Effect of thermal treatment and storage on bioactive compounds, organic acids and antioxidant activity of baobab fruit (Adansonia digitata) pulp from Malawi

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Original Research Article

Effect of thermal treatment and storage on bioactive compounds, organic acids and antioxidant activity of baobab fruit (Adansonia digitata) pulp from Malawi

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Highlights

- HPLC analysis of bioactive compounds in baobab fruit

- Fresh baobab fruit pulp is rich source of vitamin C and flavan-3-ols

- Baobab juice shows very high antioxidant activity

- Effect of storage on quality of baobab juice was investigated

- Baobab fruit is potential raw material for novel functional foods

ABSTRACT

Bioactive compounds of baobab (Adansonia digitata) pulp from Malawi were investigated. The effect of thermal treatment and storage on selected quality attributes of the juice was also evaluated. Organic compounds were analysed by HPLC; total phenol content (TPC) and total antioxidant activity (FRAP, ABTS and DPPH) were measured by spectrophotometry. Malawi baobab pulp contains high levels of procyanidin B_2 (533 ± 22.6 mg/100 g FW), vitamin C (AA + DHA) (466 ± 2.5 mg/100 g FW), gallic acid (68.5 ± 12.4 mg/100 g FW) and (-)-epicatechin (43.0 ± 3.0 mg/100 g FW) and showed a maximum TPC of $1.89 \times 10^3 \pm 1.61$ mg GAE/100 g FW. The maximum antioxidant activity was $2.81 \times 10^3 \pm 92.8$ mg TEAC/100 g FW for FRAP, $1.52 \times 10^3 \pm 17.1$ mg TEAC/100 g FW for ABTS and 50.9 ± 0.43% DPPH for DPPH. Thermal pasteurisation (72 °C, 15 s) retained vitamin C which further showed extended half-life under refrigeration temperature (6 °C). Procyanidin B_2, (-)-

epicatechin, TPC and antioxidant activity fluctuated during storage. Antioxidant activity was significantly correlated ($p \leq 0.05$) with bioactive compounds and TPC.

Keywords: Adansonia digitata; Food composition; Food analysis; Bioactive compounds; Vitamin C; (-)-Epicatechin; Procyanidin B_2; Antioxidant activity; Thermal processing; Storage.

Chemical compounds studied in this article

Ascorbic acid (PubChem CID: 54670067); (-)-Epicatechin (PubChem CID: 72276); Gallic acid (PubChem CID: 370); Procyanidin B_2 (PubChem CID: 122738); (-)-Epigallocatechin-3-O-gallate (PubChem CID: 65064)

1. Introduction

Fresh fruits and derived products are a source of food and provide essential dietary micronutrients and bioactive compounds including vitamins, minerals and polyphenols, with several human health benefits (Gamboa-Santos et al., 2014; Igual et al., 2010). Bioactive compounds influence cellular activities that modify and reduce the risk of chronic diseases associated with oxidative stress because of their antioxidant (Aron and Kennedy, 2008; Fennema, 1996; Martínez-Flores et al., 2015). Antioxidants delay or prevent oxidation of a substrate (Kris-Etherton et al., 2004). In foods these compounds preserve quality by inhibiting enzymatic browning, lipid autoxidation (Shahidi and Zhong, 2015) as well as acting as antimicrobials (Chandrasekara and Shahidi, 2011; Govardhan Singh et al., 2013; Masola et al., 2009). However micronutrients and bioactives are affected by several factors including species, processing and storage (Parada and Aguilera, 2007; Zhang et al., 2010). Huge quantities of fresh fruits are wasted in sub-Saharan Africa due to gaps in knowledge of processing and postharvest handling techniques and inadequate opportunities for industrial processing. Post harvest losses in fresh fruits are estimated to be 5 to 35% in developed countries and 20 to 50% in developing countries (Ahmad and Siddiqui, 2015). Direct and indirect losses in fruits result in a reduction in quantity and quality for family consumption and sale. Best postharvest handling and processing practices minimise losses and ensure consumption of quality foods that can readily improve nutritional status of the indigenous population (Thiong'o et al., 2000).

Ripe baobab fruits are large, egg shaped, 15 to 20 cm long, with a hard woody outer shell covered with yellowish brown hairs and are filled with a dry white powdery pulp that covers brownish bean-like seeds. The pulp, which is usually eaten fresh, is acidic (pH 3.2) and has a slight lemon taste (Saka et al., 2007). Several studies (Besco et al., 2007; Coe et al., 2013; Shahat, 2006) have shown that baobab fruit pulp is very rich in

vitamin C with a content of up to 540 mg/100 g on fresh weight basis. Furthermore, the seeds contain high levels of polyphenols (epicatechin and procyanidin), provitamin A carotenoids and fatty acids (linoleic and oleic acids) and show high antioxidant activity (Rio et al., 2013; Vermaak et al., 2011). In Malawi, baobab fruits are processed into different products including juice, yoghurt, gruel, sour dough, oil, a coffee-like drink and dried as food reserves (Saka et al., 2002). Baobab products are achieved in Malawi through local processing knowledge and techniques which inevitably result in a fast deterioration of essential nutrients.

Processing conditions (temperature, time and mechanical agitation) selected during preparation and preservation affect quality attributes including ascorbic acid (AA) content, the main component of vitamin C in final plant products (Gamboa-Santos et al., 2014; Igual et al., 2010). Degradation products of AA include dehydroascorbic acid (DHA), 2-furoic acid (FA), 2-furaldehyde (F), 2,3-diketogulonic acid (DKG), 3-deoxypentosone (DP), and low molecular weight (LMW) compounds resulting from lactone ring opening. While DHA formation is reversible, lactone ring opening is irreversible and compounds have no vitamin C acitivity and may contribute to change in flavour and odour (Fennema, 1996). DHA and dicarbonyls may react with amino acids to form sorbanic acid, which can polymerise to form coloured compounds including 5-hydroxymethylfurfural (HMF) in anaerobic conditions. The loss of AA through oxidation is influenced by pH, oxygen and water. The rate of oxidation of ascorbic acid is accelerated by heat and light catalysed by metal ions especially Cu^{2+} and Fe^{3+} and enzymes (ascorbate oxidase and peroxidase) (Fennema, 1996). Thus understanding and controlling these factors is important for retention of vitamin C and overall quality of the final product.

