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Stability of concentrated extracts of *Hibiscus sabdarifa L*. calyx during storage at different temperatures

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***Hibiscus sabdariffa* L. (Malvaceae) calyx is a valuable food resource in Africa. This herb is rich in phenolic compounds and a good source of natural antioxidants. Concentrated extract prepared with *Hibiscus sabdariffa* L. calyx is commonly used in traditional African and Asian diets and medicines. However, the organoleptic properties of these extracts are degraded during storage. Therefore, the stability of these products is one of the problems faced by roselle calyx extracts users. Stabilization of the *H. sabdariffa* extract to prevent or reduce quality changes and to extend shelf-life of these products is a challenge to food processors. In this study, strategies for minimizing or eliminating these alterations were evaluated. Concentrated extract of hibiscus calyx (60 °Brix) was divided into three parts: control, pasteurized and addition of potassium sorbate. The samples were stored at 4, 30 or 45°C for three months. Reducing sugars, pH, titratable acidity and total anthocyanins were determined during 0, 15, 30, 45, 60, 75 and 90 days of storage. The results showed that hibiscus calyx extracts can be stored at 4°C for three months without any changes in organoleptic properties. Pasteurization and addition of potassium sorbate did not improve significantly, the stability of the concentrated extracts during storage periods at high temperature. However, these treatments extended the shelf-life at cooling temperature (4°C) without serious changes.**

Key words: *Hibiscus sabdariffa* L., calyx extracts, stability, storage, temperature.

INTRODUCTION

Nowadays, food quality is no longer limited to organoleptic, nutritional and functional quality, but also entails that foods can be transported over long distances, stored and distributed without changes. Products must therefore have sufficient shelf-life to reach consumers in good quality. Thus, novel techniques have to be developed to increase the time to keep food products intact (Singh, 2018).

Roselle, *Hibiscus sabdariffa* L, is an annual herbaceous plant that belongs to the Malvaceae family with important health properties (Ali and El-Anany, 2017). *Bissap* (*H. sabdariffa* L.) is cultivated throughout the Senegalese territory and more particularly in the central and northern regions (Cisse et al., 2009a). Red *H. sabdariffa* calyx is

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used to produce a variety of food products like: jam, jelly and red soft drinks with tangy taste depending on the variety (Cisse et al., 2009b). The red color is due to anthocyanins present in the calyx. Anthocyanins are known to have nutritional and health properties (Chiu et al., 2015; Worawattananutai et al., 2014; Abdul Hamid et al., 2014; Rodriguez-Perez et al., 2017) and can be used as natural colorants in food, pharmaceutical and cosmetic products (Pinsuwan et al., 2010). The hibiscus based juices are known for their color instability during storage in correlation with changes in anthocyanin contents (Vankar and Shukla, 2011). The instability of these anthocyanins could be influenced by the quantity of solids in suspension and dissolved oxygen (Cisse et al., 2009c; Kane et al., 2015). Anthocyanins degradation during storage depends also on extraction methods and storage temperatures (Cisse et al., 2012).

Concentration process by water evaporation makes hibiscus extracts more stable. Pasteurization and food additives are methods for preservation to stabilize food products during storage. Sorbic acid and its salts kill yeasts, molds and bacteria by accumulating in microorganisms' cytoplasm, causing acidification of the cytosol (Van Beilen et al., 2014). Therefore, they preserve the organoleptic, nutritional and microbiological properties of food during storage. These changes cause a lot of losses to the value chain hibiscus.

Therefore, the objective of this study is to evaluate the impacts of pasteurization and potassium sorbate (E202) on the stability of concentrated extracts of *H. sabdariffa* during storage at 4, 30 and 45°C for 3 months.

