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Research Article

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Bioactive Contents, Antioxidant Activities, and Storage Stability of Commercially-Sold Baobab Fruit (*Adansonia digitata* L) Juice in Malawi

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Abstract

Baobab fruit (*Adansonia digitata* L) is rich in micronutrients and bioactive compounds, which are vital for healthy living. Boabab juice are produced locally and stored under different storage conditions in Malawi. However, storage of baobab juice under certain conditions can lead to degradation of its bioactive compounds. This study therefore investigated the bioactive compounds in Commercial baobab juice (CBJ) in Malawi, and determined the stability of the compounds under different storage conditions. The organic acids, flavan-3-ol aglycones, total phenolic contents and antioxidant activities of the CBJ were determined using standard methods. The stability of the bioactive compounds in CBJ were determined in the samples stored at 6, 15 and 30 ºC for 49 days. The ferric reducing antioxidant power of CBJ was 71.27±.04 TEAC/100 g FW, and the ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) was 112.62± 0.45 mg TEAC/100 g FW. The 2,2-diphenyl-1-picrylhydrazyl was 80.94 ± 0.72 %. CBJ bioactive compounds are stable at 15 ºC, and contains high concentrations of total phenolic compounds, antioxidant activities and procyanidin B_2. In conclusion, commercially sold baobab juice in Malawi is rich in bioactive compounds and have high antioxidant properties, thus, regular consumption of the juice can improve the health and wellbeing of Malawians. Information provided in this study will provide consumers with nutritional profile of CBJ and help juice processors establish adequate storage conditions for the juice.

Keywords

Commercial baobab juice, Bioactive compounds, Phenolic compounds, Vitamin C, Antioxidant activity

Introduction

Foods especially fruits contain bioactive substances which exert beneficial effects to humans, and are important for cellular and physiological activities in human body [1]. Bioactive compounds such as carotenoids, phenolic compounds and flavonoids have been widely studied for their health benefits [2]. The flavonoids including flavan-3-ols have been reported to have chelating, and radical scavenging properties, in addition to acting as enzymes mediator/inhibition, and having anti-viral activities [3].

Studies have shown that foods containing bioactive compounds (phenolic acids, pro-anthocyanidin) has the potential to enhance vascular health benefits such as reduction in the oxidation of low-density lipoproteins, reduced platelet aggregation and vasodilation among others [4]. *In vivo* studies showed that foods

that are rich in bioactive compounds such as procyanidins and flavan-3-ol can decrease deoxyribonucleic acid damage and increased antioxidant status of humans [5]. However, stability of bioactive and antioxidant compounds may be degraded during processing, storage or by enzymes activities, resulting in de-polymerisation or epimerisation of the compounds [6].

Bioactive compounds are present in fruits such as baobab, apple, strawberries, blackcurrant, vegetables, tea, coffee etc., [7]. Baobab known scientifically as *Adansonia digitata L,* is a tropical fruit rich in phenolic compounds including flavonoids which have health promoting activities [8]. The fruit and its products are reported to contain high concentrations of flavan-3-ols [9]. The fruit also contains high levels of organic acids, vitamins especially vitamin C, flavonoids, and other compounds such as tannins, sugars, glycosides, fibres etc.,[10, 11].

Baobab fruits (*Adansonia digitata L*) are consumed in different forms, examples of which include gruel, sourdough, yoghurt and juice [12]. Baobab juice is a common drink in Malawi and other East African Countries, however, there is a dearth of information regarding the bioactive composition and stability of the phytochemical properties of Commercially-sold Baobab Juice (CBJ) in Malawi. In addition, CBJ does not have nutritional label and expiry date. Hence, this study aimed to quantify the bioactive compounds in commercially-sold baobab juice in Malawi, evaluate the antioxidant properties and determine the effect of different storage conditions on the bioactive compounds stability of commercially sold baobab juice in Malawi.

Materials and Methods

Chemicals and reagents

Reagents and chemicals of analytical and HPLC grades used in the experiments were purchased from reputable chemical companies from the United Kingdom, Germany and United States of America.

Commercial Baobab Juice (CBJ)

Commercial baobab juice was obtained from local supermarkets in Malawi and transported by air in cooler box containers with ice blocks to University of Leeds, UK. Juice samples were immediately transferred to a cold room (-18 ^{0}C) at the Food Technology Laboratory, School of Food Science, until analysis.

Bioactive compounds in the CBJ

Identification and quantification of chemical compounds in baobab samples was done using RP-HPLC. Other quality attributes such as total phenolic contents and antioxidant activity were also quantified. The extraction, identification and quantification procedures were optimised following the method of Pimpão et al. [13]. Phenolic aglycones were fingerprinted and quantified according to the procedure described by Tembo et al. [11]. The HPLC analysis was done by injecting a 20 µL sample into a Gemini C18 column (250 x 4.6 mm, 5 µm) placed at 35 °C. The mobile phase was 0.1% (v/v; formic acid: deionised water) formic acid (solvent A) and 80% (v/v; deionised water) methanol (solvent B). The flow rate of the solvent was 0.5 mL/min. The gradient elution begins with 10% B to a maximum of 30% B at 15 min and remained 30% B until 45 min, and finally, 10% solvent B was attained and maintained at this level for 10 min until equilibration. Compounds identification was achieved by the spectra and retention time of the compounds with that of their corresponding standards, dissolved in 80% methanol and 20% water. Procyanidin B_2 and (-)-epicatechin were detected at 284 nm. Quantification of the compounds was achieved using calibration curves of the standards at different concentrations.

