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Research Article

Influence of Sweeteners on the Phytochemical and Physicochemical Quality and Consumer Acceptability of Roselle Beverage

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Bissap is prepared from Roselle calyxes (*Hibiscus sabdariffa* L.) by hot infusion and marketed as a health drink. To improve the tart attributes, sucrose is usually added. However, because of nutrition and health concerns, processors explore other types of sweeteners, but the impact on the phytochemical and physicochemical quality and sensory properties of Bissap is not reported despite the potential influence on consumer acceptability. In this study, Roselle calyx extract was prepared to which sugarloaf pineapple pulp, Roscoe ginger, Negro/Ethiopian pepper, and cloves were added to obtain the Bissap stock (control). Then, either sucrose, caramel, honey, or sucralose was added to the Bissap to achieve a comparable sweetness (13.1°Brix), and the effects were assessed during storage. The results showed that caramel and honey significantly increased the nonenzymatic browning of Bissap from 0.49 ± 0.04 to 0.66 ± 0.07 and 0.64 ± 0.02, and the cloud value from 0.14 ± 0.01 to 0.23 ± 0.01 and 0.28 ± 0.02, respectively. The use of honey increased the ascorbic acid from 2.58 ± 0.17 to 3.35 ± 0.19 mg AE/mL, phenols from 11.25 ± 0.90 to 17.66 ± 1.07 mg GAE/mL, flavonoid from 15.33 ± 1.12 to 27.02 ± 1.69 mg QE/mL, and antioxidant capacity from 16.59 ± 1.34 to 25.36 ± 1.00 mg GAE/mL. During storage, ascorbate content decreased, but at a rate lower for honey-Bissap than the other sweeteners whilst the flavonoid and antioxidant activity of honey- and caramel-Bissap improved. The physicochemical changes led to a shelf life of 10 days at 6°C storage. Sensory analysis revealed the highest consumer ($n = 75$) acceptability scores for sucrose (5.89 ± 0.17), sucralose (5.43 ± 0.17), caramel (5.07 ± 0.17), honey (4.30 ± 0.20), and unsweetened Bissap (2.59 ± 0.22). Although honey enhanced the functional quality of Bissap, sucralose showed the highest potential as an alternative sweetener.

1. Introduction

Roselle beverage, also known as Bissap in Benin, Ghana, and Senegal, Wonjo or Zobo in Nigeria, or Sobolo in Ghana, and by several other names in some parts of Africa, is an indigenous beverage mainly prepared as a hot infusion extract of dried Roselle (*Hibiscus sabdariffa* L.) calyxes [1, 2]. In Ghana and several West African countries, ingredients such as ginger (*Zingiber officinale* Roscoe), pepper (*Capsicum annuum* L.), and spices including spice tree (*Xylopia aethiopica* Dunal), climbing black pepper (*Piper guineense* Schum. et Thonn), and grains of paradise (*Aframomum melegueta* [Rosco] K. Schum.) may be added to enhance the flavor or aroma. For imparting fruity tones to the beverage, pineapple and/or orange juice extracts are commonly added [2]. The beverage, which is dark red-magenta in color, could be served chilled or hot, with or without sweeteners. A survey conducted by Owureku-Asare et al. [3] showed that 26% of Ghanaian respondents consume Bissap at least 2-4 times a week, showing that the beverage is widely consumed among the populace. Bissap is considered a regular health drink and marketed for its ability to reduce oxidative stress and improve lipid profile and management of hypertension and atherosclerosis, among other benefits because of the high content of phenolic compounds and flavonoids [4, 5]. Additionally, the Roselle calyx is known to have a high vitamin C content, and the regular consumption is reported to help alleviate cold symptoms, boost the immune system, facilitate wound healing, and improve digestion among others [6]. Patronage of natural/herbal therapies in the treatment and management of diseases has increased over the years, more so because of the economical and effective therapeutic outcomes [7]. Thus, utilization of Roselle and other plant types for both nutritional and medicinal purposes could be a way of improving general public health.Several consumers prefer sweetened Bissap, although a few may choose unsweetened Bissap for health, figure, and fitness reasons [8]. For preparing sweetened Bissap, sucrose (refined table sugar) is usually added, which helps to milden the sharp and sometimes tart tones from the calyx extract [9]. Sucrose is more frequently used partly because it is relatively cheap and gives no perceptible aroma to the beverage. However, sucrose offers limited advantages in terms of overall nutritive or glycemic impact, and overdose has been implicated in metabolic syndrome, type 2 diabetes, cardiovascular disease, some cancers, Alzheimer's disease, and cellular aging among others [10]. Many consumers explore other alternatives for various reasons. For example, caramel has been used as a colorant but also as a semisynthetic sweetener and flavoring agent in the beverage industry. Honey is considered a healthy natural sweetener and therapeutic for the management of diabetes and cardiovascular diseases and known to have antimicrobial, anti-inflammatory, and anticancer properties [11]. Sucralose is internationally recognized as a zero-calorie substitute, being 600 folds sweeter than sucrose and safe for all consumers including diabetics [8]. Apart from health reasons, the choice of sweetener type could be influenced by cost, which is also relevant for commercial producers of Bissap because of profit prospects. The question remains whether the type of sweetener used is relevant as far as the physicochemical and phytochemical quality of Bissap is concerned. Additionally, evaluating the impact of sweeteners on the sensory quality is important for assessing and prospecting the role of sweeteners on the consumer acceptability of Bissap. Hence, the objective of this study was to assess the impact of adding sucrose, caramel, honey, or sucralose on the physicochemical and phytochemical quality during storage and on the consumer acceptability of Bissap.