The majority of smallholder farmers and rural communities in Malawi benefit from baobab products through consumption and sale. However, utilisation is limited due to insufficient knowledge on the effects of processing conditions for quality control. For instance, the current practice of processing baobab into juice relies heavily on uncontrolled thermal treatment and this may have great influence on the content of heat sensitive micronutrients. The high vitamin C content (AA + DHA) in baobab pulp is completely lost in the final juice due to excessive heating (Tembo, 2008).

Recently there is a growing interest to provide sufficient data on nutritinal value and quality changes during processing and storage of priority indigenous fruits of Sub-Saharan Africa because these are a cheap source of food as well as essential dietary micronutrients on which the resource poor can survive. Indigenous fruits from this region are a potential source of raw material for novel functional foods. Ndabikunze and co-workers (Ndabikunze et al., 2010) reported chemical composition of Vitex mombassae (smelly-berry), Adansonia

digitata (baobab), Uapaca kirkiana (wild loquat) and Sclerocarya birrea (marula plum) indigenous fruits from various Miombo woodlands of Tanzania. Similar chemical composition were analaysed in Parinari curatelifolia, Strychnos spinosa and Adansonia digitata fruits from Buhera district of Zimbabwe (Nhukarume et al., 2010). These studies show wide variation in chemical composition including bioactive compounds within and among fruit species. Such variation may be ascribed to environment, processing and analytical methods employed (Tembo, 2008).

There is sufficient knowledge and information on the nutritional profile and utilisation of commonly consumed fruits but such information is scarce for most indigenous fruits of Malawi (Tembo, 2008). Moreover most of the compositional information reported for these fruits is based on less sensitive and selective analytical techniques. To the best of our knowledge, there is no scientific study on the influence of thermal treatment and storage on bioactive compounds and antioxidant activity of baobab fruit products from Malawi using highly sensitive and selective analytical techniques. In the present study, we quantify ascorbic acid (AA), vitamin C (AA+ DHA), (-)-epicatechin, procyanidin B_2, gallic acid, (-)-epigallocatechin-3-O-gallate, 5-hydroxymethylfurfural (HMF) and organic acids in fresh baobab pulp and investigate quantitatively changes of some of these induced by thermal food preservation and pasteurisation. The content of ascorbic acid, vitamin C (AA + DHA), organic acids (citric and malic), procyanidin B_2, (-)-epicatechin, total phenols and antioxidant activity were measured in the pasteurised juice (72 °C, 15 s) during storage at 6, 15 and 30 °C for 60 days.

2. Materials and methods

2.1 Chemicals

Only chemicals of analytical and HPLC grade were used. Standards (ascorbic acid, citric acid, dehydroascorbic acid, malic acid, tartaric acid, gallic acid, (+)-catechin, (-)-epicatechin, procyanidin B_2, (-)-epigallocatechin-3-O-gallate,, 5-hydroxymethylfurfural), formic acid, sodium carbonate, iron (III) chloride anhydrous, hydrochloric acid and sodium acetate trihydrate were purchased from Fisher Scientific (UK); Folin-Ciocalteu phenol reagent, potassium dihydrogen phosphate (KH_2PO_4), potassium persulphate, ferric chloride hexahydrate, 2,4,6-tris-2-pyridyl-s-triazine (TPTZ), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 2,2–diphenyl-1-picrylhydrazyl (DPPH) and metaphosphoric acid (HPO_3) were from Sigma (USA); 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) was from Calbiochem (Germany); acetonitrile, ethanol and methanol were from BDH chemicals (UK).

2.2. Plant materials

Fresh fruits of baobab were purchased from rural community markets at the peak of harvesting season (April and May 2013) in Chikwawa, Machinga, Balaka and Mangochi districts of Malawi. Fruits from different sites were kept separately in sack cloth and transported by air at ambient conditions to the University of Leeds, for pretreatment, processing and subsequent analysis. Only undamaged fruits with no signs of infection such as discolouration or rotting were selected for the study. Baobab fruits were cracked, and the pulp (which is in the form of a powder) was separated from the hard shell and seeds using a mortar and pestle. Pulp powder was sieved to one size (1 mm) using Endecotts test sieve shaker. A composite baobab powder sample was obtained by homogenising powder with a sieve shaker from different sites in equal proportions. Pulp powder was microwaved (Kenwood, K25MMS12) for 60 s to investigate the effect of this heat treatment on ascorbic acid. Composite pulp powder was kept in air tight plastic containers at dry ambient conditions for about 3 days until juice preparation. Dry pulp powder has a longer shelf life (up to 3 years) likely because of high acidity and low moisture content.

2.3 Preparation of baobab juice

Baobab juice was prepared following the protocol commonly used by small holder farmers in Malawi with slight modification (Fig. 1). Composite pulp powder (100 g) that was kept in air tight plastic containers was added into cooled boiled drinking water (1 L, 15 °C, Leeds, UK), and the mixture homogenised using a domestic blender (Kenwood Chef Classic, model KM 330, China) operated at medium speed for 10 min. The resultant mixture was transferred into falcon tubes (50 mL) and centrifuged at 4000 rpm for 20 min at 4 °C in a Beckman Coulter Centrifuge (Allegra, model X-22, California, USA) to separate the pulp from remaining liquid juice. Afterwards the juice was filtered through muslin cloth into containers, degassed through a vacuum box (Mill wall, UK) for 10 min and immediately refrigerated (4 °C). Samples representing time zero were immediately deep frozen (-20 °C) and the rest distributed into three storage temperatures (6, 15 and 30 °C).

2.3.1 Thermal pasteurisation of baobab juice

To obtain domestic batch thermal pasteurisation, each juice sample (10 mL) was heated (60 °C) in falcon tubes (15 mL) using a thermostatic water bath (Grant Instruments, model VFP, Cambridge, UK). The juice took 5 min to reach 60 °C and was held at this temperature for 30 and 60 min. Temperatures were constantly monitored by incorporating thermocouples in representative falcon tubes filled with same volume of the juice. Treated juice

was immediately cooled in ice-water to a final temperature of 24 °C. To obtain conventional thermal pasteurisation, juice (5 L) was heated in a plate heat pasteuriser (Alfa Laval, model P20-HB, Sweden) at 72 °C and 85 °C for 15 s as described by other authors (Awuah et al., 2007; Igual et al., 2010). The regeneration section of the pasteuriser cooled samples to 4 °C. Degassed fresh and pasteurised juice samples were transferred into falcon tubes (15 mL) covered with aluminum foil to prevent exposure to light and stored at 6 °C in a cold room (Droitwich, UK) and incubators (Sanyo incubators, model MIR-153, Japan) set at 15 and 30 °C for a maximum period of 60 days. Separate falcon tubes with specific storage conditions (temperature and time) were used to minimise exposure to ambient conditions during analysis.

