citric acid and malic acid), flavonoids, polysaccharides, and vitamins [9]. Anthocyanins, in particular, are responsible for the characteristic red color of the calyx and exhibit antioxidant properties.

The combination of anthocyanins and tannins can have a significant impact on the sensory characteristics and chemical properties of foods and beverages. The objective of his work is to reveal the effect of tannins in the bark of the fruit of *Saba Senegalensis* and *Combretum Micranthum* on the stability of anthocyanins in beverages made from red calyxes of *hibiscus*. The tests will be carried out with batches of samples of beverages prepared at the rate of a chalices/water ratio of 1/40 ($kg.kg^{-1}$). Monitoring of color parameters and residual anthocyanin concentration will be done every week over a period of eight weeks.

2. MATERIAL AND METHODS

2.1. Material

The stabilization tests were carried out with the powder of red chalices Figure 1 (powder Figure 2) of the horticultural variety known as Vimto. The *Saba Senegalensis fruit* comes from the Ziguinchor region of Senegal harvested in 2022 Figure 3 and bark powder fruits of Saba.S (Figure 4). The dried leaves of *Combretum Micranthum* come from the Thies region of Senegal harvested in 2022 Figure 5.

Figure 1. Red chalice.

Figure 2. Red chalice powder.

Figure 3. Fruits of Saba senegalensis.

Figure 4. Bark powder fruits of Saba.S.

Figure 5. Leaves of combretum micranthum.

2.2. Methods

2.2.1. Manufacture of Powder from the Calyx and Peel of the Fruit of Saba Senegalensis

The red chalices as well as the bark of *Saba Senegalensis* are first sorted and washed then dried in an oven (70°C/4 hours). A grinding and sieving follows to have the powders of the two products (Figure 6, 7).

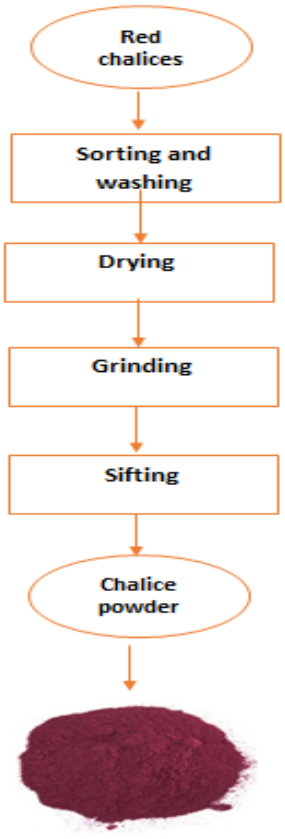

Figure 6. Making red chalice powder.

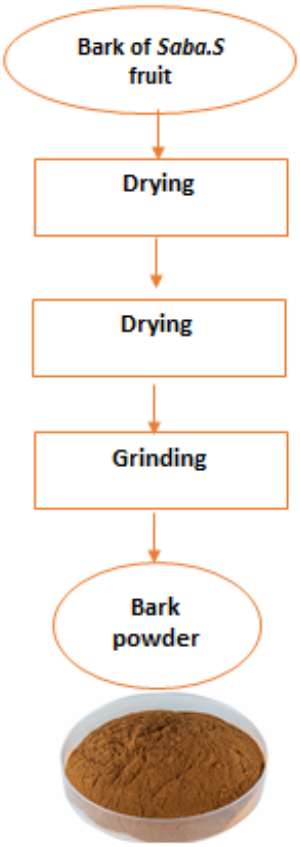

Figure 7. Manufacture of *S. Senegalensis* bark powder.

2.2.2. Beverage Production

The calyx powder of *H. Sabdariffa* is obtained using an electric grinder (Thermomix Vorwerk, France). The dried leaves are crushed and sieved then macerated in demineralised water at the rate of a calyx/water ratio of 1/40 ($kg.kg^{-1}$) with 25 g of crushed leaves of *Combretum Micranthum* and another preparation with 25 g *Saba. S* fruit husk powder. Manual stirring is carried out every 10 minutes for 1 hour. The final extract is recovered and filtered for each case.