Determination of organic acids

The organic acids [ascorbic acid (AA), tartaric acid (TA) dehydroascorbic acid (DHA), citric acid (CA), and malic acid (MA)] were analysed according to the procedures described by Pimpão et al. [13]. The vitamin C and dehydroascorbic acid content of the baobab juice was quantified following the procedure of Chebrolu et al. [14]. Briefly, about 20 mL of the juice was added to 40 mL metaphosphoric acid (0.3 g/L) and the mixture was thoroughly mixed to obtain a homogenised solution. Supernatant obtained by centrifugation at 4 °C for 10 min at 4000 rpms was used for the determination. The determination of AA and DHA were done following a pre-column derivatisation. 2-carboxy ethyl phosphine hydrochloride (TCEP, 5 mg/L) was used for the derivatisation. DHA was measured by difference between total ascorbic acid and ascorbic acid measured before reduction.

Identification of organic acids was done using HPLC equipment (UFLCXR, Shimadzu, Japan). A C18 column (250 x 4.6 mm, 5 µm; Gemini, UK) conditioned at 25 °C was used. 20µL sample was injected into the column. An isocratic condition with flow rate of 0.5 mL/min for 15 min was employed and the mobile phase was potassium dihydrogen phosphate (10 mM, pH 2.6). Identification of the compounds namely; tartaric acid, malic acid, citric acid and ascorbic acid AA were achieved through their chromatogram at 210 and 254 nm. The organic acids were quantified by the use of calibration curve obtained from standard concentrations between 5 to 100 mg L^{-1}

Determination of (-)-epicatechin and procyanidin B2 (flavan-3-ol)

The determination of flavan-3-ol [(-)-epicatechin and procyanidin B_2] was done using a reversed phase high performance liquid chromatography. The solvents were solvent A; formic acid: (0.1%) and solvent B; methanol (80%), flow rate was 0.5 mL/min. The HPLC programme started with gradient elution with 10% B to 30% B for 15 min and was maintained at this constant rate for 45 min, after which a final washing with 10% solvent B was was done for 10 min. Detection of the compound chromatogram was done at 284 nm. The retention time and the UV spectra of (-)-epicatechin and procyanidin B_2 were compared with that of spiked standards, dissolved in 80% methanol. The compounds were quantified through the use of standard curves obtained from different concentrations

of each standard.

Determination of phenolic compounds

The phenolic compounds of the CBJ were extracted following the method described by Tembo et al. [11]. The TPC (Total Phenolic Content) was determined using Folin-Ciocalteu assay [15]. The blank and sample absorbance were read on UV spectrophotometer at 765 nm. Quantification was done using a calibration curve plotted with different concentrations of gallic acid.

Determination of antioxidant activities

The FRAP (ferric reducing antioxidant power) was determined according to the procedure of Benzie and Strain [16] with some modifications. Aliquot amount (0.2 mL) of the juice extract samples were added to 6 mL FRAP reagent. The solution was vortexed and allowed to stand (10 min) at ambient condition, afterwhich absorbance was read at 593 nm in a UV spectrophotometer. Total equivalent antioxidant capacity (TEAC) was determined using ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid) [17]. Aliquot amount (0.1 mL) of the juice extract samples were mixed with 3.9 mL ABTS, and the solutions were allowed to stand for 106 min at 25 °C. Absorbance of the samples were read at 734 nm. For both FRAP and ABTS assays, quantification were achieved using Trolox calibration curves as described before [8].The DPPH radical scavenging activity was determined by the assay described by Bolarinwa and Muhammad [18], with some modifications. The juice extract samples (0.1 mL) were homogenised with DPPH methanol (0.1 mM, 3.9 mL) solution, allowed to be the dark at ambient condition for 30 min to prevent oxidation of the compounds. Absorbance of the samples were read at 517 nm

Storage

Effect of storage conditions on the bioactive components of the CBJ was done by hygienically dispensing 15 mL of the juice into a screw capped tubes. The tubes were divided into three groups; group one were refrigerated (6 °C), group two and three were kept in incubators set at 15 and 30 °C. All the tubes were left in this storage conditions for 49 days.

Statistical analysis

Results for phenolic compounds, organic acids and antioxidant activities were compared reading using using Tukey's test at $p \leq 0.05$ significant level. Pearson's Correlation Coefficient Test at $p \leq 0.05$ was employed to establish relationship between the quality attributes of the commercially-sold baobab juice.

Results and Discussion

RP-HPLC identification and quantification of chemical compounds in baobab samples

The organic compounds structures in the CBJ samples are showed in supplementary material 1. The results of the recommended validation techniques; linearity, limit of detection, recoveries, limit of quantification, for quantitative chemical analysis [19] employed to validate the HPLC method showed $R^2 \geq 0.999$, indicating higher linearity (supplementary material 2). The selectivity and sensitivity of the method was determined through the limit of detection while the minimum amount of compound to be quantified in the sample extracts was determined through limit of quantification.

Organic acids

Organic acids composition in food products especially fresh fruits and derived juices is among the most important criteria used by customers when choosing products in the markets. This is because organic acids are essential for nutrition and health, as well as stability and safety of food products. The level of AA, DHA, CA, MA and TA in the CBJ is shown in table 1.

The level of AA in the commercial baobab juice (50.9 mg/100 g FW) was lower than that of fresh baobab fruits pulp (352 mg/100 g FW) and HTST pasteurised baobab juice (309 mg/100 g FW) reported by Tembo et al. (2017) and USDA (2015), respectively. The level of dehydroascorbic, a component of vitamin C was 35.80 mg/100 FW in CBJ. This value is lower than the dehydroascorbic value (115 mg/100 g FW) reported for fresh baobab pulp [11]. This could be due to thermal degradation of the heat labile vitamin C components, as a result of high pasteurisation temperature employed for CBJ by commercial processors. The contents of CA, MA and TA in the commercial baobab juice were 1046.45, 1384.02 and 1178.26 mg/100 mL, respectively (Table 1). The mean citric and malic acid contents recorded in CBJ were lower than the malic acid (3300 mg/100 g FW) and citric acid (2360 ± 28.8

Table 1: Organic acids, flavan –3-ol, phenolic compounds and antioxidant activity of commercial baobab juice in Malawi.