2. Materials and Methods

2.1. Preparation of Bissap. Bissap was prepared according to the method proposed by Bolade et al. [12] with some modifications: A weighed (60 g) quantity of predried (60°C, 5 days) Roselle calyxes (*Hibiscus sabdariffa* L.), obtained from Abura Market, Cape Coast, in the Central Region of Ghana, was washed with distilled water and transferred to a 5.0 L thermos-resistant plastic bowl. Then, 2.0 L hot water (100°C) was added to the calyxes, covered with a lid, and allowed to steep at ambient temperature (26-28°C) overnight (12 hours). The resulting extract was filtered using a cheesecloth, and the residue was reextracted with an additional 2.0 L hot water for 20 min. Slices of freshly peeled pineapple (*Ananas comosus* Sugarloaf, 890 g), ginger (*Zingiber officinale* Roscoe, 100 g), pepper (Xylopia aethiopica Negro/Ethiopian pepper, 5 g), or pieces of cloves (*Syzygium aromaticum*, 1.0 g) were milled into a pulp using a Kult pro mixer (WMF AG, Geislingen, Germany), or a powder using an MR9100 mount fly electric grinder mill (Morphy Richards Ltd., South Yorkshire, England), and mixed with 4.0 L of the calyx extract at 70°C [2]. Then, the mixture was allowed to sediment at ambient temperature for 2 hours and decanted to obtain Bissap, which served as the stock.

2.2. Addition of Sweeteners. To determine the effect of sweetening agents on the quality of Bissap, commonly available sweeteners on the Ghanaian market including sucrose, caramel, honey, and sucralose (E-number: E 955, BOC Sciences, London, UK) were experimented. To establish a basis for the sugar content of the Bissap drinks, samples of commercial Bissap were procured from eight different vendors in the Ghanaian local market, and the sugar content (°Brix) was measured using a digital refractometer (MA871, Milwaukee Instruments, USA). Based on the average sugar content, sweetened Bissap samples for the experiment were prepared to achieve 13.1°Brix as follows: 300 g sucrose was added to 3.0 L Bissap stock to obtain sucrose-sweetened Bissap "sucrose-Bissap." For caramel-sweetened Bissap, 600 g sucrose was transferred into an aluminum pan and heated with constant stirring on a hot plate for 90 min to obtain a brown caramel, which was dissolved in 600 mL Bissap stock. Then, the mixture was added to a fresh Bissap stock until the desired Brix to obtain the "caramel-Bissap." Honey-sweetened Bissap was prepared by adding pure honey until the desired Brix to obtain "honey-Bissap." According to the manufacturer's recommendations, 1.0 g sucralose was required to achieve sweetness that is equivalent to 8 g sucrose. On that basis, 37.5 g sucralose was added to 3.0 L Bissap stock to obtain sucralose-Bissap. The sweetened Bissap was then heated to 80°C for 20 min, transferred into 500 mL per bottle, and refrigerated (6°C) until analyses.

2.3. Physicochemical Analysis. The pH, Brix, and color of Bissap were measured using an electronic pH meter (B10P Benchtop), a digital refractometer (MA871, Milwaukee Instruments, USA), and a color meter (CS-10, CHN Spec, China), respectively, as described by Ampofo-Asiama et al. [13]. Nonenzymatic browning (NEB) and cloud value of the supernatant were determined after centrifuging (SIGMA 3–30 K, Laborzentrifugen GmbH, Osterode, Germany) Bissap at $3000 \times g$ for 10 min. Cloud value was analyzed by measuring the absorbance at 660 nm (Jenway 6400, Bibby Scientific Ltd). For NEB, 5 mL absolute ethanol was added to an equal volume of the supernatant and centrifuged at $3000 \times g$ for 10 min [14]. Distilled water served as the blank for all measurements.

2.4. Antioxidant Activity and Phytochemical Analysis. The antioxidant activity and phenolic content were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and Folin-Ciocalteu assays, respectively [13, 15]. Briefly, ethanolic extract was prepared by mixing 1 mL Bissap with an equal volume of ethanol and centrifuging at $10,000 \times g$ for 20 min. For the antioxidant capacity, 100 μL of the supernatant was added to 900 μL Tris-HCl buffer and incubated with 2 mL, 0.1 mM DPPH in methanol solution in the dark for 30 min. DPPH solution without the sample served as the control, and gallic acid (GA) was used as the calibration standard. After measuring the absorbance of the samples at 517 nm, the antioxidant capacity was estimated based on the calibration curve and expressed as mg gallic acid equivalent per mL (mg GAE/mL) Bissap.

For the total phenolic content, 100 μL portion of the Bissap sample was added to 750 μL Folin-Ciocalteu reagent and 750 μL 6% sodium bicarbonate. The mixture was then incubated at 25°C for 90 min, and the absorbance was measured at 725 nm. Gallic acid served as the calibration standard, and the total phenolic content was expressed as mg GAE/mL Bissap.

The flavonoid content was determined using the aluminum chloride colorimetric assay [16]. To 1 mL of the Bissap, 2 mL of distilled water was added followed by 300 μL of 5% sodium nitrite and an equal volume of 10% aluminum chloride. After incubating the mixture at ambient temperature (26-28°C) for 10 min, 1.0 mL of 1 M sodium hydroxide was added, and the absorbance was measured at 420 nm. Quercetin was used as the calibration standard, and the flavonoid content was determined using the calibration curve and reported as mg quercetin equivalent per mL (mgQE/mL) Bissap.

Ascorbate content was measured based on the 2,4-dinitrophenylhydrazine assay as described by Dadzie et al. [17] using ascorbic acid as a standard. To 1.0 mL of Bissap, 5.0 mL of metaphosphoric-acetic solution was added and centrifuged at $10,000 \times g$ for 15 min. Exactly 9.0 μL bromine water was added to 1.54 mL of the supernatant, after which 50 μL of 10% thiourea and 390 μL of 2% 2,4-dinitrophenylhydrazine were added. After incubation at ambient temperature for 3 hours, 1.93 mL of chilled 4.5 M H_2SO_4 was added, and the absorbance was measured at 521 nm. Ascorbic acid content was determined based on the calibration curve and was expressed as the mg ascorbate equivalent per mL (mg AE/mL) Bissap. The arithmetic mean of values related to physicochemical and phytochemical analyses are based on triplicate experiments.