2. 4 Physicochemical properties of baobab pulp powder

All analyses were undertaken as described in AOAC (2005). Moisture content (%) was determined by the dry oven method. Accurately weighed pulp powder (5 g) was enclosed in a convection oven (Intertechnica, model Fev 18, Reading, UK) set at 105 - 110 °C overnight, cooled in a desiccator for 30 minutes and reweighed. pH measurements were accomplished using a pH meter (Hanna Instruments, model HI2211, Bedfordshire, UK). Pulp powder (10 g) was added to deionised water (100 mL), which was previously boiled and cooled to room temperature. The contents were homogenised for 60 s and transferred into a small beaker and the pH measured directly after equilibration at room temperature.

2.5 Extraction of soluble phenolic compounds from baobab products

Extraction of phenolics from fresh pulp powder, untreated and pasteurised juice was undertaken as described by Sun et al. (2013). Pulp powder (1 g) or juice (1 mL) was dissolved in methanol/water (80:20, v/v) to a final volume of 10 mL in a falcon tube (15 mL). The resultant mixture was vortexed (20 s), centrifuged (4000 rpm, 4 °C, 10 min) and filtered using Whatman No.1 paper. Sample extract was deep frozen (-18 °C) until determination of antioxidant activity or further extraction. For HPLC analysis, methanolic extract (5 mL) was evaporated to dryness using the EZ-2 Genevac (Genevac Limited, Ipswich, UK) applying the low boiling point method (32 °C for 5 hrs) and reconstituted to 2.5 mL with methanol/water (80:20, v/v) representing a final five-fold dilution of the original sample.

2.5.1 HPLC analysis of phenolic compounds and 5-hydroxymethylfurfural

Reversed-phase high performance liquid chromatography (HPLC) was used to identify and quantify phenolic compounds, and 5-hydroxymethylfurfural (HMF) using a liquid chromatograph (UFLCXR, Shimadzu, Japan) equipped with a degasser (DGU-20 A5), a pump system (LC-20 AD XR), an auto sampler (SIL-20 AC XR), a column oven (CTO-20 AC), a diode array detector system (DAD; SPD-M20A) and a communications bus module (CMB-20A) coupled with LC chromatographic software (Version 1.25). Five-fold diluted extracts were filtered through a Millipore 0.20 µm PTFE filter into amber glass HPLC vials. A sample volume of 20 µL was injected into the HPLC and separations were achieved on a Gemini C18 column (250 x 4.6 mm, 5 µm; Phenomenex, UK) operated at 35 °C. The method was performed as described by Pimpão et al. (2013) with some modification. The mobile phase consisted of 0.1% (v/v) formic acid in deionised water (solvent A) and 80% (v/v) methanol in deionised water (solvent B), employed at a flow rate of 0.5 mL/min. The gradient elution programme started at 10% B to reach 30% B at 15 min and remained 30% B until 45 min. In a final reequilibration step, 10% of solvent B was reached and maintained for 10 min. Compounds were identified by retention time and UV spectra with their corresponding standards as well as carrying out the peak spiking of samples with their standards. Chromatograms of (-)-epicatechin, procyanidin B_2, (-)-epigallocatechin-3-O-gallate, gallic acid and HMF were detected and recorded at the wavelength of 284 nm for quantification. All standards were dissolved in methanol/water (80:20, v/v) and employed as the stock solutions. Standard calibration curves (peak area against concentration) were plotted for quantification. Results were expressed as milligram per 100 gram of fresh weight (mg/100 g FW). Retention time (min), limit of detection (LOD), limit of quantification (LOQ), linearity (R^2), wavelength (nm) and recoveries (%) obtained and used are presented in Table 1.

2.5.2 Determination of total phenol content

Total phenol content (TPC) was determined using Folin-Ciocalteu assay (Singleton et al., 1999). Within 3-8 minutes, Folin reagent (5 mL) and sodium carbonate solution (1.0 M, 4 mL) were added to sample extract / standards (1 mL), vortexed and left to stand for 2 hours at 26 °C. Absorbance of samples and blank were measured at 765 nm using a Cecil CE3021 3000 Series Spectrophotometer (Talbot Scientific Limited, Cheshire, UK). A calibration curve with gallic acid concentration ranging from 25 to 150 mg L^{-1} and high linearity ($R^2 \geq$ 0.999) was used for quantification. Total phenol content was expressed as milligram gallic acid equivalent per 100 gram of fresh weight (mg GAE/100 g FW).

2.5.3 Antioxidant activity (FRAP, DPPH and ABTS)

The ferric reducing antioxidant power (FRAP) assay was employed according to Benzie and Strain (1996). Sample extract (0.2 mL) and freshly prepared FRAP reagent (6 mL) were transferred into a falcon tube, vortexed and left at room temperature for 10 min. The absorbance of the samples was measured at 593 nm using a Cecil CE3021 3000 Series Spectrophotometer (Talbot Scientific Limited, Cheshire, UK). The 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical cation method was employed according to Re et al. (1999). Sample extracts (0.1 mL) were added to $ABTS^{.+}$ aqueous solution (3.9 mL) that had achieved an absorbance of 0.7 ± 0.02 at 734 nm after dilution with deionised water. The mixture was kept at 25 °C for 106 min and absorbance was measured at 734 nm using a Cecil CE3021 3000 Series Spectrophotometer (Talbot Scientific Limited, Cheshire, UK). Calibration curves, with Trolox concentration ranging from 10 to 250 mg L^{-1} and high linearity ($R^2 > 0.999$) were used for quantification of FRAP and ABTS antioxidant activity. Results were expressed as milligram Trolox equivalent antioxidant capacity per 100 gram of fresh weight (mg TEAC /100 g FW).