Compound	Content (mg/100 mL)	Antioxidant Activity	Concentration mg/100 mL)
Ascorbic acid	50.93 ± 3.99	FRAP	71.27±0.04
Dehydroascorbic acid	35.80 ± 4.00	DPPH	80.94±0.72
Citric acid	1046.45±150.77	ABTS	112.62±0.45
Malic acid	1384.02±135.20	**Others**	
Tartaric acid	1178.26±28.61	TPC	1112.22 ±3.47
Procyanidin B_2	98.47 ±11.33		
(-)-Epicatechin	6.072 ± 0.63		

FRAP= Ferric reducing antioxidant power; DPPH = 2, 2–diphenyl-1-picrylhydrazyl; ABTS =2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); TPC= Total Phenolic. Values are means of three independent samples ± SD.

mg/100 g FW) reported for fresh baobab pulp [11]. However, the level of tartaric acid in CBJ was higher than that of fresh baobab pulp [11]. This could be attributed to added tartaric acid during juice processing, as this is the common practice of juice processors in Malawi.

Flavan-3-ol

The most common and major flavonoids in foods is flavan-3-ol [20]. The (-)-epicatechin and procyanidin B2 contents of CBJ is presented in table 1. Interestingly, CBJ contains more procyanidin B2 than (-)-epicatechin. The procyanidin B_2 content of CBJ is higher than that of other fruits products [13]. Thus, commercial baobab juice is a rich source of dietary flavonoids especially flavan-3-ol. The concentration of (-)-epicatechin in CBJ is within the range reported for millet [21]. Consumption of CBJ can therefore complement known rich sources of dietary flavonoids, including tea and coffee which are relatively expensive and unaffordable to most rural communities of Malawi, due to wide spread poverty.

Total phenolic contents (TPC) and antioxidant activities of the CBJ

Phenolic compounds are implicated in health promoting and disease preventive roles [8]. Preliminary screening of nutraceutical, herbal juices and other functional foods for bioactive compounds is generally done using TPC and antioxidant activity assays [22]. The TPC serves as an indicator for total antioxidant capacity [23]. The TPC and antioxidant capacity results (Table 1) showed that the CBJ contains higher TPC (1112.22 mg/100 g FW) than the TPC concentration 68.42 mg GAE/100 mL in orange juice [24],as well as finger millet (589.12 mg/100 g) [21], which is a known rich phenolic source. The level of TPC in CBJ is comparable with well-known medicinal plants (*Moringa oleifera*) [25] and health promoting leafy vegetables (*Solanum macrocarpon, Talinum fruticosum, Corchorus olitorius and Amaranthus spp.)* [26]. In addition, CBJ is among top 15 out of the 100 foods that are common polyphenols sources [27].

As shown in table 1, CBJ contains high FRAP, DPPH, and ABTS levels. The FRAP antioxidant activity of the juice is 71.27 mg TEAC/100 g FW, the DPPH is 80.94% and ABTS is 112.62 mg TEAC/100 g FW. Mean DPPH value was higher in CBJ than in fresh baobab pulp 50.93% [11]. On the other hand, FRAP and ABTS values were lower than those reported in fresh baobab pulp; 4.66 x 10^3 and 1.68 x 10^3 mg TEAC/100 g FW [11].Total antioxidant activity values obtained in CBJ are comparable with values reported for pure orange juice (71 mg TEAC /100 g FW) and asparagus (75 mg TEAC / 100 g FW), but higher than values for pure grapefruit (54 mg TEAC /100 g FW) and potato vegetables (54 mg TEAC /100 g FW), quince and jujube juice [28, 29]. Higher antioxidant activities in the CBJ could be due to high concentration of ascorbic acid and flavan-3-ol in the juice. Variation in levels of antioxidant activity between CBJ and fresh baobab pulp is ascribed to processing techniques and quality of raw materials used. In addition, bioactive and nutritive value of baobab pulp could be affected by environmental factors, land, and geographical condition as well as storage conditions of the fruits [10]. Fruit processors commonly store baobab pulp on the shelf at room temperature 27±2 ◦C for longer periods after harvest. This may lead to degradation of the phytochemicals and loss of quality attributes.

Influence of storage conditions on the ascorbic acid contents of the CBJ

Retention studies of micronutrients and phenolic compounds are important to food technologists for quality control or selection of a suitable processing technique. The results of the effects of storage conditions on the organic acids content of the CBJ are presented in table 2.

Ascorbic acid contents of the CBJ samples was stabled at 15 °C, but unstable in the refrigerated samples (6 °C) and the samples stored at 30 °C. After 49 days of storage, the percent retention of ascorbic acid in the stored samples were 12.60% and 16.10% for samples stored at 6 °C and 30 °C, respectively, and 91.1% for samples stored at 15 °C. This indicates that storage at 15 °C retains vitamin C of the samples better. However, reverse effect was observed for dehydroascorbic acid (DHA). Percent retention of DHA in the stored samples were 28.58% and 16.59 % for samples stored at 6 °C and 30 °C, respectively, and 10.92% for samples stored at 15 °C. This was not surprising as major loss of ascorbic acid is through oxidation to dehydroascorbic acid. Loss of AA is associated with evolution of DHA at the first instance. Higher retention of vitamin C in the samples stored at incubation temperature of 15 °C could be due to the concentration of the total solute in the samples, which was probably responsible for protection against vitamin C (AA and DHA) degradation at this storage condition. It is important to note that in addition to sugar and vitamin C, the Malawian fruit processors add additives such as sodium benzoate to stabilise the juice after pasteurisation. Protective mechanism might be temperature dependent which is optimum at 15 °C storage temperature.