2.5. Sensory Analysis and Storage Quality. Consumer acceptability of Bissap sweetened with the various types of sugars was determined using a 7-point hedonic scale [18]. For this, a sensory panel of 75 members (males, 45; females, 30; mean age, 21.7 years) was randomly recruited for the study and presented 50 mL of Bissap sweetened with either sucrose, caramel, honey, or sucralose in counterbalanced order. Sensory attributes including appearance, aroma, taste, aftertaste, and overall acceptability were rated on a scale of dislike extremely (1) to like extremely (7). Bissap without the sweetener was included for comparison. Sensory experiments were done once.

The effect of sweetener type on the storage quality of Bissap was determined by withdrawing samples and analyzing the physicochemical (pH, Brix, color, cloud value, and nonenzymatic browning) and phytochemical (ascorbate level, phenolic content, flavonoid content, and total antioxidant capacity) content at regular intervals (0, 3, 7, 10, 14, and 18 days) during refrigerated (6°C) storage. The arithmetic mean of values related to physicochemical and phytochemical analyses are based on triplicate experiments.

2.6. Data Analysis. Statistical analysis was carried out using SPSS (IBM, SPSS Statistics 20, version 2020). Significant differences in physicochemical and phytochemical parameters were determined using ANOVA at a significance level of $p < 0.05$. Multiple comparisons of the mean test were carried out using the Tukey test. Principal component analysis (PCA) and Pearson's correlation analysis were performed to explore associations and correlations between the differently sweetened Bissap and the measured physicochemical parameters and consumer sensory scores.

3. Results

3.1. Effects of Sweeteners on Physicochemical Properties. Physicochemical properties of Bissap prepared using different sweeteners shown in Table 1 depict no significant differences in pH between the freshly prepared, unsweetened Bissap (control) and the sweetened Bissap analogues. The Brix of sucrose-, caramel-, and honey-Bissap were comparable but significantly higher than that of sucralose-Bissap (4.37 ± 0.22), which was close to the unsweetened Bissap (3.17 ± 0.10). In terms of color, $L*$ values increased for sucrose-Bissap, but no significant effects were observed for honey-, caramel-, and sucralose-Bissap. Furthermore, $a*$ values were not affected when honey or sucralose was used as opposed to the increase or decrease with sucrose or caramel, respectively. Again, $b*$ values were lower than the unsweetened Bissap when sucrose or sucralose were used, but significantly higher values were recorded when honey or caramel was used as sweeteners. The hue decreased for sucrose- and sucralose-Bissap but increased for caramel- and honey-Bissap. Whilst the Chroma showed higher values for sucrose-Bissap and lower for caramel-Bissap, no changes for honey- or sucralose-Bissap were observed. The change in color of sweetened Bissap compared to the unsweetened Bissap was marginal with the highest recorded for sucrose-Bissap and the lowest for sucralose-Bissap. Sweetening Bissap with caramel or honey significantly increased the NEB and cloud values; however, the use of sucrose or sucralose showed no significant effects.

3.2. Effects of Sweeteners on the Physicochemical Stability of Bissap during Storage. During storage, a trend of increasing pH (Figure 1(a)) and a decrease of Brix (Figure 1(b)) was observed after 10 days of storage. But the changes were not significantly different from that of the freshly prepared samples (day 0) (Figure 1). Chroma (Figure 2(a)) significantly decreased within the first 3 days and remained lower than the fresh sample during 18 days of storage (Figure 2). A significantly higher hue (Figure 2(b)) was recorded only on day 3 than other storage days for honey and caramel-sweetened Bissap, but unsweetened Bissap remained relatively unchanged.

Table 1: Physicochemical and phytochemical composition of unsweetened and sweetened Bissap.

Parameter[x]	Unsweetened	Sucrose	Caramel	Honey	Sucralose
pH	2.76 ± 0.12^a	2.78 ± 0.15^a	2.76 ± 0.14^a	2.88 ± 0.18^a	2.67 ± 0.09^a
Brix	3.17 ± 0.10^a	13.07 ± 0.14^b	13.19 ± 0.15^b	13.07 ± 0.11^b	4.37 ± 0.22^c
Color					
L^*	23.01 ± 0.47^a	25.16 ± 0.37^b	23.33 ± 0.16^a	25.50 ± 2.02^{ab}	23.96 ± 0.02^a
a^*	12.17 ± 0.20^a	14.43 ± 0.47^b	10.65 ± 0.16^c	12.02 ± 0.96^a	12.04 ± 0.18^a
b^*	4.30 ± 0.31^a	3.99 ± 0.93^a	4.45 ± 0.02^a	4.89 ± 1.22^a	3.91 ± 0.25^a
Color change	—	3.13 ± 0.61^a	1.57 ± 0.50^c	2.57 ± 0.23^b	1.03 ± 0.35^c
Hue	19.44 ± 0.18^a	15.46 ± 0.18^b	22.68 ± 0.22^c	22.15 ± 0.10^c	18.01 ± 0.10^d
Chroma	12.91 ± 0.14^a	14.97 ± 0.31^b	11.54 ± 0.20^a	12.98 ± 0.10^a	12.66 ± 0.30^a
NEB	0.49 ± 0.03^a	0.44 ± 0.05^a	0.66 ± 0.07^b	0.64 ± 0.02^b	0.47 ± 0.03^a
Cloud value	0.14 ± 0.01^a	0.16 ± 0.02^a	0.23 ± 0.01^b	0.28 ± 0.02^b	0.14 ± 0.01^a
Ascorbate (mg AE/mL)	2.58 ± 0.17^a	2.68 ± 0.46^a	2.77 ± 0.26^a	3.35 ± 0.19^b	2.86 ± 0.27^a
Phenol content (mg GAE/mL)	11.25 ± 0.90^a	11.12 ± 1.03^a	16.80 ± 1.85^b	17.66 ± 1.07^b	10.31 ± 0.97^a
Flavonoids (mg QE/mL)	15.33 ± 1.12^a	14.65 ± 0.90^a	21.64 ± 1.14^b	27.02 ± 1.69^c	16.35 ± 0.99^a
Antioxidant (mg GAE/mL)	16.59 ± 1.34^a	18.37 ± 1.03^a	22.37 ± 1.05^b	25.36 ± 1.00^b	16.52 ± 1.09^a

[x]Results are based on arithmetic mean ± standard deviation from triplicate determinations. Values in the same row marked by different superscript letters differ significantly at $p < 0.05$.