2.2.3. Pasteurization and Storage

The drinks are packaged in bottles disinfected with bleach diluted at 100 ppm for thirty minutes, drained and dried in the oven. After filling and cold capping, pasteurization is carried out for each batch with the scale of 75°C/30 min.

Analyzes are carried out every week over a period of two months in order to monitor the physico-chemical parameters and the evolution of the red color due to anthocyanins.

2.2.4. 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, et al. [10]. After dilution in two buffer solutions at pH 1.0 and pH 4.5. The absorbance is measured with a UV spectrophotometer (Spec cord 200 plus, Germany) at 510 and at 700 nm.

2.2.5. 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].

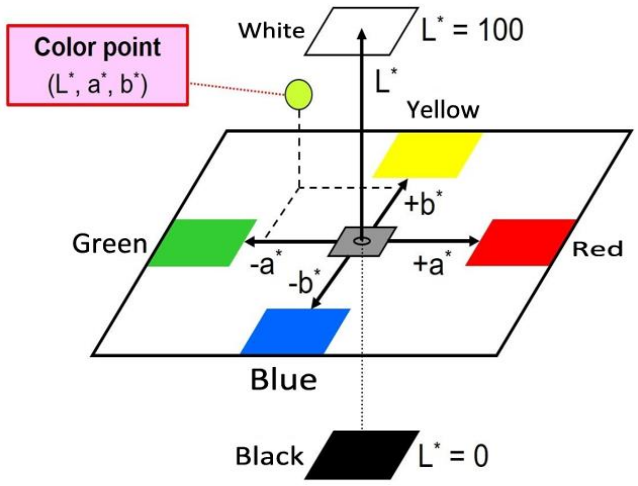

Figure 8. Illustration of color parameters (a, b, L).
Note: International Lighting Vocabulary [11].
L* indicates lightness, a* is the red/green coordinate, and b* is the yellow/blue coordinate.

2.2.6. Brix Determination

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/100 g), 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 (Analysis of variance) with R software version 3.2.4 Revised (2018-03-16, R-70336) and Minitab 18 software. 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. Monitoring of Anthocyanin Concentration during Storage at 4 °C and 37 °C

Figures 6 and 7 give the evolution of the anthocyanin concentration on the first day and during the storage of the beverage batches for 8 weeks at 4 °C and 37 °C. Figures 9 and 10 show the loss rates obtained at the first, fourth and eighth week of storage at 4 °C and 37 °C. There Figure 13 shows the correlations between the different anthocyanin concentrations in beverages at the first two dimensions of the PCA. Figure 14 presents the projection of beverage classes according to anthocyanin concentration on the PCA (Principal component analysis) factorial plane.

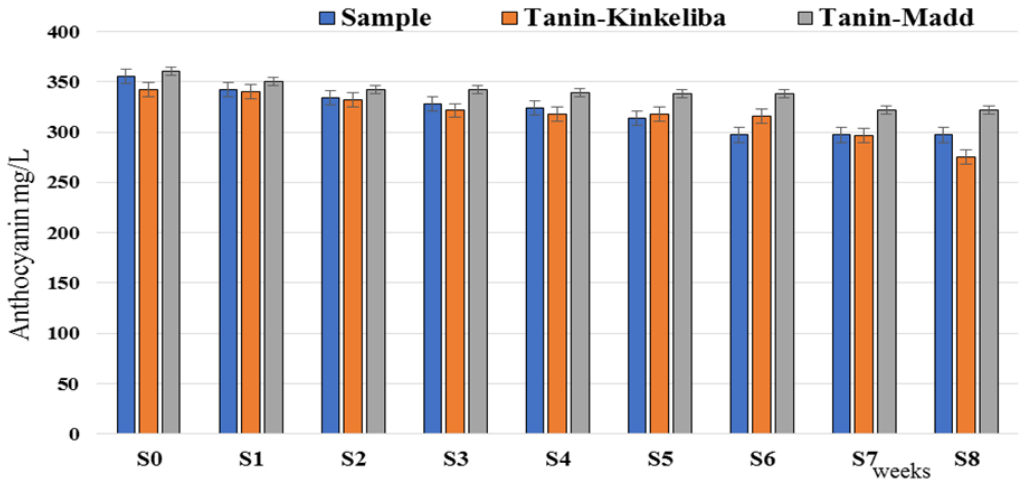

Figure 9. Evolution of anthocyanin concentration during storage at 4 °C/ 8 weeks.