Vitamin C degradation kinetics in CBJ is presented in figure1. The degradation of vitamin C in CBJ samples stored at refrigeration condition (6 °C) is showed a first ($R_6^2 \geq 0.9794$; k = 0.0348 day^{-1}) and second ($R_{15}^2 \geq 0.6156$; k = 8.18 x 10^{-4}(mg. $Day)^{-1}$) order kinetics of degradation with half-lives (ln 2/k, first order), and $1/kC_0$; second order of 19.9 days and 10.23 days, respectively. At 15 °C, vitamin C loss better fitted zero order kinetics ($R_{15}^2 \geq 0.6156$; k = 0.8272 $mgday^{-1}$) of degradation compared to first ($R_{15}^2 \geq 0.6069$; k= 0.0082 day^{-1}) and second ($R_{15}^2 \geq 0.5711$; k = 0095 $(mgday)^{-1}$ kinetics. Vitamin C (ascorbic acid) was more stable and the equation predicted a much longer half-life ($C_0/2k$; 73 days) where C and k denote initial concentration and rate constant, respectively, which was not attained during the study. At 30 °C, data best fitted zero order kinetics of degradation ($R_{30}^2 \geq 0.9613$; k = 1.977 $mg-day^{-1}$). It is not surprising that the half-life ($C_0/2k$; 30 days) of ascorbic acid is less than 2-fold compared to half-life at 15 °C. Temperature increases rate of degradation by increasing frequency of colliding species. However, the half-life of ascorbic acid at 30 °C is higher than half-life at refrigeration condition (6 °C). It is possible that other chemical compounds including organic acids and phenolic compound are responsible for better retention/stability of ascorbic acid at elevated temper-

ature than under refrigeration temperature. However, storage at high temperature for long periods may have negative effects on the organic acids content of food samples. In addition, rate of non-enzymatic degradation of vitamin C (Vitamin C browning) could be influenced by the sugar and amino acids contents of the juice, plus acidic conditions (alanine and arginine) [30]. Past studies also reported zero-order vitamin C non-enzymatic degradation kinetics in fruits juices and model systems [31].

Data from this study showed that competitive kinetic models are temperature dependent. Apart from vitamin C, past studies indicated that other vitamins and phytochemicals in food and model systems follow first order kinetic model of degradation [32]. For example, degradation kinetics of carotene and vitamin E under four different temperatures followed a first-order kinetic model [33]. The difference could be the magnitude of rate constants which influence half-lives of all models and overall stability of micronutrient. However, mixed models of zero, first and second degradation kinetics of vitamin C in the same food or model systems, which are oxygen concentration and temperature dependent was also described by Gambo-Santos et al. [34]. Thus, the findings of this research are in congruent with past studies. According to the data obtained in this study, the quality of CBJ in terms of vitamin C, is better retained at 15 ˚C than refrigeration (6 ˚C) and storage at 30 ˚C. This information may be important to baobab fruit processors in Malawi and elsewhere. Phenolic antioxidants compounds which are relatively stable and available at higher temperature may be responsible for retarding vitamin C degradation synergistically.

Effect of storage conditions on the organic acids content of the CBJ

The levels of organic acids in fruits depend on the fruit categories and varieties. Table 2 shows the organic acids content of the CBJ stored at different conditions for 49 days. In general, there are variations in the levels of the organic acids during storage. Citric and malic acids were mostly affected at higher storage temperatures (15 and 30 ˚C) compared to tartaric acid. The level of all acids was fairly stable at refrigeration temperature. At refrigeration condition (6 °C), the mean CA value was 1077.85 mg /100 mL and values ranged from 799.41 to 1220.97 mg/100 mL. The level of CA increased significantly on day 21, but the increment was constant till day 49. The mean CA level at 15 °C was 1022.05 mg/100 mL and values ranged from 917.47 to 1181.98 mg/100 mL. The mean CA level remained unchanged for the initial 14 days, then decreased on day 21 after which the reduction was gradual until day 49. Finally, at 30 °C, the mean CA concentration was 941.66 mg/100 mL and with values in the ranges of 909.54 to 1046.45 mg/100 mL. The level of CA decreased on day 7, and remained in that level till day 49. These results indicate that the level of CA was affected by storage conditions.

The concentration of malic acid also varied during storage, and was similar to the observation reported during storage of citric acid. During refrigeration condition (6 °C), the mean level of MA was1063.03 mg/Land values ranged from 170.47 to 1936.63 mg/100 mL. A significant decrease ($p \leq 0.05$) in the concentration of MA to 198.32 mg (100 m L)$^{-1}$ was observed on day 7, which then stabilised till day 14; rose again on day 21 and increased significantly at the end of the storage periods. After a significant decrease ($p \leq 0.05$) on day 7, the concentration of MA was fairly stable during storage at 15 and 30 °C. Unlike citric acid, the mean concentration of malic acid was generally not significantly affected by temperature towards the end of the storage time.

Tartaric acid (TA) was the least influenced by storage temperature. Tartaric acid had highest values among all the organic acids, and was more stable compared to citric and malic acid. The overall mean value of TA was 1152.35 (100 m L)$^{-1}$and values ranged from 1055.51 to 1202.66 (100 m L)$^{-1}$. A very small percentage difference (12.24%) between highest and lowest value is a clear indication that it was stable over a wider temperature and storage periods. This indicates that TA can effectively serve as a preservative to prevent oxidative and microbiological degradation of food due to its stability.

Evolution of organic acids in stored foods have been associated with fermentation, esterification and oxidation processes in most plant foods [35]. Hydrolysis of carbohydrates and degradation of phenolic compounds may be responsible for fluctuations and high levels of organic acids reported at the end of the storage period, especially in the samples stored at 15 and 30 ˚C. In general, tartaric acid did not degrade markedly during storage at all temperatures. Results from this study indicate that despite fluctuations of CA and MA, levels still remained higher in the juice at all storage temperatures. It is important to note that ascorbic acid did not degrade much at 15 °C compared to refrigeration temperature (6 °C) contrary to past reports for fruit juices. This could be due to other protective mechanisms against ascorbic acid degradation employed by other antioxidants such as phenolic compounds which tend to be readily available at elevated temperatures.