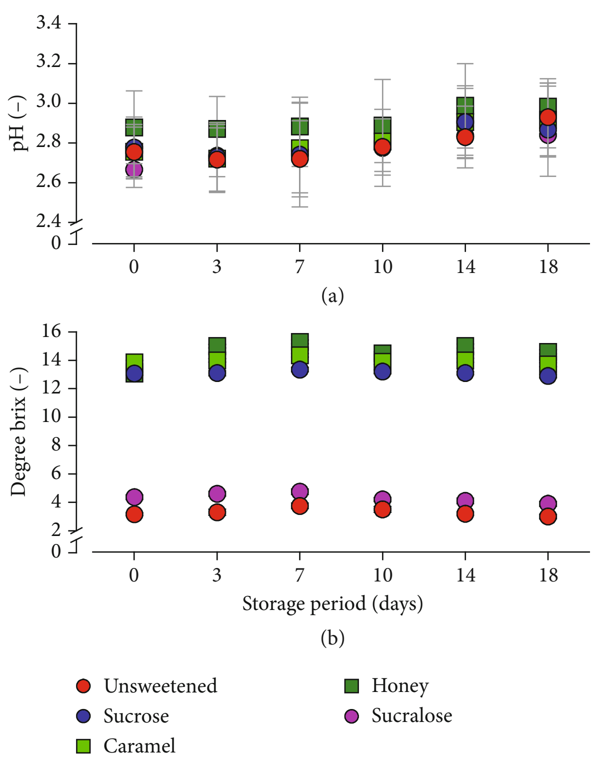

Figure 1: The effect of storage at 6°C on (a) pH and (b) Brix of unsweetened (control, red circle), sucrose (blue circle), caramel (green square), honey (olive green square), and sucralose-sweetened (pink circle) Bissap during 18 days. ∗Significantly different at $p < 0.05$.

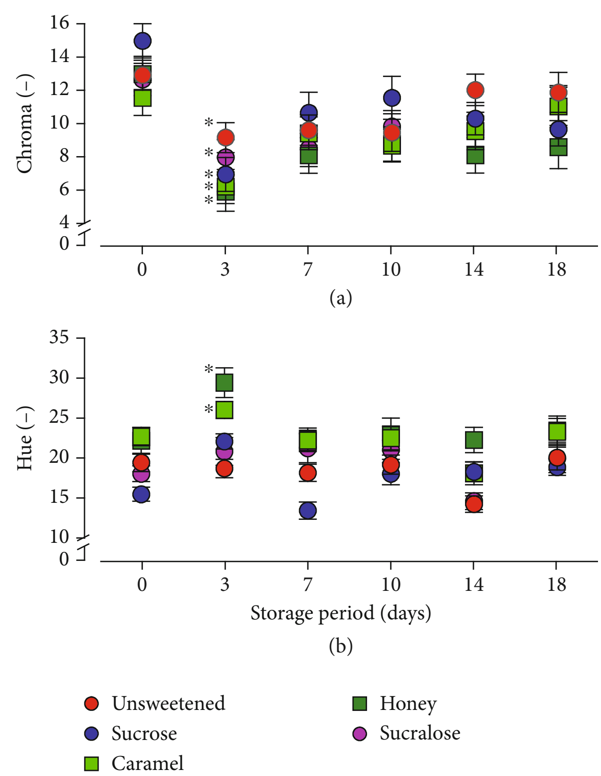

Figure 2: The effect of storage at 6°C on (a) Chroma and (b) hue of unsweetened (control, red circle), sucrose (blue circle), caramel (green square), honey (olive green square), and sucralose-sweetened (pink circle) Bissap during 18 days. ∗Significantly different at $p < 0.05$.

A general increase in nonenzymatic browning (NEB) was observed during storage (Figure 3). Honey-Bissap showed a significant increase of NEB after 10 days (Figure 3(a)) whilst caramel-Bissap showed the lowest increase (15%) after 18 days of storage. On the contrary, cloud values of unsweetened and sweetened Bissap significantly decreased after 3 days (Figure 3(b)), reaching the lowest values after 7 days (Figure 3). Sucrose-Bissap showed the

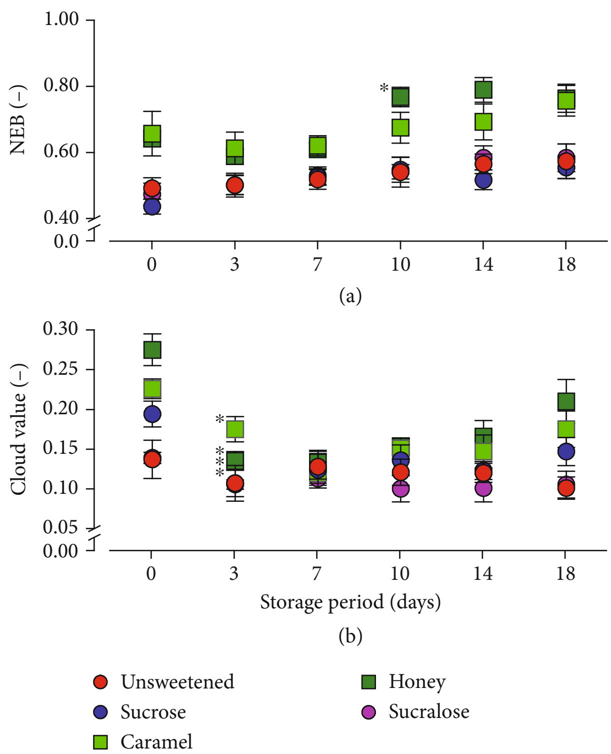

Figure 3: The effect of storage at 6°C on nonenzymatic browning ((a) NEB) and (b) cloud value of unsweetened (control, red circle), sucrose (blue circle), caramel (green square), honey (olive green square), and sucralose-sweetened (pink circle) Bissap during 18 days. ∗Significantly different at $p < 0.05$.