The radical scavenging activity was measured using the DPPH assay according to Brand-Williams et al. (1995). Sample extracts (0.1 mL) were added to methanol DPPH solution (0.1 mM, 3.9 mL), vortexed and kept in the dark for 30 minutes at room temperature. The absorbance were measured at 517 nm using a Cecil CE3021 3000 Series Spectrophotometer (Talbot Scientific Limited, Cheshire, UK) and results expressed as % DPPH free radical scavenging activity according to equation (i).

$$\% \text{ DPPH} = (1 - A_s/A_c) \times 100$$ ……………………………………………………................................ (i)

Where A_s and A_c are absorbance of sample and control respectively.

2.6 Extraction and quantification of vitamin C and organic acids

Main organic acids including ascorbic acid (AA), citric acid (CA), malic acid (MA) and tartaric acid (TA) were extracted and quantified using HPLC according to the method of Pimpão et al. (2013) with some modification. Baobab juice (20 mL) was dissolved in metaphosphoric acid (0.3 g/L, 40 mL), vortexed for 20 s at medium speed and centrifuged (4000 rpm, 10 min, 4 °C). The supernatant was filtered using Whatman No.1 paper to ensure clear filtrate. The solution was passed through a Millipore 0.45 µm PTFE filter into amber glass HPLC vials. Vitamin C (AA + DHA) was also determined after reduction of dehydroascorbic acid to ascorbic acid using 2-carboxy ethyl phosphine hydrochloride (TCEP, 5 mg/L) as described by Chebrolu et al. (2012). Thus DHA was measured by difference because it is unstable and its peak splits in the C18 column when measured

directly. Quantification of organic acids was conducted using the UFLCXR HPLC equipment described in previous section. A sample volume of 20 µL was injected and separations were achieved on a Gemini C18 column (250 x 4.6 mm, 5 µm; Phenomenex, UK) operated at 25 °C. Separation was carried out under isocratic conditions (0.5 mL/min; 15 min) using 10 mM potassium dihydrogen phosphate (pH 2.6) as the mobile phase. Chromatograms of organic acids (CA, MA and TA) and AA were recorded at 210 and 254 nm respectively. Quantification was achieved using external standard method (Klimczak et al., 2007). A mixture of organic acid standards with a concentration ranging from 5 to 100 mg L^{-1} and high linearity ($R^2 \geq 0.999$) was used for calibration and the result expressed in mg/100 g FW. Spiked samples were included to determine sensitivity and reproducibility of the method, account for the percent loss and to monitor any shift in retention time of individual organic acids (Harris, 2007). The loss of vitamin C in baobab juice during storage was predicted using integrated laws of zero (2.1) and first (2.2) order reactions (Atkins, 2010).

$$C = C_0 - kt \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \quad (2.1)$$

$$\ln C = \ln C_0 - kt \dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots\dots \quad (2.2)$$

Where C, the concentration at time t; C_0, the concentration at time zero; k, the rate constant; t, the storage time

2.7 Statistical analysis

Results are presented as means of at least three determinations of independent samples ± standard deviation (SD). Analysis of variance (ANOVA) using Tukey's test ($p \leq 0.05$) was performed to evaluate the significance of differences between treatments and storage time using IBM SPSS statistical software version 22. Correlations between antioxidant activity and variables (vitamin C (AA + DHA), procyanidin B_2, (-)-epicatechin and total polyphenol content) were determined using Pearson's Correlation Coefficient Test to ascertain relationships.

3. Results and discussion

3.1 Physicochemical properties, vitamin C and organic acids

The quality of raw materials plays a significant role in overall product quality (Rössle et al., 2011) and determine postharvest handling, processing and preservation techniques to be undertaken. For this reason, baobab pulp was screened using HPLC for the presence of organic acids and bioactive compounds (Fig. 2). Physicochemical properties including organic acids, pH and moisture content influence sensory and microbiological quality characteristics while vitamin C determines nutritional quality and the antioxidant

properties of fruit products. Quantitative information of vitamin C, organic acids and bioactive compounds of baobab pulp is provided in Table 2. The pH of fresh baobab pulp was 3.11 ± 0.01, which is attributed to high levels of organic acids including vitamin C (AA + DHA), citric (CA), malic (MA) and tartaric acid (TA). Baobab fruit pulp contains very low moisture ($10.0 \pm 0.30\%$) and is likely a major contributing factor for extended AA shelf life in the pulp. Higher moisture content and water activity influence hydrolysis of DHA leading to formation of 2, 3-diketogulonic acid which has no vitamin C activity. This may be attributed to increased availability of water to act as a solvent for reactants and catalysts (Fennema, 1996). Coe et al. (2013) and Saka et al. (2007) reported a pH of 3.11 and 3.33 respectively in fresh baobab pulp. Results obtained from this study are therefore within the range reported by others.

The mean vitamin C content (AA + DHA) measured in the fresh baobab pulp (467 ± 8.1 mg/100 g FW) is more than eight fold higher than commonly consumed raw navel oranges (56.1 ± 1.0 mg/100 g) and raw lemon juice (42.9 ± 0.4 mg/100 g) reported by Scherer et al. (2012). However several authors have reported a wide range of vitamin C content (67 to 500 mg/100 g) in fresh baobab pulp (Chadare et al., 2009; Ibrahima et al., 2013; Kamatou et al., 2011; Tembo, 2008). Variation in composition of plant foods is attributed to several factors including species, environmental conditions, ripening stage at harvest, postharvest handling, storage condition and analytical techniques used (Aron and Kennedy, 2008; Tembo, 2008). For instance, Chadare et al. (2009) reported an average vitamin C content of 283 mg/100 g pulp but also noted the tree-to-tree variability ranging from 150 – 500 mg/100 g. Titrimetric or spectrophotometric methods of measuring vitamin C are unstable and not accurate due to inaccuracies in determining end points and interferences in sample matrix (Scherer et al., 2012). The use of a selective and sensitive method (RP-HPLC-PDA) in the present study ensures accurate quantification of quality attributes in baobab fruit products.