Effects of storage conditions on flavan-3-ol contents of the CBJ

The flavonoids; oligomeric and polymeric flavan-3-ols have been reported to possess different antioxidant capacities [36]. Phytochemical evolution is not uniform due to several factors such processing procedures and use of preservatives employed for food products and storage conditions. Thus, it is essential to evaluate changes in chemical attributes in order to assess effectiveness of processing and preservation techniques on product quality. Such information is necessary to food technologists and consumers. Results of the effects of storage condition on procyanidin B_2 and (-)-epicatechin contents of the stored CBJ samples are presented in Table 3. The results showed that storage time and temperature affected levels of both procyanidin B_2 and (-)-epicatechin. Samples stored at 15 ˚C showed significantly ($p \leq 0.05$) higher levels of procyanidin B_2 and (-)-epicatechin compared to other stored samples. At 6 ˚C, the mean values of procyanidin B_2 and (-)-epicatechin were 89.20 mg/100 mL and 15.63 mg/100 mL, respectively. Procyanidin B_2 values ranged from 67.75 to 98.47 mg/100 mL while (-)-epicatechin values ranged from 9.58 to 20.92 mg/100 mL. By day 49, procyanidin B_2 and (-)-epicatechin retention were 68.8% and 45.8%, respectively. It seems

Table 2: Organic acid content (mg/100 g FW) in commercial baobab juice stored at three different temperatures.

Temp (°C)	Days	AA	DHA	CA	MA	TA
6	0	84.89 ± 0.67^{a}	35.80 ± 2.31^{ab}	1046.45±150.77^{abcdefg}	1384.02±135.20^{bcd}	1107.68±101.30^{ab}
	7	53.36 ± 0.67^{abcd}	26.01±4.37^{abcdefg}	865.65±123.02^{gh}	198.32±66.42^{g}	1178.26±28.61^{ab}
	14	32.10 ± 0.80^{cde}	28.66 ± 4.37^{abc}	799.41±51.62^{h}	170.47± 13.11^{g}	1186.93±6.36^{ab}
	21	12.44 ± 0.6^{de}	31.15 ± 3.40^{abc}	1220.97±30.09^{ab}	948.60±71.97^{cdef}	1181.82±48.89^{ab}
	28	11.75 ± 0.88^{e}	17.44±4.60^{bcdefg}	1248.16±87.56^{a}	1025.25±286.56^{bcdef}	1187.00±32.22^{ab}
	35	10.93 ± 0.90^{e}	17.06± 4.10^{bcdefg}	1169.80±34.74^{abcde}	1404.42±38.49^{bc}	1189.89±11.50^{ab}
	42	11.81 ± 0.90^{e}	13.36± 3.76^{bcdefg}	1099.96±22.63^{abcdef}	1436.54±256.03^{b}	1091.78±45.22^{ab}
	49	10.70 ± 0.90^{e}	10.23± 4.36^{cdefg}	1172.36±23.27^{abcd}	1936.63±64.74^{a}	1116.94±13.18^{ab}
15	7	77.36 ± 1.64^{ab}	46.31 ± 5.14^{a}	1181.98±15.90^{abc}	974.67±20.44^{bcdef}	1055.51±15.11^{c}
	14	84.52 ± 2.00^{a}	13.74 ± 0.00^{bcdefg}	1052.57±17.25^{abcdefg}	936.90±9.40^{cdef}	1123.08±38.14^{ab}
	21	84.15 ± 1.78^{a}	ND	991.90±56.86^{cdefgh}	916.31±70.43^{def}	1143.66±53.87^{ab}
	28	76.60 ± 1.92^{ab}	2.21 ± 1.82^{g}	999.98±48.20^{bcdefgh}	756.89±27.70^{f}	1153.97±35.21^{ab}
	35	74.79 ± 2.38^{ab}	5.22 ± 0.88^{efg}	1039.69±54.38^{abcdefg}	822.46±110.52^{f}	1202.66±12.88^{a}
	42	76.80 ± 2.02^{ab}	16.80 ± 5.83^{bcdefg}	917.47±37.53^{fgh}	1350.25±458.41^{bcde}	1097.30±33.45^{ab}
	49	77.36 ± 2.05^{ab}	3.91± 0.63^{fg}	946.36±7.89^{efgh}	955.60±44.57^{bcdef}	1202.66±12.88^{a}
30	7	60.10 ± 4.00^{abc}	27.28 ± 3.49^{abcde}	909.54±12.47^{fgh}	778.81±27.43^{f}	1178.26±28.61^{ab}
	14	65.86 ± 4.34^{abc}	12.75 ± 12.75^{bcdefg}	882.77±31.33^{fgh}	770.85±50.44^{f}	1186.93±6.36^{ab}
	21	51.36 ± 3.04^{abcd}	16.66 ± 3.90^{bcdefg}	915.31±22.99^{fgh}	978.10±319.64^{bcdef}	1181.82±48.89^{ab}
	28	39.18 ± 4.85^{bcde}	9.87 ± 4.31^{cdefg}	972.47±54.37^{cdefgh}	716.61±27.03	1187.00±32.22^{ab}
	35	18.76 ± 2.41^{de}	25.36 ± 2.31^{abcdefg}	950.68±70.03^{defgh}	715.50±131.50	1189.89±11.50^{ab}
	42	11.18 ± 0.76^{e}	9.75 ± 2.53^{cdefg}	919.06±66.53^{fgh}	746.92±38.32^{cdef}	1091.78±45.22^{ab}
	49	13.64 ± 1.35^{e}	5.94 ± 3.22	937.02±41.55^{fgh}	884.31±23.97^{ef}	1116.94±13.18^{ab}