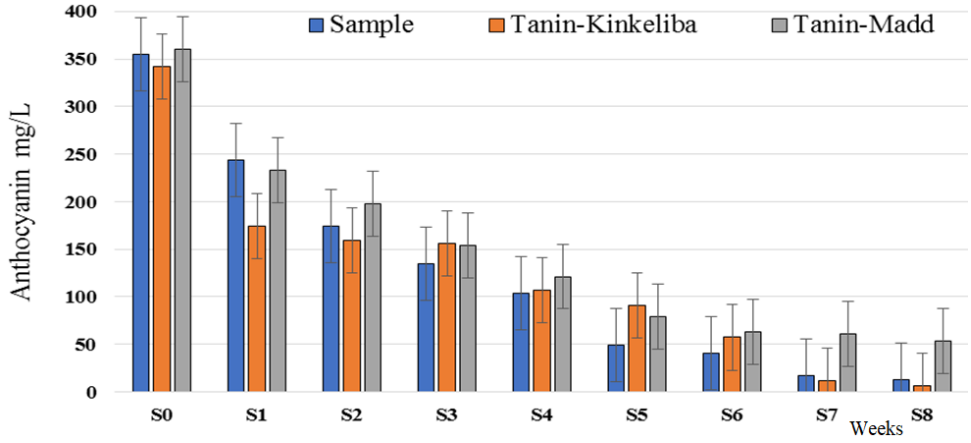

Figure 10. Change in abthocyanin concentration during storage at 37 °C.

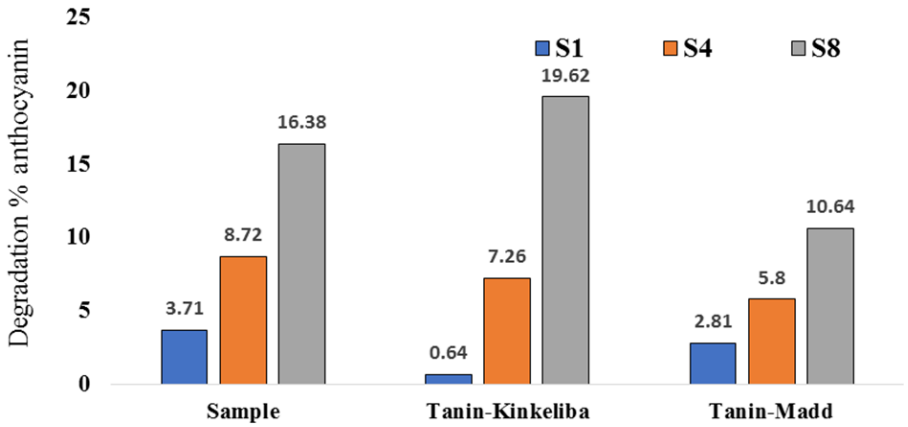

Figure 11. Evolution of the rate of degradation of anthocyanins in weeks 1,4 and 8 at 4 °C.

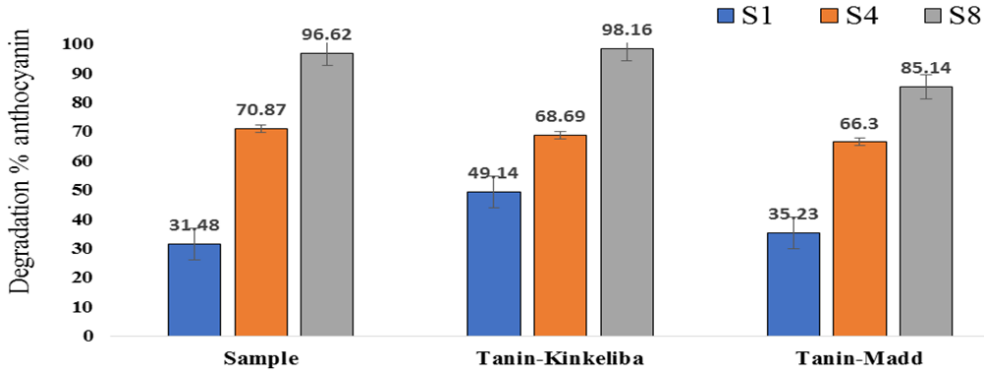

Figure 12. Evolution of the percentage of degradation of anthocyanins (Weeks 1,4 and 8) at 37 °C.