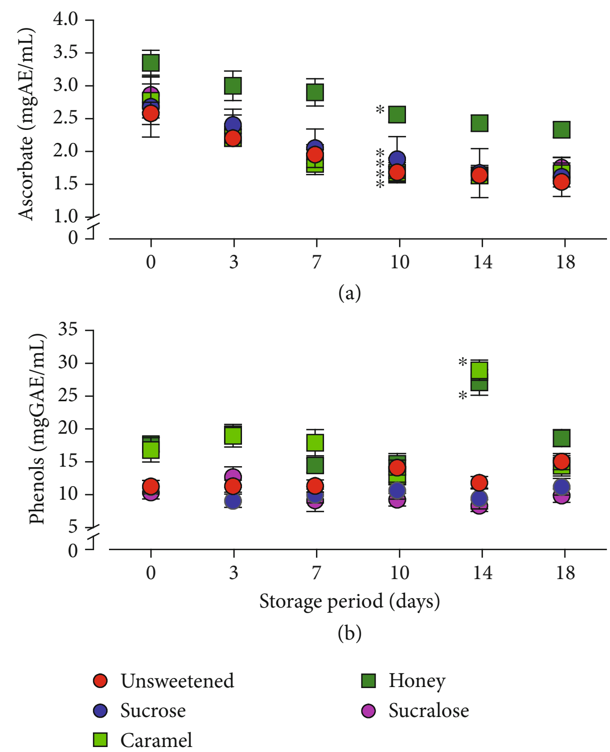

Figure 4: The effect of storage at 6°C on (a) ascorbate and (b) phenols of unsweetened (control, red circle), sucrose (blue circle), caramel (green square), honey (olive green square), and sucralose-sweetened (pink circle) Bissap during 18 days. ∗Significantly different at $p < 0.05$.

most relative decrease after 3 days, and towards the end of storage, the relative change in cloud value remained almost constant for all sweetened Bissap.

3.3. Effect of Sweeteners on the Phytochemical Content of Bissap during Storage. Ascorbate levels of Bissap were not significantly affected when sucrose, caramel, or sucralose were added; however, the addition of honey significantly increased the ascorbate content. Additionally, the phenolic and flavonoid contents and antioxidant activity of Bissap significantly increased with the addition of caramel and honey, but the effect was insignificant when sucrose or sucralose was used (Table 1).

During storage, ascorbate levels of unsweetened Bissap progressively decreased from 2.58 ± 0.17 mg AE/mL to 1.50 ± 0.094 mg AE/mL, representing an approximately 42% decrease (Figure 4). Similarly, an approximately 40% decrease of ascorbate levels of Bissap for sucrose, caramel, and sucralose and a 30% decrease for honey-Bissap were recorded after 18 days of storage (Figure 4(a)). The phenolic content of Bissap did not follow any clear trend. For example, whereas unsweetened Bissap showed comparable phenolic contents on other days, higher values on 10 days and 18 days were recorded (Figure 4(b)). Similarly, honey-Bissap revealed higher phenolic contents on 3 days and 18 days and lower contents on other days. Caramel-Bissap had the highest on 14 days and lower content on other days (Figure 4). Similar to phenolic contents, flavonoid content of unsweetened Bissap revealed higher contents on 10 days with no significant change on other days during storage (Figure 5(a)). After the addition of sweeteners, honey-Bissap and caramel-Bissap showed the highest flavonoids on 7 days whilst other days showed no significant differences when compared to the control. No significant effects were observed for the other types of sweeteners. Although no significant differences in the antioxidant capacity of unsweetened Bissap were observed, that of caramel, sucrose, and honey-Bissap showed significant increases after 3 days (Figure 5(b)).

3.4. Effect of Sweeteners on the Consumer Acceptability of Bissap. Figure 6 shows that the addition of sweeteners to Bissap did not affect the consumer scores for appearance, which showed a range of scores from 5.08 ± 0.19 (caramel-Bissap) to 5.53 ± 0.17 (honey-Bissap). Sucrose-Bissap showed the highest consumer-acceptability scores for aroma (5.58 ± 0.17), aftertaste (5.55 ± 0.17), and overall acceptability (5.90 ± 0.17) whilst that of unsweetened Bissap (aroma, 4.20 ± 0.25; aftertaste, 2.24 ± 0.20; overall acceptability, 2.59 ± 0.22) gave the lowest among the beverages. Sucralose-Bissap gave the highest score of 5.84 ± 0.80 for taste. The order of the overall consumer-acceptability scores for Bissap was sucrose-Bissap > sucralose-Bissap > caramel-Bissap > honey-Bissap > unsweetened Bissap.