FW = Fresh weight; AA = Ascorbic acid; DHA = Dehydroascorbic acid; CA = Citric acid; MA= Malic acid; TA = Tartaric acid; ND = Not detected. Values are means of three independent samples ± SD. Mean values within a column with different superscript letters indicate significant differences (Tukey's test $p \leq 0.05$).

procyanidin B_2 and (-)-epicatechin were lower but generally remain unchanged at 6 °C considering the fact that most changes were not significance. At 15 °C, overall mean values of procyanidin B_2 and (-)-epicatechin were 213.53 mg/100 mL (22.01 to 245.11 mg/100 mL) and 45.41 mg/100 mL (16.34 to 73.03 mg/100 mL), respectively. The level of procyanidin B_2 decreased significantly ($p \leq 0.05$) between day 0 and day 7, rose back to initial levels between day 7 and day 14, increased steadily and significantly until day 42, and finally decreased slightly on day 49. However, (-)-epicatechin values remained unchanged between day 0 and day 14, increased significantly ($p \leq 0.05$) between day 21 and day 28, and remained stable until the end of storage (day 49). By the end of the storage periods, procyanidin B_2 and (-)-epicatechin increased by 106% and 133%, respectively. Samples stored at 30 °C varied in the levels of procyanidin B_2 (16.60 to 224.89 mg/100 mL) and (-)-epicatechin (0 to 41.94 mg/100 mL). This indicate high level of degradation of the bioactive compounds at this storage temperature. By the end of storage period, procyanidin B_2 and (-)-epicatechin showed a decrease of 83 and 100%, respectively.

The increase in procyanidin B_2 and (-)-epicatechin concentration has been reported to be enhanced by the solute contents of the food sample [37]. In this study, the high level of sugar in the juice may be responsible for the increase in the bioactive compounds during storage. On the other hand, decrease in the bioactive compounds could be due to addition of benzoic acid as preservative. This results in reduction in the pH of the juice, consequently promoting the hydrolysis of polymers to oligomeric or monomeric compounds. Drastic loss of flavan-3-ols at 30 °C suggested decomposition or breakdown to smaller organic molecules including phenolic acids, carboxylic acids, aldehydes and ketones [38]. Thus, storage of CBJ at 15 °C is favourable, as levels of the bioactive compounds (flavan-3-ols) were higher and vitamin C was more stable compared to other temperatures.

Effects of the storage conditions on the total phenol and antioxidant activities of the CBJ

The changes in total polyphenolic content of the stored CBJ is presented in table 3. Sample stored at refrigeration condition (6 °C) and 30 °C were more affected compared to those at 15 °C. This trend is similar to other attributes (vitamin C, organic acids and flavan-3-ols) described previously. Fluctuation in the level of TPC was common in all samples. At 6 °C, the mean TPC was 997.17mg GAE/100 g FW and values ranged from 852.78 to 1122.78 mg GAE/100 g FW. There was a continuous significant decreased ($p \leq 0.05$) in the level of TPC until day 14, and the values increased on day

Table 3: Flavan-3-ol, total phenolics and antioxidant activity in commercial baobab juice stored at different temperatures.

Temp (°C)	Days	PRO (mg/100 g FW)	EC (mg/100 g FW)	TPC (mg/100 g FW)	FRAP (mg/100 g FW)	DPPH (%)	ABTS (mg/100 g FW)
6	0	98.47±11.33 ^{ef}	20.92±2.18 ^{f}	1112.22±3.47^{c}	71.27±0.04^{g}	80.94±0.72 ^{bc}	112.62±0.45 ^{abcde}
	7	105.70±9.26 ^{ef}	15.07±0.70 ^{fgh}	947.22±19.32^{g}	76.78±0.09^{ef}	80.66±0.28 ^{cde}	116.05±0.45 ^{ab}
	14	107.51±8.22 ^{ef}	18.29±0.89 ^{fg}	882.78±2.55^{h}	63.01±0.57 ^{jk}	85.18±0.05^{b}	114.88±0.45 ^{abcde}
	21	99.41±8.45 ^{ef}	17.81±1.42 ^{fg}	1029.44±5.85^{f}	75.02±0.29^{f}	78.93±0.16 ^{fgh}	117.53±0.39 ^{a}
	28	101.99±10.61 ^{ef}	18.68±1.04 ^{fg}	1071.11±0.96^{de}	71.90±0.27^{fg}	87.19±0.28 ^{a}	115.85±0.30 ^{abc}
	35	88.98±14.20 ^{ef}	15.11±0.65 ^{fgh}	1122.78±25.51^{c}	87.46±0.99 ^{b}	81.06±0.72^{bc}	117.21±0.11 ^{a}
	42	71.49±8.04 ^{f}	12.80±1.18 ^{gh}	1122.78±38.49^{c}	81.72±0.92^{cd}	79.70±0.21^{def}	115.33±0.30 ^{abcd}
	49	67.75±11.89 ^{f}	9.58±1.03 ^{hi}	852.78±3.47 ^{h}	65.96±0.23^{ij}	79.12±0.09 ^{efgh}	111.00±0.30 ^{bcde}
15	7	22.01±1.84 ^{g}	19.49±2.05 ^{f}	760.00±3.00 ^{ij}	52.74±0.63^{m}	76.39±0.35^{ij}	105.90±0.51 ^{fghi}
	14	93.40±12.82 ^{ef}	16.34±2.71 ^{fg}	935.00±4.41^{g}	60.14±0.44^{kl}	82.02±0.05^{c}	115.27±0.59 ^{abcd}
	21	355.35±53.30 ^{a}	73.03±7.68^{a}	955.00±3.33^{g}	69.36±0.29 ^{hi}	85.89±0.76 ^{ab}	111.46±0.40 ^{bcdef}
	28	314.96±1.12 ^{a}	64.65±0.40 ^{b}	1160.00±3.33^{b}	80.86±0.44^{cd}	81.06±0.43^{bc}	109.71±1.07 ^{defg}
	35	261.46±3.31 ^{b}	45.77±0.08 ^{cd}	1092.22±3.47 ^{cd}	83.88±0.31^{bc}	79.55±0.44 ^{defg}	110.62±0.11 ^{bcdef}
	42	245.11±0.33 ^{b}	50.85±0.23 ^{c}	1075.00±3.33^{d}	81.87±0.15 ^{cd}	80.82±0.57 ^{cde}	109.19±0.19 ^{efgh}
	49	202.45±0.31 ^{cd}	47.75±0.30 ^{cd}	1227.22±0.96 ^{a}	84.03±0.17 ^{bc}	77.63±0.65 ^{hi}	103.70±0.40 ^{hi}
30	7	224.89±11.55 ^{c}	41.94±0.14 ^{d}	1100.56±2.55 ^{cd}	75.73±0.3^{f}	77.88±0.19 ^{ghi}	110.16±0.51 ^{cdef}
	14	178.09±0.36 ^{d}	32.18±0.13 ^{e}	1020.56±0.96 ^{f}	93.65±4.86 ^{a}	78.03±0.28 ^{fghi}	104.41±0.30 ^{ghi}
	21	118.89±3.38 ^{e}	5.93±0.07 ^{ij}	1041.39±1.73 ^{ef}	79.50±0.49^{de}	75.06±0.67^{j}	100.40±0.22 ^{ij}
	28	21.07±0.62 ^{g}	2.10±0.06 ^{j}	790.00±3.00^{i}	61.73±0.24^{k}	65.32±0.51^{k}	94.71±6.11 ^{j}
	35	3.93±0.99 ^{g}	1.46±0.10 ^{j}	751.67±0.8 ^{j}	57.42±0.19^{l}	55.38±0.62^{l}	74.87±6.23 ^{k}
	42	10.82±8.09 ^{g}	ND	575.28±3.849^{k}	43.45±1.86^{n}	44.62±0.39^{m}	70.48±0.30 ^{k}
	49	16.60±0.00 ^{g}	ND	503.89±1.27 ^{l}	36.88±0.12^{o}	36.45±1.24^{n}	69.51±0.30 ^{k}