The results reveal that storage at low temperature (4 °C) slows down chemical reactions and anthocyanin degradation processes in beverages stored at 4 °C Figure 9. A loss of 16.38 % is observed with the batches of control beverages, 19.62 % for the *Combretum micranthum tannins* and 10.64 % for the *Saba Senegalensis tannins* over a period of eight weeks of storage Figure 11.

In contrast, storage at higher temperatures, such as 37 °C, results in faster degradation of anthocyanins. Heat conditions accelerate chemical reactions, oxidation and degradation of sensitive compounds. Thus significant loss rates are recorded respectively on the control batches (96.62 %), the batches with *Combretum* tannins (98.16 %) and the *Saba S* batches. (85.14 %) Figure 12. Anthocyanin losses are lower at 4 °C and 37 °C with *Saba Senegalensis* tannins. This justifies that tannins can interact with anthocyanins to form complexes, this interaction protects them from degradation caused by oxidation, light or other environmental factors [12, 13]. Tannins can vary widely in structure and molecular size. Different types of tannins may have different interactions with anthocyanins, which may influence their stability in varying ways [14-19]. The stability of anthocyanins can vary depending on several factors, such as pH, temperature, light, oxidation, interactions with other compounds, and storage time. The longer the storage time, the more the anthocyanins are likely to undergo degradation. However, the stability of anthocyanins can vary depending on their specific type and storage conditions [20-23]. Exposure to light, especially ultraviolet light (UV), can cause anthocyanins to break down. Storage in opaque containers or protected from light can help preserve their stability [24, 25].

It should be noted that the stability of anthocanins can also be influenced by other factors specific to each food or matrix, such as the presence of natural antioxidants, enzymatic compounds or matrix factors.

Figure 13 presents the correlations between the different anthocyanin concentrations of beverages in the first two dimensions of the PCA. The eigenvalue plot suggests that the first two axes altogether explain 100 % of the inertia. The first factorial plane (Dim 1) contains 57.72 % inertia and the second (Dim 2) 42.28 % inertia. The variables anthocyanin concentration 37 °C weeks 2, 4, 7 and 8 are strongly correlated and positively with the first

dimension (Madd tannins (*Saba Senegalensis*)). Dimension 2 is characterized by the variable anthocyanins 37 °C, weeks 3 and 5 which is positively correlated to it (*Combretum M* tannins) Figure 13.