MATERIALS AND METHODS

Production of extracts of *H. sabdariffa* L

The concentrated extracts were made with calyx of a local variety of *H. sabdariffa* L called *Bissap Vimto*. Twenty kilograms of calyx purchased from local market in Dakar, Senegal were mixed with 100 L of water and soaked for 4 h. The resulting mixture was filtered with a 0.2 mm diameter steel filter and a cotton filter to obtain a clear extract. The extract with 8.8 °Brix was then concentrated with an evaporator (AURIOL, France) to 60 °Brix. The concentrated extracts were divided into three: concentrated extract without treatment (NTC), concentrated extract with addition of 0.16% potassium sorbate (CSP) and the pasteurization of the concentrated extract at 70 C for 30 min (PC). Samples were stored in glass bottles wrapped in aluminum foil and stored at 4, 30 and 45°C. From zero time to day-90, every 15 days, up to 90 days, the concentrated calyx extracts were determined for: reducing sugars, pH, titratable acidity and total anthocyanins.

pH was determined with a pH-meter (Hanna HI 223, USA) dipped into the bottles (AOAC, 2005). The amount of titratable acid was determined by potentiometric titration using sodium hydroxide solution with a potentiometer titrator (Titroline Schott T23230 N° M 011287, Germany) (AOAC, 2005). Reducing sugars were measured with the Luff-Schoorl method (Kowalski et al., 2013). The sugars were extracted in aqueous ethanol. After eliminating the ethanol, the solutions were clarified and the sugars were determined before and after inversion. All analyses were performed in triplicate.

The anthocyanins concentrations of the hibiscus extracts were also determined during storage (Lee et al., 2005). The principle of determination was based on the properties of anthocyanins to change color depending on the pH (pH differential method). After dilution of the extract in two buffer solutions at pH 1.0 and 4.5 purchased from Fisher Scientific, USA, the absorbance was measured at 510 and 700 nm in triplicate. The anthocyanin's concentration was calculated using the following formula:

$$Ca = \frac{MW \times A \times dF \times 1000}{\varepsilon}$$

Ca: Anthocyanin concentration (mg/L); MW: molecular weight of Delphinidin 3-sambubioside, the major anthocyanin in *H. sabdariffa* extract, (597 g/mol); ε: molar extinction coefficient (26 000 L mol-1. cm-1); dF: dilution factor; A: absorbance, calculated using the formula:

A = (A1 - A2) - (A3 - A4)

Where, A1 = Absorbance measured at pH = 1 and 510 nm; A2 = absorbance measured at pH = 1 and 700 nm; A3 = absorbance measured at pH = 4.5 and 510 nm; A4 = Absorbance measured at pH = 4.5 and 700 nm.

Statistical analysis

Results are expressed as mean ± SD. The Fisher's protected least significant difference (PLSD) for multiple comparisons after one-way ANOVA was used to analyze the data (SPSS Version 14.0J, SPSS, Chicago, IL). Differences were considered significant at $P < 0.05$.

RESULTS

Characterization of the *H. sabdariffa* extracts

The effect of concentration process on some physiochemical properties of the hibiscus extracts are shown in Table 1. The results showed that the evaporation process increased the Brix from 8.8 to 60 °Bx. Non-concentrated extract (NC) had a significantly higher pH (2.1±0.01) than the concentrated extract (1.4±0.04). The concentration process decreased the pH and significantly increased reducing sugars and anthocyanins in the samples.

pH variation during storage

The pH of the samples decreased during storage (Table 2). This decrease was significant ($P < 0.05$) for CSP samples stored at different temperatures at day-90 as compared to zero time. There were no differences between the pH of NTC, CSP and PC at day-90 of storage at 4, 30 and 45°C.

Titratable acidity variation during storage

Table 3 shows the changes of the titratable acidity

Table 1. Effect of the concentration process on some physico-chemical properties of *Hibiscus sabdariffa* L. extracts.

Parameters	NC	NTC
pH	2.1±0.01[a]	1.4±0.04[b]
Titratable acidity (mEq/l)	457.66±0.01[a]	3885.595±604.476[b]
Ruducing sugars (g/l)	74.66±42.15[a]	113.140±46.465[b]
Total anthocyanins (g/l)	1.58±0.30[a]	14.602±0.402[b]

Values are means ± SD, n= 3. Values in the same row with different superscripts are significantly different, P<0.05.

Table 2. pH variation during storage of *Hibiscus sabdariffa* L. extracts at 4, 30 and 45°C.

Treatment	Storage temperatures (°C)			
	Zero time	4	30	45
		After 90 days		
NTC	1.430±0.040[a]	1.415±0.007	1.400±0.014	1.455±0.007
CSP	1.600±0.010[b]	1.355±0.007	1.41±0.042	1.410±0.014
PC	1.460±0.010[a]	1.355±0.049	1.405±0.007	1.425±0.035

Values are means ±SD, n=3, Values in the same column with different superscripts are significantly different, P<0.05.