FW = Fresh weight; PRO = Procyanidin B2; EC = (-)-Epicatechin; TPC= Total Phenolic; FRAP= Ferric reducing antioxidant power; DPPH = 2,2–diphenyl-1-picrylhydrazyl; ABTS =2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); ND = Not detected. Values are means of three independent samples ± SD. Mean values within a column with different superscript letters indicate significant differences (Tukey's test $p \leq 0.05$).

21, remained stable until day 42 and decreased on day 49. At 15 °C, the mean TPC was 998.60 mg GAE/100 g FW and values ranged from 7760.00 to 1227.22 mg GAE/100 g FW.

The level of TPC dropped significantly on day 7, and increased steadily between day 14 and 35, dropped on day 42, and increased at the end of storage period. However, initial level of TPC increased by 10% at 15 °C by day 49. At 30 °C, the mean TPC (828.67 mg GAE/100 g FW) was lowest compared to other storage temperatures (6 and 30 °C). TPC values ranged from 503.89 to 1112.22 mg GAE/100 g FW. The level of TPC decreased steadily with storage time at 30 °C, a direct indication of continuous degradation of phenolic compounds. Only 45% of initial TPC remained by the end of storage period (49 days, 30 °C). Increase in TPC during storage was reported by other authors [28, 38]. According to Piljac-Žegarac *et al.* [39], reaction between sugars and TPC could results in reduction in TPC concentration in foods. However, CBJ contains high level of sugar added as sweetener during processing. The decrease in TPC of the juice at higher storage temperature (30 °C) could be attributed to loss of heat sensitive phenolic compounds which are susceptible to chemical oxidation at high temperature [40]. An increase in TPC that was evident at 15 °C may be ascribed to transformation of proanthocyanidins to formation of varied phenolic compounds or hydrolysis of the compounds into monomeric or dimers during long period of storage [41]. This is well supported and is in agreement with high values of procyanidin B_2 and (-)-epicatechin observed in the CBJ juice stored at 15 °C compared to samples stored at 6 and 30 °C.

Effects of storage conditions on the antioxidant activities of the CBJ

Storage conditions influence stability of natural flavonoids and their resultant antioxidant activities in food. Foods including juice may not be consumed immediately after purchase, thus knowledge of changes in the functional properties during storage is necessary for selection of storage conditions. Bioactive compounds present in food or biological systems have different antioxidant activity mechanisms which are based on structural conformation (lipophilic or hydrophilic) [22]. Lipophilic and hydrophilic antioxidants were assayed using ABTS, and DPPH assay was employed for lipophilic antioxidants activities [42].

The mean FRAP value of the samples stored under re-

frigeration condition (6 °C) was 71. 41 µM TEAC/100 g FW and levels ranged from 65.96 to 87.46 µM TEAC/100 g FW. While at 15 °C, the mean FRAP value was 70.70 µM TEAC/100 g FW and levels ranged from 52.74 to 87.46 µM TEAC/100 g FW. Finally at 30 °C, the mean FRAP value was 63.56 µM TEAC/100 g FW and levels ranged from 36.88 to 87.46 µM TEAC/100 g FW. It is clearly evident that mean values were decreasing with storage temperature. The values of FRAP decreased by 7.45 and 48% by the end of 49 days storage period at refrigeration (6 °C) and 30 °C, respectively. However, in the case of the samples stored at 15 °C, there was an increase of 17.9% in the FRAP values by day 49. The increment in the FRAP levels could be due to high concentration of ascorbic acid and phenolic compounds in the CBJ.