3.2 Total phenol content and antioxidant activity of fresh baobab pulp

Although total phenol content (TPC) and antioxidant activity assays (FRAP, ABTS and DPPH) have some limitations including interferences, effect of incubation time and the use of artificial radicals not found in food or biological systems, they are widely used for preliminary screening for the presence of phenolic compounds and antioxidant activity in foods or biological systems (Apak et al., 2016; Shahidi and Zhong, 2015). Moreover, these assays have high throughput and are widely used for ranking antioxidant activity of similar substrates under comparable conditions (Kaur and Kapoor, 2001; Shahidi and Zhong, 2015). Results show that fresh baobab pulp from Malawi contains higher total phenol content ($1.87 \times 10^3 \pm 1.61$ mg/100 g FW) than some of

the commonly consumed fruits considered as rich source of dietary polyphenols including black chokeberry (1.75×10^3 mg GAE/100 g), blackcurrant (821 mg GAE/100 g) and apple (205 mg GAE/100 g) according to Pérez-Jiménez et al. (2010). Ibrahima et al. (2013) reported lower TPC (1.09×10^3 mg GAE /100 g) for baobab fruit pulp from Madagascar while Lamien-Meda et al. (2008) reported a much higher level of TPC (3.52×10^3 – 4.06×10^3 mg GAE/100 g) for baobab pulp from Burkina-Faso.

Results show that fresh baobab pulp has a very high antioxidant activity/capacity (FRAP, ABTS and DPPH). The ferric reducing antioxidant power (FRAP) and 2, 2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical quenching ability in fresh baobab pulp were $4.66 \times 10^3 \pm 11.8$ and $1.68 \times 10^3 \pm 17.0$ mg TEAC/100 g FW) respectively while the 2, 2–diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was $50.93 \pm 0.4\%$ DPPH. FRAP values fell within the range (4.17×10^3 – 5.43×10^3 mg/100 g) reported by other authors (Coe et al., 2013) in fresh baobab pulp obtained from different locations. High level of antioxidant activity is attributed to vitamin C and phenolic compounds present in fresh baobab pulp. Antioxidant activity in baobab fruit pulp cited in literature is vastly variable; from 2.70×10^3 mg TEAC/100 g (Ibrahima et al., 2013) to 1.50×10^5– 1.75×10^5 mg TEAC/100 g (Kamatou et al., 2011). The relatively low antioxidant activities of baobab pulp reported by Ibrahima et al. (2013) could be attributed to the lower content of ascorbic acid quoted in the same paper. Lamien-Meda et al. (2008) demonstrated differences in antioxidant activity when samples are extracted with different solvent systems. In the present work, methanol/water (80:20, v/v) was selected for extraction of phenolic compounds after preliminary optimisation experiments. It is also possible that the study which reported exceptionally high antioxidant capacities for baobab pulp employed a longer incubation period for these assays. Incubation time affects the extent of reaction between the oxidant, also known as probe ([Fe (III) $(TPTZ)_2]^{3+}$, $ABTS^{·+}$ or DPPH·) and the antioxidants. Further, the kinetic pattern of phenol oxidation is dependent on several factors (concentration, oxidation state) such that the reaction between phenolic antioxidants and probe may not reach completion within the time span of the protocol leading to underestimated TEAC values (Apak et al., 2016). Kaboré et al. (2011) described variations in chemical composition of baobab pulp with soil type, geographical location, age of fruit, processing and storage conditions, which may also explain the differences in antioxidant activity reported in literature. Baobab fruits collected from different sites in Malawi showed significant ($p \leq 0.05$) variation in the level of micronutrients including vitamin C, provitamin A and minerals (Tembo, 2008) which influence antioxidant activity of derived products.

3.3 Bioactive compounds of fresh baobab pulp

The determination of bioactive compounds in food systems is necessary to fingerprint and track compounds responsible for functional properties both in vitro and in vivo. Such information is necessary to provide reliable dietary intake requirement of fresh foods and derived products. RP-HPLC-PDA analysis of phenolic compounds and 5-hydroxymethylfurfural was conducted after optimising chromatographic conditions in order to fingerprint predominant phytochemicals and account for the high TPC and antioxidant activity values observed in fresh baobab pulp from Malawi. Although a few researchers have reported the presence of polyphenols including procyanidins (Coe et al., 2013; Kamatou et al., 2011; Shahat, 2006) in baobab fruit, limited quantitative information on several phenolic compounds is available for baobab fruits from Malawi. Therefore this study analysed the levels of procyanidin B_2, (-)-epicatechin, (-)-epigallocatechin-3-O-gallate and gallic acid in fresh baobab pulp and resultant juice. The LOD and LOQ were obtained according to Gosetti et al. (2007). Recoveries were conducted to account for matrix effect and monitor any shift in retention times (Harris, 2007). High recoveries were achieved for compounds that were present in fresh baobab pulp and ensured accurate analysis of these phytochemicals.

Procyanidin B_2 and (-)-epicatechin were the most predominant flavan-3-ols in methanol/water (80:20, v/v) extract of baobab extract (Fig. 2B). The content of procyanidin B_2 (533 ± 22.6 mg/100 g), gallic acid (68.5 ± 12.4 mg/100 g) and (-)-epicatechin (43.0 ± 3.08 mg/100 g) determined in fresh baobab pulp were significantly higher ($p \leq 0.05$) than levels reported for many fruits. For instance Prior and Gu (2005) reported that choke berry, cranberry, blueberry and plum contain 664 ± 47.7, 419 ± 75.3, 256 ± 108 and 216 ± 50 mg/100 g of total proanthocyanidin content respectively. Pimpão et al. (2013) reported that blueberry and blackberry contain 16.3 ± 3.1 and 6.08 ± 0.25 mg/100 g gallic acid respectively. Fresh baobab pulp from Malawi is thus an excellent source of procyanidin B_2 and gallic acid and may be considered as a potential source of raw materials for novel functional foods. Moreover the level of (-)-epicatechin (43.0 ± 3.08 mg/100 g) was higher than levels reported by Pimpão et al. (2013) for blueberry, raspberry, blackberry and Portuguese crowberry. It can therefore be envisaged that procyanidin B_2, (-)-epicatechin and gallic acid contribute significantly to the total phenol content and antioxidant activity values observed in fresh baobab pulp.

3.4 Effect of thermal treatment on ascorbic acid (AA) content of baobab pulp and juice.

Several studies show that ascorbic acid is highly thermo labile compound easily degraded upon application of heat (Rawson et al., 2011). Therefore the effect of microwaving on ascorbic acid content of fresh baobab pulp was evaluated. Results show that AA was not significantly affected ($p > 0.05$) by this heat treatment. Fresh pulp

and microwaved pulp showed mean AA content of 309 ± 2.7 and 281 ± 0.9 mg/100 g FW respectively representing more than 91% retention (Fig. 3). Similarly, Brewer et al. (2003) observed that microwave treatment at any power level for as little as 1 min reduced peroxidase activity and retained ascorbic acid content in broccoli, green beans and asparagus.