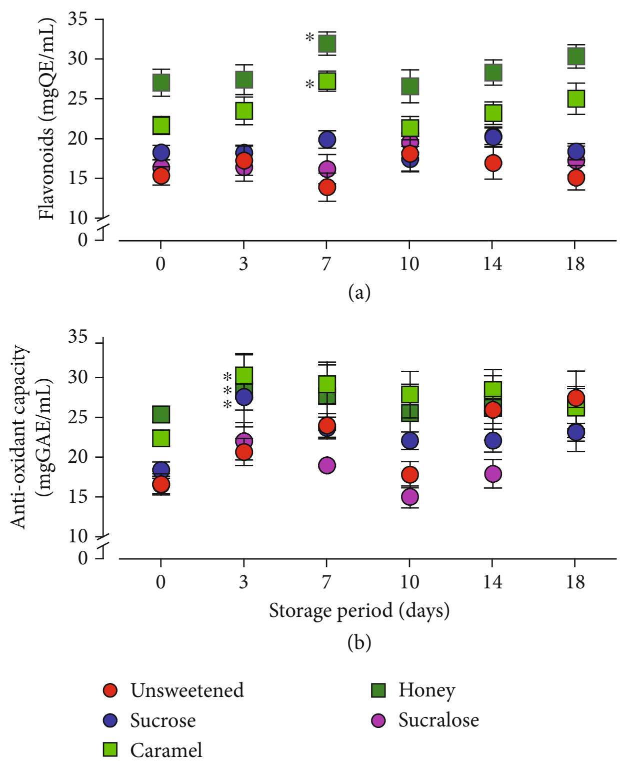

Figure 5: The effect of storage at 6°C on (a) flavonoid and (b) antioxidant capacity of unsweetened (control, red circle), sucrose (blue circle), caramel (green square), honey (olive green square), and sucralose-sweetened (pink circle) Bissap during 18 days. ∗Significantly different at $p < 0.05$.

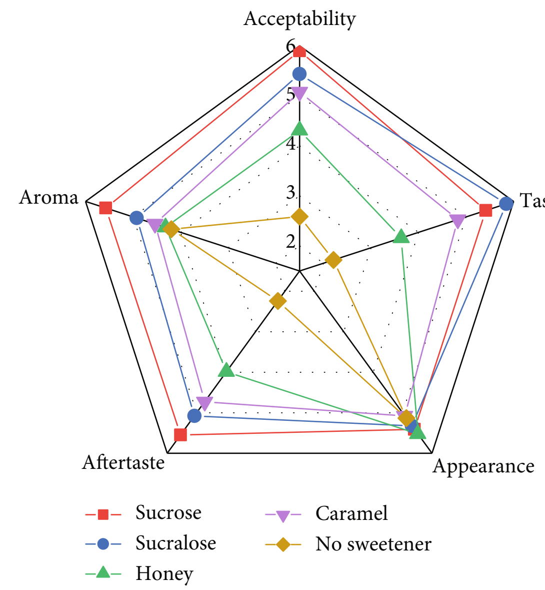

Figure 6: Effect of using different sweeteners on the sensory scores of Bissap.

3.5. Correlation between the Physicochemical Parameters and Sensory Scores. Principal component analysis (PCA) exploring relationships between the physicochemical and phytochemical properties and consumer sensory scores (samples' scores) for the various types of sweeteners (loadings) shows that the first two principal components (PC 1 and PC 2) accounted for 44% of the explained variance (Figure 7). Distinct patterns showed that depending on the type of sweetener used, caramel- and honey-Bissap clustered along the positive x-coordinates in the 1st and 4th quadrants whilst sucrose, sucralose-Bissap, and unsweetened Bissap clustered along the negative x-coordinates in the 2nd and 3rd quadrants. Positive associations were observed between sucrose-Bissap and all the sensory attributes (taste, appearance, aftertaste, aroma, and overall acceptability) and between honey-Bissap and nonenzymatic browning, flavonoids, and antioxidant activity. The Pearson correlation analysis between the physicochemical properties and scores of the sensory attributes of Bissap (Figure 8) showed a significant ($p < 0.05$, $r > 0.8$) positive relationship between appearance and ascorbate content but a negative correlation between aroma and nonenzymatic browning. Among the attributes, significant positive relationships were observed between acceptability, aroma, taste, and aftertaste. Regarding the physicochemical properties, positive correlations were observed among antioxidant activity, Brix, nonenzymatic browning, flavonoids, cloud value, and ascorbate content.

4. Discussion

The production and consumption of Roselle beverage have been reported in several parts of the world, where table sugar (sucrose) was the more frequently used type of sweetener, helping to mask the tart and sharp taste of the extract [19]. Moderating the consumption of sugar is, however, a major worldwide challenge despite the reported health issues associated with the high caloric content and sugar-related diseases such as obesity and diabetes [20].

Previous studies have reported the pH of Roselle extract to be less than 3 [19, 21, 22]. A similarly low pH was observed in this study, although factors such as the cultivar, extraction conditions, and recipes could influence the final pH. Addition of sweeteners did not significantly influence the pH, which was within the values reported previously [23]. The low pH of Roselle beverage has been attributed to the presence of organic acids such as ascorbic, malic, citric, and phenolic acids [24].

Roselle calyx is reported to contain 3-5% sugars [25]. The low sugar content could partly account for the low Brix of unsweetened Bissap, which remained low despite the addition of the base ingredients. Sucralose is a calorie-free, nonnutritive artificial sweetener, which is about 600 times sweeter than sucrose [8]. Thus, for imparting a comparable sweetness, a lower Brix of the sucralose-Bissap than that of caramel-, honey-, or sucrose-Bissap was recorded (Table 1), which can be beneficial for reducing the total caloric content. The $L*$, $a*$, and $b*$ color primaries revealed disparities, affecting the hue and Chroma depending on the type of sugars used. The higher $a*$ observed for sucrose-Bissap than caramel-Bissap could be explained by the protective effects of sucrose on anthocyanin degradation [24, 26], which is responsible for the red-magenta tint of Bissap. Additionally, caramel itself has several brown-pigmented compounds that could impact Bissap's color. In