Table 3. Variation of titratable acidity (mEq/l) during storage of *Hibiscus sabdariffa* L. extracts at 4, 30 and 45°C

Treatment	Storage temperatures			
	Zero time	4	30	45
		After 90 days		
NTC	3885.595±604.476	4228.950±6.152	4276.760±36.883	4880.895±18.434[a]
CSP	3959.480±306.749	4198.540±36.854	4328.915±24.586	4563.615±61.469[b]
PC	3924.710±944.386	4233.295±61.469	4237.640±30.731	4650.54±61.462[b]

Values are means ±SD (n=3). Values in the same column with different superscripts are significantly different, P<0.05.

Table 4. Variation of reducing sugars (g/l) during storage of *Hibiscus sabdariffa* L. extracts at 4, 30 and 45°C.

Treatment	Storage temperatures (°C)			
	Zero time	4	30	45
		After 90 days		
NTC	113.140±46.465	123.36±0.01	112.56±0.01	117.955±0.007[a]
CSP	128.220±0.583	121.20±0.01	118.00±0.01	113.635±0.007[b]
PC	114.920±44.213	128.75±0.01	123.36±0.01	117.955±0.006[a]

Values are means ±SD (n=3). Values in the same column with different superscripts are significantly different, P<0.05.

between samples during storage at different temperatures. At day-90, the titratable acidity in samples stored at 4°C was non-significantly different from that on zero time. During storage at 45°C, titratable acidity significantly (P <0.05) increased in CSP and PC samples at day 90.

Evaluation of reducing sugars during storage

The concentration of reducing sugars in the hibiscus concentrates during storage is shown in Table 4. At day 90, CSP samples stored at 45°C showed the lowest concentration of reducing sugars (113.635±0.007 g/L) as

Table 5. Changes of anthocyanins levels in NTC, CSP and PC samples during storage at 4°C.

Treatment	Zero time	Day-15	Day-30	Day-45	Day-60	Day-75	Day-90
NTC	14.602±0.402^{a}	12.766±1.645	13.394±0.956	11.180±0.644	12.941±0.707	12.288±0.827	13.708±0.981
CSP	13.828±2.654^{b}	12.181±1.642	14.427±1.187	12.302±1.750	12.992±1.485	12.655±1.022	13.111±1.517
PC	12.340±0.360^{b}	11.818±1.166	14.059±0.369	12.408±1.500	12.985±1.021	12.800±1.077	13.800±1.225

Values are means ±SD (n=3). Values in the same column with different superscripts are significantly different, P<0.05.

Table 6. Changes of anthocyanins levels in NTC, CSP and PC samples during storage at 30°C.

Treatment	Zero time	Day-15	Day-30	Day-45	Day-60	Day-75	Day-90
NTC	14.602±0.402^{a}	11.597±1.823	10.922±0.955	7.473±0.443^{a}	9.871±2.064	5.748±0.323	7.222±4.836
CSP	13.828±2.654^{b}	10.030±0.889	11.613±1.886	8.724±1.101^{b}	8.337±2.695	5.504±0.648	3.852±1.619
PC	12.340±0.360^{b}	10.881±0.553	11.851±1.012	9.362±1.133^{ab}	6.765±1.562	4.082±1.656	4.487±0.815

Values are means ±SD (n=3). Values in the same column with different superscripts are significantly different, P<0.05.

Table 7. Changes of anthocyanins levels in NTC, CSP and PC samples during storage at 45°C.

Treatment	Zero time	Day-15	Day-30	Day-45	Day-60	Day-75	Day-90
NTC	14.602±0.402^{a}	3.327±0.974^{a}	2.110±0.251	2.110±0.251^{a}	1.536±0.558	0.795±0.413	-
CSP	13.828±2.654^{b}	4.486±0.378^{ab}	2.816±1.252	2.816±1.252^{b}	2.135±1.857	-	-
PC	12.340±0.360^{b}	3.894±0.637^{b}	3.288±1.006	3.288±1.006^{b}	1.454±0.367	-	-

Values are means ±SD (n=3), Values in the same column with different superscripts are significantly different, P<0.05.

compared to NTC and PC stored at the same temperature (117.955±0.007 and 117.955±0.006 g/L, respectively).