For acidic fruit products, conventional thermal pasteurisation (72 °C, > 15 s) is recommended but domestic batch thermal pasteurisation (60 °C, 30 min; 60 °C, 60 min) is also widely used (Chen et al., 2013). Both methods may degrade taste, colour, flavour and nutritional quality of juice. Therefore the effect of different thermal pasteurisation on quality attributes of baobab juice was investigated in order to account for loss of AA. Fig.3 shows AA percent retention of pasteurised juice and untreated control (assigned 100%). Pasteurised juice (72 °C, 15 s) showed significantly higher ($p \leq 0.05$) AA than other treatments. For pasteurised juice (85 °C, 15 s), elevated temperature might have contributed to an increased rate of degradation of AA while for domestic batch thermal pasteurisation regimes (60 °C, 30 min; 60 °C, 60 min) both temperature and time will have influenced degradation of AA. Igual et al. (2010) observed no significant difference in the content of AA between freshly squeezed grapefruit juice and conventionally pasteurised juice (80 °C, 11 s). Higher solute concentration including organic acids and sugars in grapefruit juice may be responsible for protective effect against AA oxidation by binding metal ions and reducing their catalytic potency (Fennema, 1996). AA or vitamin C (AA + DHA) degradation may vary in different fruit products due to differences in plant matrices, composition, concentration, pH and water activity (Fennema, 1996; Gamboa-Santos et al., 2014). For instance the presence of metal ions including Fe^{3+} and Cu^{2+} in the matrix may catalyse oxidation of ascorbate. The oxidation of AA to DHA is reversible and relative yield depends on reaction conditions. The pH dependence of oxidation of AA is governed mainly by the relative concentration of fully protonated ascorbate (AH_2), ascorbate monoanion (AH^-) and ascorbate dianion (A^{2-}) species hence pH or pK_a of the system (Fennema, 1996). AA is stable at pH < 2.5 while DHA is stable at pH 2.5 - 5.5 (Fennema, 1996; Sanmartin et al., 2000). Juice formation increases water activity and may raise the pH shifting the AA/DHA equilibrium, favouring DHA formation which may easily undergo irreversible degradation with increased temperature during thermal pasteurisation (Fennema, 1996; Rawson et al., 2011).

3.5 Effect of storage temperature on vitamin C (AA + DHA)

To investigate the effects of storage, pasteurised juice (72 °C, 15 s) that retained high AA was kept under isothermal conditions (6, 15 and 30 °C) for a maximum period of 60 days. These temperatures represent

refrigeration (6 °C), medium (15 °C) and higher ambient temperature (30 ºC) usually attained in Malawi where raw materials were collected.

Retention studies of vitamins to assess the effects of food processing and storage on the nutritive value of foods are of great importance to food technologists. Vitamin C is an important component of our nutrition and used as additive in many foods because of its antioxidant capacity (Burdurlu et al., 2006). Unlike fat soluble vitamins (A, D, E and K) and B vitamins, vitamin C is unstable and under less desirable conditions decomposes easily therefore in fruit and vegetables it provides an indication of the loss of quality (Fennema, 1996). Since several decomposition reactive products of vitamin C may combine with amino acids forming brown pigments including 5-hydroxymethylfurfural which is the other problem of quality loss in juices during storage it is necessary to describe vitamin C degradation and investigate kinetics of vitamin C loss in stored juice (Burdurlu et al., 2006).

In the present study, the change in vitamin C concentration (AA + DHA) in baobab juice was evaluated as a function of storage time and temperature. Fig. 4 shows the vitamin C concentration (mg L^{-1}) over time at a fixed temperature. The loss of vitamin C increased with both storage time and temperature. Juice stored at 6 °C showed significantly longer ($p \leq 0.05$) vitamin C retention than other temperatures.

Changes in vitamin C (AA + DHA) and AA and are shown in Table 3. AA was more affected than total vitamin C (AA + DHA) during storage, suggesting the loss of AA to DHA was greater than the loss of DHA to DKG. This is attributed to a higher pH of the juice (pH 3.11) than the recommended pH range (< 2.5) for AA stability compared to DHA which is most stable between pH 2.5 and 5.5 (Fennema, 1996). For instance at 6 °C, the initial concentration of AA decreased by about 72 and 91% by day 14 and 28 respectively while vitamin C decreased by about 38 and 69% respectively during the same period. Similarly, at 15 °C, the initial level of AA dropped significantly ($p \leq 0.05$) until day 28 then became stable until the end of storage time. However, the level of vitamin C did not change significantly ($p > 0.05$) during the same period. At 30 °C, both AA and vitamin C (AA + DHA) were affected and reached minimum values within 28 days. Higher storage temperature was likely more responsible for increased degradation of both AA and DHA. In general, the loss of AA and vitamin C (AA + DHA) was gradual towards the end of storage at 6 and 15 °C. The loss of vitamin C in pasteurised juice during storage is likely attributed to non-enzymatic pathways since enzymatic degradation is eliminated during processing (Burdurlu and Karadeniz, 2003). DHA as well as dicarbonyl compounds formed during its degradation (DKG, erythrulose, 3-deoxythreosone, xylosone) can undergo ascorbic acid browning (non-enzymic browning) via a Strecker-like degradation reaction with amino acids forming brown pigments

(Fennema, 1996), including HMF. Brown pigmentation was quite visible in baobab juice stored at 30 °C. Burdurlu et al. (2006) also reported that AA degradation in citrus juice increased with both storage temperature and time. Vitamin C degradation kinetics enables accurate prediction of vitamin C loss and shelf-life determination of juice at a specific temperature. Vitamin C degradation for juice samples stored at 6 and 15 °C best fitted first order reaction kinetics of degradation ($R_6^2 \geq 0.99$ and $R_{15}^2 \geq 0.85$) with rate constants (-slope) of 0.044 and 0.0538 day^{-1} and half-lives (ln 2/k) of 16 and 13 days respectively (Fig. 5). Vitamin C stored at 30 °C showed very short shelf life and dropped by more than 88% in 14 days and may best fit zero order reaction kinetics of degradation. Elevated temperature increased rate of vitamin C degradation. The rate of degradation of vitamin C is largely dependent on activation energy (Ea) of AA which may vary widely. The activation energy, Ea (33.4 kJ/mol) of AA in baobab pulp was calculated from Arrhenius plot $\left(\ln k = - \frac{E_a}{RT} + \ln A\right)$ and was lower than values reported in other fruit juices. Other researchers reported Ea values for AA ranging from 53.4 to 106 kJ/mol in citrus juice concentrates (Karadeniz, 2004) and 51 to 135 kJ/mol for orange juice (Remini et al., 2015). Higher Ea means degradation of AA is more favourable with increasing temperature. Therefore results from this study suggest that temperature had less influence on AA degradation compared to citrus juices.