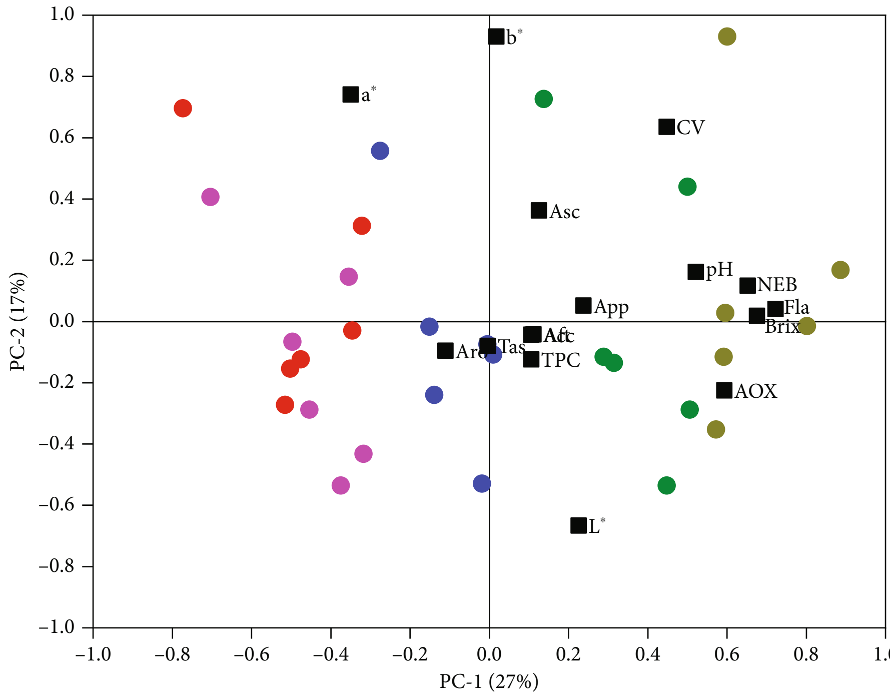

Figure 7: PCA biplot (PC1 is plotted against PC2) of Roselle beverage sweetened with different agents. The samples' scores (unsweetened (red circle), sucrose (blue circle), caramel (green circle), honey (olive green circle), sucralose (pink circle)) are superimposed on the loadings (°Brix; pH; AOX: total antioxidant capacity; ASC: ascorbate; CV: cloud value; NEB: nonenzymatic browning; Fla: flavonoid content; TPC: total phenolic content; $L*$ value; $a*$ value; $b*$ value; Tas: taste; Aft: aftertaste; Aro: Aroma; App: appearance; Acc: acceptability).

principle, the overall color change was insignificant, which is probably caused by the masking effect of the intense red-magenta pigmentation typical of Roselle calyx [3]. The increase in cloud value of caramel- and honey-Bissap could be attributed to the dark-brown nature of the sweeteners due to the presence of brown color compounds, which could have influenced turbidity. Probably, products of nonenzymatic browning in caramel and floral pigments of honey [27] could additionally contribute to the increase in nonenzymatic browning of Bissap.

A low pH and Brix are relevant for improving shelf life by decreasing microbial proliferation, which could lead to spoilage during cold storage. Minor increase of pH and decrease of Brix after 10 days of cold storage of sweetened Bissap (Figure 1) could be due to the commencement of submicrobial activity, leading to the production of basic compounds such as ammonia from protein degradation, and thus, altering the pH, decreasing Brix, and increasing nonenzymatic browning and the cloud value [13, 14]. It is possible, however, that the low pH of Bissap and the potential antimicrobial activity of the phytochemicals from the spices which were added during the formulation of the beverage coupled with the low-temperature storage (6°C) could inhibit microbial growth and thus maintain a relatively stable pH.

On one hand, honey is reported to contain floral pigments including carotenoids, xanthophylls, anthocyanins, several flavonoid and phenolic compounds [26], and vitamin C [28], explaining how the use of honey could contribute to the higher vitamin C, phenolic, flavonoid contents, and, consequently, antioxidant activity of Bissap. Again, caramel is known to contain melanoidin compounds [29], which can additionally contribute to flavonoids, phenolic, and antioxidant activity of Bissap as observed in the study. On the other hand, the presence and interaction of the floral pigments with the anthocyanins of Bissap could form brown-colored complexes during storage [30], contributing to the higher difference in nonenzymatic browning of honey-Bissap than caramel-Bissap, which additionally contained brown pigments from the onset of storage. The decrease of cloud value could be ascribed to aggregation and sedimentation of suspending soluble compounds of Bissap during storage, and the higher rate of ascorbate decrease during the storage of unsweetened, sucralose-, caramel-, or sucrose-Bissap than honey-Bissap could be caused by the high content of antioxidant compounds of honey, which are known to shield ascorbate degradation by ascorbic acid oxidases [31]. The fluctuations in phenolic and flavonoid content of Bissap during storage could be related to oxidation reactions, and reactions between oxidized polyphenols and formation of new compounds of antioxidant character or by microbial activity as reported for fruit beverages during storage [32].

Bissap with approx. 13°Brix, used in this study, is commonly found on the Ghanaian market, and the soluble solids are comparable to the reported values from Nigeria [20] and Mexico [20, 33], showing that consumers prefer sweetened Bissap. Indeed, the sensory acceptability of Roselle beverage has been observed to be affected by the sugar-to-acid ratio, which influences the balance between sweetness and acidity perception [13, 21, 34]. It seems possible, therefore, that an approximate sugar content of 13°Brix provides the optimal