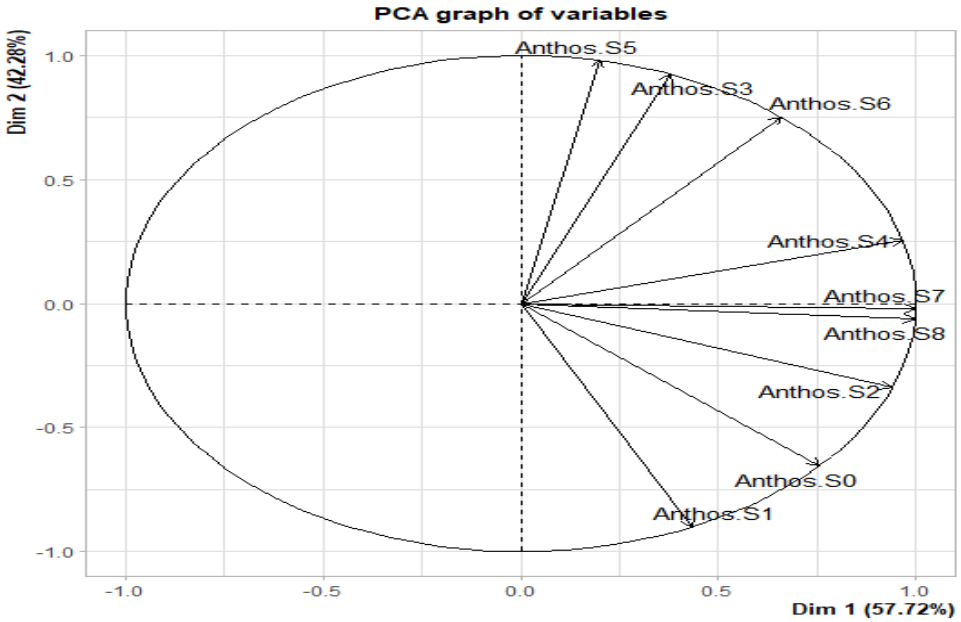

Figure 13. Correlation between the different anthocyanin concentrations of beverages in the first two dimensions of the PCA.

Figure 14 presents the projection of beverage classes according to anthocyanin concentration on the PCA factorial plane.

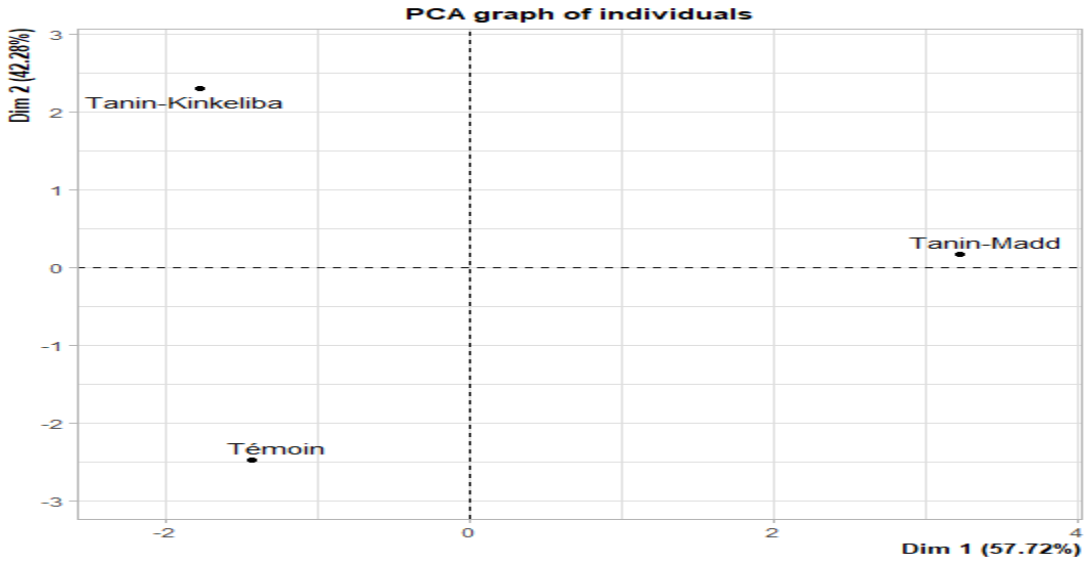

Figure 14. Projection of beverage classes according to anthocyanin concentration on the PCA factorial plane.

Beverage batches are grouped into three classes Figure 14. Class 1 is characterized by the control drink, the second class characterized by the drink with *Saba S* tannins and the class of drink batches with *Combretum .M* tannins.

3.2. Monitoring of Changes in Color Intensity during Storage for 60 Days at 4 °C and 37 °C

Figures 15 and 16 give the evolution of the coloring intensity of the color parameters (l, a, b) during the storage of the beverage batches for 60 days at 4 °C and 37 °C.