Variation of anthocyanin during storage

As shown in Table 5, at 4°C, all the treatments kept most of the anthocyanin levels [13.708 g/L (NTC), 13.111 g/L (CSP) and 13.800 g/L (PC)]. Table 6 shows the effect of temperature storage (30°C) on the anthocyanin levels of hibiscus extract samples. From the results, all the treatments affected the level of anthocyanin. The residual level of anthocyanin was 49, 31 and 26% for NTC, PC and CPS, respectively. Table 7 shows that the highest destruction of anthocyanin among the storage temperatures was at 45°C in all the samples.

DISCUSSION

The concentration process decreased pH. This process also significantly increased titratable acididy, reducing sugars and anthocyanins in the samples. These results are in accordance with Youssef and Shatta (2006). Samples of hibiscus concentrated extracts had a very low pH (1.4 to 1.6). This acidity was due to organic acids such as the succinic and oxalic acids contained in the calyces of *H. sabdariffa* (Cisse et al., 2009b). Hibiscus extracts also contain other organic acids such citric, hydroxycitric, hibiscus, malic, tartaric and ascorbic acids (Da-Costa-Rocha et al., 2014). The increase in titratable acidity during storage at 45°C did not influence significantly, the final pH of the samples. The low pH of these concentrates makes it possible to avoid growth of certain microorganisms because the minimal pH of growth for most bacteria is 4.5 with some exceptions like lactic and acetic acid bacteria which can grow at pH lower than 4. The yeasts and molds have a minimum growth pH of 1.5 to 3.5 (Oyarzabal and Backert, 2011). Pasteurization and addition of food preservatives like potassium sorbate are means to inhibit the multiplication of these microorganisms which are the main agents influencing the conservation of food products (Yang et al., 2017).

The calices also contain sugars with glucose as the major sugar present (Peng-Kong et al., 2002). Reducing sugars were shown to decrease during storage. This reduction tended to increase during storage at 30 and 45°C than at 4°C (Table 4). Microorganisms can grow rapidly at favorable temperature and use these sugars in their metabolism, leading to the reduction of the sugar content (Pokusaeva et al., 2011). Changes in reducing sugars can influence sensory acceptability of hibiscus drinks by the consumers (Bechoff et al., 2014).

Hibiscus calyces are rich in anthocyanin used as

natural colorant and functional food ingredient (Idham et al., 2012). These anthocyanins are responsible for the red color of *H. sabdariffa* calyx products. The concentration of anthocyanin is influenced by the storage conditions and has an impact on the shelf-life (Sinela et al., 2016). Samples stored at 45°C for three months lost more than 99% of their anthocyanin content, while those kept at 4°C retained almost all their anthocyanins. Temperature is an essential factor in the degradation of anthocyanins. Indeed, temperature was also the determining factor in the deterioration of the phenolic pigments (Fernandez-Lopez et al., 2013; Al-Sanabani et al., 2016). In the case of anthocyanin, high temperatures favor the opening of the heterocyclic anthocyanins with the formation of chalcones which are colorless products (Dziezak, 1986). The degradation of these pigments causes disappearance of the chromatic characteristics since they determine the color of the products (Camelo-Mendez et al., 2016). Pasteurization, addition of potassium sorbate and the pH of hibiscus extracts affected anthocyanin stability. Food preservatives such as potassium sorbate were shown earlier to have a slight influence on the anthocyanins stability (Moldovan and David, 2014). High pressure processing and thermal pasteurization were reported to influence polyphenols and anthocyanins stability during storage (Marszalek et al., 2017). West and Mauer (2013) showed that the stability of anthocyanins in solution was inversely correlated to increasing pH and temperature and was related to the common destruction of anthocyanins and ascorbic acid in solution. Finally, the use of low temperature (4°C) besides pasteurization and/or addition of potassium sorbate could extend the shelf-life of these concentrates without changes.

In conclusion, concentrated hibiscus extracts can be stored at an optimal temperature of 4°C for 3 months without serious changes in the extracts. Pasteurization and addition of potassium sorbate to the concentrated extracts of *H. sabdarifa* did not improve significantly, the stability of these products during storage at higher temperature.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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