Results of the effect of storage conditions on the DPPH radical scavenging activity of CBJ are shown in table 3. The DPPH value of 80.94% recorded for the CBJ was higher than the radical scavenging activity of some common fruits [27]. This could be due to high phenolic and sugar contents of the CBJ. However, long storage period resulted in reduction in the DPPH values of all the samples at various storage conditions (Tables 3). Lowest DPPH values recorded at higher storage temperature (30 ^{0}C) could be due to losses in the flavonoids contents of the CBJ at this temperature.

The mean values of ABTS were 110.70 ± 2.15 , 106.02 ± 3.53 and 89.68 ± 17.35 mg TEAC/100 mL at 6, 15 and 30 °C respectively. ABTS activity reduced from 117.53 to 111.00 at 6 °C; 115.27 to 103.70 at 15 °C and 112.62 to 69.51 mg TEAC/100 mL at 30 °C, indicating percentage reduction of 1.4, 7.5 and 38.3%, respectively in the samples stored at refrigeration (6 °C), 15 °C and 30 °C conditions. Thus, evolution of the ABTS activity was consistent with other antioxidant activity measurements (FRAP and DPPH).

In general, the stability of the nutritive value of the CBJ was compromised by the processing condition characterised by prolonged heat treatment and excessive usage of sugar and benzoic acid as potential preservatives. This calls for alternative pasteurisation methods and techniques to fully benefit from the rich natural nutrients in baobab fruit, i.e. the bioactive compounds.

Pearson's correlation

The results of the correlation coefficients between the quality parameters of CBJ are presented in table 4. The results indicate that the antioxidant activities (FRAP, DPPH and ABTS) and bioactive compounds (AA, TPC, Procyanidin B_2 (-)-Epicatechin) were positively correlated ($p \leq 0.01$). The correlation coefficients between antioxidant activities: FRAP and DPPH; FRAP and ABTS; and DPPH and ABTS were 0.70, 0.66 and 0.95, respectively. As indicated in table 4, AA was significantly correlated with TPC, procyanidin B2, (-)-epicatechin, FRAP, DPPH and ABTS. The TPC showed high correlation with procyanidin B_2 ($r = 0.58$, $p \leq 0.01$), epicatechin ($r = 0.55$, $p \leq 0.01$), FRAP ($r = 0.88$, $p \leq 0.01$), DPPH ($r = 0.80$, $p \leq 0.01$) and ABTS ($r = 0.76$, $p \leq 0.01$). The correlation coefficients of procyanidin B_2 with FRAP, DPPH and ABTS were 0.82, 0.51 and 0.41, respectively. The correlation between (-)-epicatechin and FRAP, DPPH and ABTS were 0.51, 0.51 and 0.41, respectively.

Procyanidin B_2 had strong positive correlation with the antioxidant activities compared to (-)-epicatechin. However, procyanidin B_2 and (-)-epicatechin had strong positive correlation ($r = 0.96$, $p \leq 0.01$). Concentrations of (-)-epicatechin and procyanidin B_2 increased proportionally ($R^2 > 0.92$) during storage at 15 °C (Figure 2). This suggest that depolymerisation of higher proanthocyanidins to procyanidin B_2 and (-)-epicatechin was optimum for both compounds at this temperature. The correlation between TPC and AA was lower than with flavan-3-ols. This could be due to thermal degradation of ascorbic acid. On the other hand, phenolic compounds are relatively stable at the relatively high temperature (15 and 30 °C) storage conditions. This could be responsible for higher antioxidant activities recorded for the samples stored at these high temperatures. Past studies also reported high positive correlation between AA, TPC, flavan-3-ols and antioxidant activi-

Table 4: Pearson correlation coefficients (r) between the quality attributes of commercial baobab juice in Malawi.

		DHA	CA	MA	TA	TPC	FRAP	DPPH	ABTS
AA		0.32	-0.184	0.005	-0.134	0.389**	0.238*	0.384*	0.276*
DHA			0.114	0.00	-0.158	0.018	-0.138	0.154	0.203
CA				0.569**	-0.18	0.166	0.041	0.247*	0.318**
MA					-0.70**	0.263*	0.149	0.163	0.204
TA						0.201	0.336**	0.126	0.079
TPC							0.879**	0.796**	0.760**
FRAP								0.703**	0657**
DPPH									0.953**
ABTS									
PRO									

AA = Ascorbic acid; DHA = Dehydroascorbic acid; CA = Citric acid; MA= Malic acid; TA = Tartaric acid; TPC = Total phenol content; FRAP = Ferric reducing antioxidant power; DPPH = 2, 2–diphenyl-1-picrylhydrazyl; ABTS = 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid); PRO = Procyanidin B_2; EC = (-)-Epicatechin. Significant at $p \leq 0.05$ (*) and $p \leq 0.01$ (**).

ties (FRAP, DPPH and ABTS) in fruits at lower temperature [29, 43] close to the one reported in this study. Hence, the results obtained in this study are consistent with those of previous findings and published data. The present study therefore, suggests that storage of CBJ at 15 °C enhances the level and better retain the bioactive compounds (total phenols, procyanidin B_2 and (-)-epicatechin) and thus, enhanced antioxidant activities of the juice.

Conclusion

Data obtained in this study showed that CBJ in Malawi contains high levels of bioactive compounds and have high antioxidant activities. The levels of phenolic compounds and antioxidant activities of CBJ were higher than that of some common fruits and vegetable products. However, significant losses of the CBJ bioactive compounds occurs when the juice is stored at 6 and 30 °C. In order to benefit maximally from the bioactive compounds in CBJ, the juice is better stored at 15 °C. Future research should investigate the effect of non-thermal preservation such as ultrasound, high hydrostatics pressure processing, membrane filtration, pulsed electric field, and natural preservatives methods on the stability of baobab juice bioactive compounds.

Conflict ofInterests

Authors declared no conflict of interest.

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