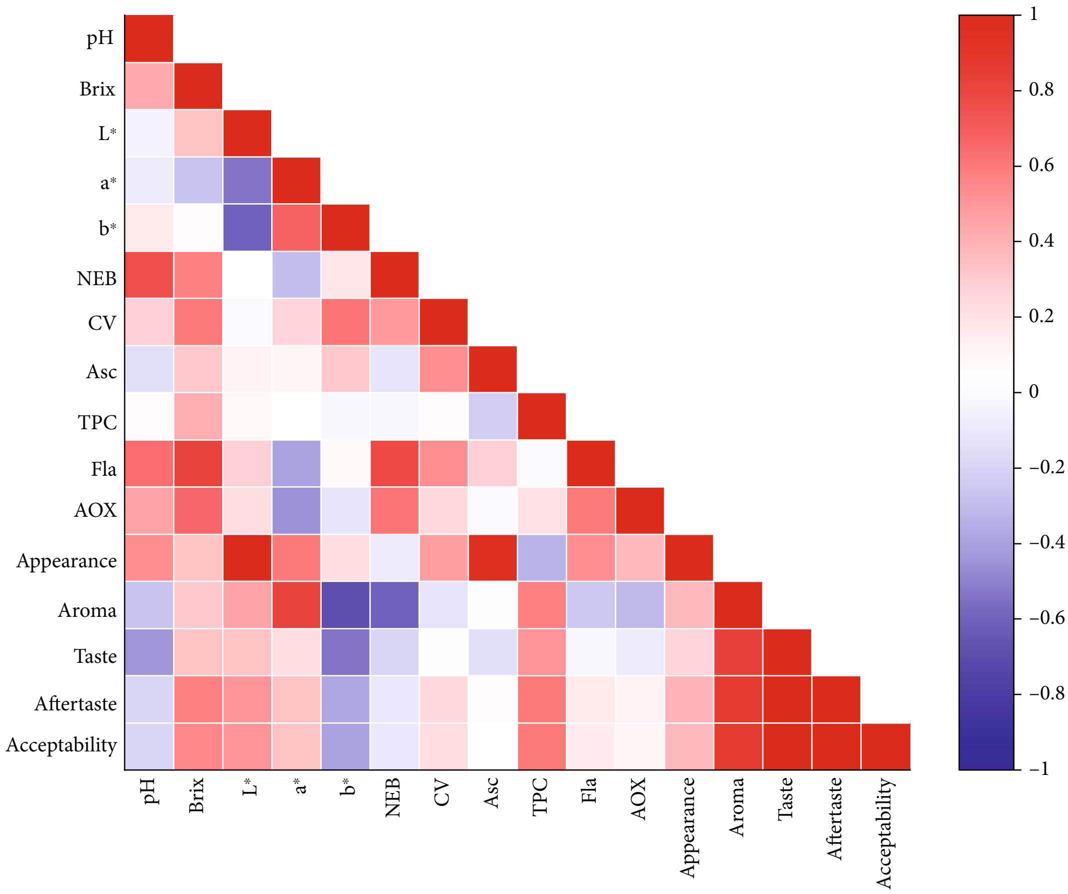

Figure 8: The Pearson correlation analysis between the physicochemical parameters and sensory scores of the differently sweetened Bissap (AOX: total antioxidant capacity; ASC: ascorbate; CV: cloud value; NEB: nonenzymatic browning; Fla: flavonoid content; TPC: total phenolic content).

sensory perception for Roselle beverage and could have led to the reduced sensory acceptability of unsweetened Bissap, which was high in acidity but low in sweetness.

The lower taste and aftertaste scores of honey- and caramel-Bissap than sucrose-Bissap could be attributed to the flavor and mouthfeel imparted by the sweeteners. On one hand, the PCA plot (Figure 7) provided an acceptable account of the analyzed parameters and showed clustering of the coordinates of honey- and caramel-Bissap along with some physicochemical properties such as nonenzymatic browning, flavonoid, antioxidant activity, cloud value, and ascorbate content that were high in the respective samples (Figure 7). On the other hand, clustering of the coordinates of sucrose, sucralose, and unsweetened Bissap with aroma may point to a rather mild impact of the sweeteners on the aroma of Bissap. From Figure 8, the positive relationship between appearance and ascorbate levels could be ascribed to the added honey or caramel, which showed high ascorbate content. The significant correlation between acceptability, aroma, taste, and aftertaste suggests that the three latter factors are the main drivers of Bissap acceptability. Aroma appeared to be negatively impacted by nonenzymatic browning. Probably, compounds that contribute to nonenzymatic browning in honey or caramel could have negatively impacted the aroma of Bissap, leading to the lower acceptability among consumers when compared to that of sucrose or sucralose. Monteiro et al. [19] showed that Roselle beverages prepared by extracting the pigments by means of vacuum concentration showed red color intensity, aroma, and balance between sweetness and acidity as the main drivers of consumer acceptability. The observed trend could also be related to familiarity of the sensory panel to the taste and aftertaste of Bissap prepared using sucrose rather than honey or caramel. The positive correlation among the physicochemical properties shows that the increase of the antioxidant activity of Bissap during storage was mainly due to the flavonoid content, which was high in the honey- and caramel-Bissap analogues. The comparable sensory scores of sucrose- and sucralose-Bissap provide an avenue for sucralose use in the production of sugar-free Bissap, which could appeal to consumers who show some restrictions to the consumption of sucrose-Bissap either for personal or health reasons. The results could provide consumers the opportunity to enjoy Bissap whilst benefiting from the health and medicinal properties.

5. Conclusion

The study showed that the type of sweetener for preparing Bissap was important, as the use of sucrose, caramel, or honey, rather than sucralose, caused an increase in the °Brix without any significant changes in the pH. The use of honey and caramel increased the cloud value, nonenzymatic browning, and phenolic and flavonoid compounds and improved the antioxidant capacity. During storage, minor increases in pH and decrease of Brix, Chroma, and cloud values of Bissap samples were observed. The ascorbate content of Bissap samples decreased, but the rate was lower when honey was used as a sweetener. The phenolic and flavonoid content followed no clear trend although higher flavonoids were recorded for honey- and caramel-Bissap, probably contributing to the higher antioxidant activity during storage. The physicochemical changes led to a shelf life of 10 days during refrigerated (6°C) storage of Bissap. Sensory analysis revealed the highest and comparable scores for Bissap beverage sweetened with sucrose or sucralose whilst the use of caramel and honey resulted in lower consumer-acceptability scores for taste, aroma, aftertaste, and overall acceptability. Future studies focusing on the use of other types of sweeteners such as beet sugar and the impact of the spices on the microbiological quality of Bissap would be relevant for expanding Bissap consumption and the storage quality.

Data Availability

All data is included in the manuscript.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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