3.6 Effect of pasteurisation and storage on organic acids (citric and malic acid)

Quantification of organic acids is important for proper comprehension of organoleptic and microbiological quality of fruits and derived products. Specifically organic acids influence flavour, colour and microbial stability in foods. Moreover organic acids are widely used as preservatives for a wide range of fruit products.

Table 3 shows levels of citric (CA) and malic acid (MA) in conventionally pasteurised juice (72 °C, 15 s) stored at 6, 15 and 30 °C for 60 days. In general results showed that pasteurisation and storage had little influence on the level of both organic acids. Pasteurised juice (72 °C, 15 s) showed no significant difference (p > 0.05) in levels of CA (3.34 x 10^3 ± 21.2) and MA (2.41 x 10^3 ± 43.1) mg L^{-1} compared to fresh pulp or untreated juice control. The levels of CA and MA in control juice were 3.30 x 10^3 ± 18.5 and 2.36 x 10^3 ± 28.8 mg L^{-1} respectively. Similarly, Igual et al. (2010) reported that pasteurisation (80 °C, 11 s) had no influence on the levels of MA in grapefruit juice.

The level of CA was stable at 6 °C but marginally fluctuated at 15 and 30 °C during storage. At 6 °C, the mean concentration of CA was 3.24 x 10^3 ± 133 mg L^{-1} and values ranged from 3.02 x 10^3 ± 168 to 3.36 x 10^3 ± 31.8 mg L^{-1}. At 15 °C, the mean CA concentration was 3.15 x 10^3 ± 263 mg L^{-1} and values ranged from 2.76 x 10^3 ±

191 to $3.38 \times 10^3 \pm 15.3$ mg L^{-1}. There was a significant drop ($p \leq 0.05$) in the level of CA on day 42 but levels increased again on day 60. Finally, at 30 °C, the mean CA concentration was $3.26 \times 10^3 \pm 244$ mg L^{-1} and values ranged from $2.97 \times 10^3 \pm 213$ to $3.51 \times 10^3 \pm 27.9$ mg L^{-1}. There was a significant drop ($p \leq 0.05$) in the level of CA on day 28, which then remained stable until day 42 and finally increased significantly ($p \leq 0.05$) on day 60. As can be seen, the mean concentration of CA is marginally influenced by storage temperature.

Although the level of malic acid (MA) showed some pronounced fluctuations compared to citric acid, it was generally stable during storage. At 6 °C, the mean concentration of MA was $2.17 \times 10^3 \pm 270$ mg L^{-1} and values ranged from $1.84 \times 10^3 \pm 45.8$ to $2.41 \times 10^3 \pm 43.1$ mg L^{-1}. A significant drop ($p \leq 0.05$) in the concentration of MA was observed on day 14, which then stabilised until day 42 and rose again at the end of storage. At 15 °C, the mean concentration of MA was $2.00 \times 10^3 \pm 371$ mg L^{-1} with values ranging from $1.51 \times 10^3 \pm 318$ to $2.41 \times 10^3 \pm 43.1$ mg L^{-1}. The level of concentration of MA on day zero and 60 were not significantly different ($p > 0.05$). Finally, at 30 °C, the mean concentration of MA was $2.17 \times 10^3 \pm 300$ mg L^{-1} and values ranged from $1.82 \times 10^3 \pm 387$ to $2.48 \times 10^3 \pm 21.0$ mg L^{-1}. Just like CA, the overall mean concentration of MA was generally not strongly influenced by storage temperature.

Fermentation, esterification and oxidation are the most probable pathways for evolution of organic acids (Piras et al., 2014). Stability of organic acids may be attributed to inactivation and destruction of degradative enzymes and microorganisms including yeast and lactic acid bacteria during pasteurisation. The decrease in CA and MA was significantly lower ($p \leq 0.05$) under refrigeration storage (6 °C) than at elevated temperatures (15 and 30 °C). Residual enzymes and microorganisms may still facilitate loss of organic acids through fermentation and esterification at elevated temperatures. Some higher values of organic acids observed towards the end of storage at higher storage temperatures (15 and 30 °C) could be attributed to degradation of carbohydrates and other phenolic compounds in the juice. In general, citric acid did not degrade markedly during storage. The degradation of organic acids in baobab juice with storage are far less than degradation of vitamin C (AA + DHA). Nevertheless results from this study indicate that baobab juice needs to be stored under refrigeration (6 °C) to extend vitamin C and organic acids shelf-life.

3.7 Effect of pasteurisation and storage temperature on procyanidin B_2 and (-)-epicatechin content

Pasteurisation reduced the level of procyanidin B_2 by 12.6% but increased the level of (-)-epicatechin by 10.9% compared to initial levels in untreated juice. Storage time and temperature affected levels of both procyanidin B_2 and (-)-epicatechin (Table 4). For pasteurised juice stored at 6 °C, the level of procyanidin B_2 and (-)-