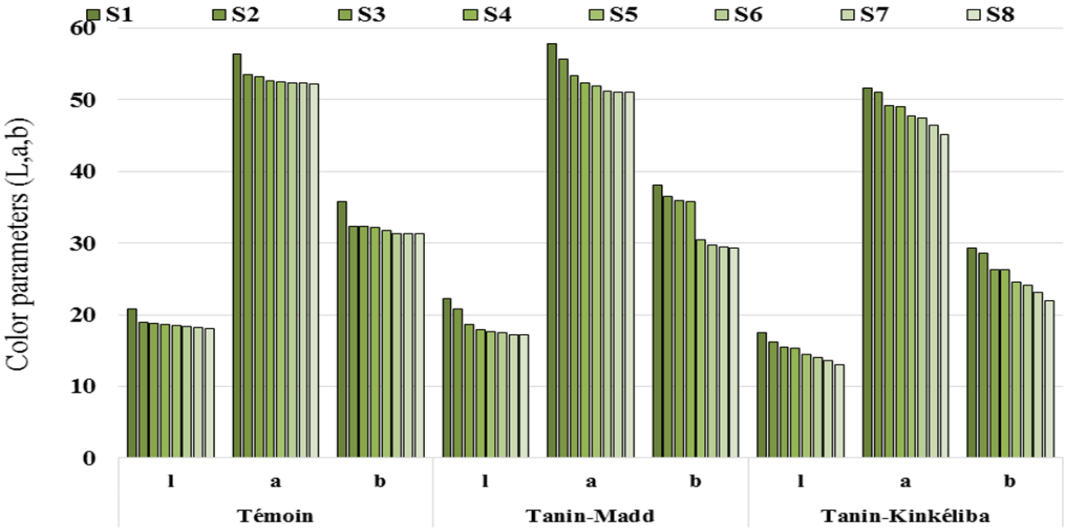

Figure 15. Evolution of color parameters (l,a,b) during storage at 4 °C/ 8 weeks.

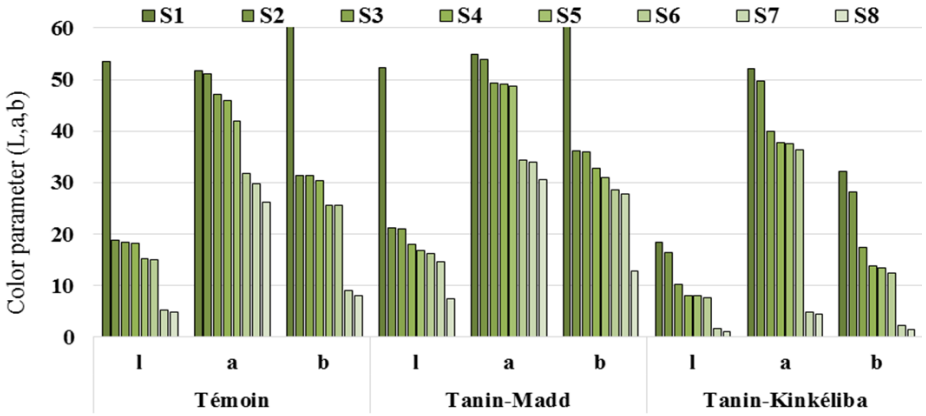

Figure 16. Evolution of color parameters (l,a,b) during storage at 37 °C/ 8 weeks.

The results show a drop in color parameters (L, a, b) during storage at 4 °C for eight weeks (Figure 15). This decrease is more accentuated with the storage temperature of 37 °C Figure 16. The color parameter (a) gives the intensity of the red coloration. This intensity decreases with the degradation of anthocyanins during storage. This degradation of anthocyanins is due to changes in the chemical groups present in the anthocyanin molecule [26-32]. Compounds, in addition to their role as cofactors in copigmentation, can be directly involved in the coloring of the medium via the formation of polymeric pigments with anthocyanins [33-35]. Some of these new pigments are more stable than monomeric anthocyanins with respect to nucleophilic attacks and other chemical modifications and thus play a major role in slowing down the color degradation of anthocyanin-based products [35].

4. CONCLUSION

Work on the stabilization tests of *hibiscus sabdariffa anthocyanins* by co-maceration of calyxes with the powder of bark of the fruit of *Saba Senegalensis* and leaf of *Combretum Micranthum* showed that the tannins of bark of the fruit of *Saba Senegalensis* have significant effects on slowing anthocyanin degradation and red color when stored at 4 and 37 °C. *Combretum* leaf tannins have no significant effect on anthocyanin red color stability and intensity. It should be noted that the interactions between tannins and anthocyanins can vary depending on many factors, such as the concentration of the compounds, the pH, the temperature and the other components present in solution. Tannins can protect anthocyanins from degradation due to oxidation or other chemical reactions. This can contribute to the color stability and longevity of food products containing anthocyanins.

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Competing Interests: The authors declare that they have no competing interests.
Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

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