epicatechin increased significantly (p ≤ 0.05) on day 42 by 269 and 107% respectively compared to initial levels. By the end of storage, the level of procyanidin B_2 and (-)-epicatechin was 120 and 21.8% respectively higher compared to levels at time zero. At 15 ℃, the level of procyanidin B_2 increased between day 0 and day 14, remained stable between day 14 and day 42 and finally dropped significantly (p ≤ 0.05) on day 60. (-)-Epicatechin increased between day 0 and day 14, remained stable until day 28, and dropped significantly (p ≤ 0.05) on day 42, and finally increased on day 60. By the end of storage, procyanidin B_2 decreased by 70.6% while (-)-epicatechin increased by 103 % compared to initial levels. At 30 °C the trend of procyanidin B_2 between day 0 and day 42 was similar to that observed at 15 °C. The level increased significantly by day 14, remained stable until day 42 then finally increased significantly (p ≤ 0.05) on day 60. While (-)-epicatechin increased between day 0 and day 14, then decreased continuously until day 42, and finally remained stable until the end of storage time. By the end of storage time, procyanidin B_2 and (-)-epicatechin showed an increase of 250 and 18% respectively compared to levels at time zero. Chen et al.(2013) reported that thermal processing may cause complex physical and chemical reactions affecting the phenolic composition, including the release of phenolic compounds from their bonded forms, degradation of polyphenols and the breakdown and transformation of phenolic compounds. Evolution of procyanidin B_2 and (-)-epicatechin during processing and storage (temperature and time) has been previously attributed to several reactions including depolymerisation of procyanidin polymers to oligomers (monomers, dimers, trimers, and tetramers), polymerisation, hydroxylation, esterification, epimerisation, thermal degradation, enzymatic and non-enzymatic oxidation and microbial degradation (Aron and Kennedy, 2008; Kaur and Kapoor, 2001; Keenan et al., 2011; Li et al., 2015). These reactions are influenced by composition and matrix of plant material. Hydroxylation, esterification, and depolymerisation are favourable under acidic and low pH conditions typical of baobab fruit juice (pH ≤ 3.11). Baobab fruit has significantly higher levels of organic acids which were stable during storage and may promote proposed reaction pathways for evolution of procyanidin B_2 and (-)-epicatechin.

3.8 Effect of pasteurisation and storage temperature on total phenolic content of baobab juice

Pasteurisation reduced the level of total phenol content (TPC) significantly (p ≤ 0.05) compared to untreated control (Table 4). The level of TPC in untreated and pasteurised juice was $1.56 \times 10^3 \pm 1.60$ and $1.08 \times 10^3 \pm 3.67$ mg GAE/100 g FW respectively representing about 31% loss. Moreover a decrease of 16% in TPC in fresh pulp ($1.87 \times 10^3 \pm 1.61$ mg GAE/100 g FW) was already observed during juice formation. The decrease in the level of TPC in untreated juice may be attributed to low solubility of some phenolic compounds in water during

juice formation while lower values of TPC in pasteurised juice is likely attributed to loss of heat sensitive phenolic compounds. Longer extraction and homogenisation time may increase the yield of total phenol content in untreated juice through release of more hydrophilic phenolic compounds from pulp matrix prior to pasteurisation. It is therefore necessary to optimise processing and preservation conditions in order to ensure a higher yield and retention of bioactive compounds in the final juice. A significant decrease in the level of TPC with pasteurisation was also reported in grapefruit juice (Igual et al., 2010).

Results showed that storage time and temperature influenced the level of TPC in the juice (Table 4). In all samples, the level of TPC was fluctuating during storage. For instance, At 6 °C, the mean TPC was $1.13 \times 10^3 \pm 109$ mg GAE/100 g FW and values ranged from $1.02 \times 10^3 \pm 5.26$ to $1.31 \times 10^3 \pm 1.60$ mg GAE/100 g FW. There was a significant increase ($p \leq 0.05$) in the level of TPC on day 28, which then dropped on day 42 and increased again towards the end of storage. At 15 °C, the mean TPC was $1.09 \times 10^3 \pm 215$ mg GAE/100 g FW and values ranged from $7.23 \times 10^2 \pm 16.1$ to $1.26 \times 10^3 \pm 4.01$ mg GAE/100 g FW. The level of TPC increased significantly ($p \leq 0.05$) on day 14, then dropped to a minimum by day 42, and increased again at the end of storage. At 30 °C, the mean TPC was $1.13 \times 10^3 \pm 93.6$ mg GAE/100 g FW and values ranged from $1.02 \times 10^3 \pm 10.2$ to $1.27 \times 10^3 \pm 5.78$ mg GAE/100 g FW. Surprisingly, the mean values of TPC at 6 and 30 °C were not significantly different ($p > 0.05$). A possible reason for the observed decrease in TPC during storage could be due to polyphenols reacting with sugars and sugar metabolites (Agbenorhevi and Marshall, 2012) present in baobab juice. For example gallic acid which was available in baobab pulp and juice can form complex sugar esters such as 2-O-digalloyl- tetra-O-galloyl-glucose during storage (Rio et al., 2013). Acidic hydrolysis of carbohydrates occur during storage of juice and decomposition products (glucose and fructose) may react with phenolic compounds by esterification in essence reducing total phenol content. It is also possible that phenolic compounds were forming complexes with proteins leading to changes in the structural, functional and nutritional properties of both compounds (Ozdal et al., 2013). The interaction may be attributed to the fact that phenolic group is an excellent hydrogen donor that forms hydrogen bonds with the carboxyl group of the protein (Mulaudzi et al., 2012). The decrease in TPC of juice kept at 30 °C could be largely attributed to loss of heat sensitive phenolic compounds which become more sensitive to chemical oxidation with increased temperature. Nevertheless, the level of TPC in pasteurised juice remained significantly higher ($p \leq 0.05$) compared to untreated juice. An increase in TPC may be attributed to transformation of proanthocyanidins (oligomeric and polymeric flavan-3-ols) to dimers or monomeric phenolic compounds or formation of other phenolic compounds

during juice storage (Piljac-Zegarac et al., 2009). The trend of evolution of TPC during storage is also dependent on fruit species besides processing and preservation conditions employed.

3.9 Effect of pasteurisation and storage on antioxidant activity (FRAP, ABTS and DPPH) of baobab juice

Preservation methods are believed to be responsible for the depletion of naturally occurring antioxidants. Although processed fruit products are expected to have lower health protecting capacity than fresh ones, the functional properties of the former may be stable during storage (Nicoli et al., 1999).

The influence of pasteurisation and storage on stability of antioxidant activity was evaluated in baobab fruit juice at three storage temperatures. Three methods (FRAP, ABTS and DPPH) were used because of multiple reaction characteristics and mechanisms involved in a mixed or complex system (Apak et al., 2016a; Du et al., 2009) in order to better reflect total antioxidant activity of baobab juice. For instance DPPH assay only measures lipophilic antioxidants while ABTS considers both hydrophilic and lipophilic antioxidants (Apak et al., 2016).

Results indicate that pasteurisation reduced the antioxidant activity of the juice (Table 4). For instance, FRAP, ABTS and DPPH was reduced by 2.4, 1.4 and 3.2% respectively compared to levels in untreated juice. The decrease in antioxidant activity is attributed to loss of bioactive compounds including vitamin C and total phenol content observed in the juice. Evolution of antioxidant activity during storage is shown in Table 4. At 6 °C, the mean FRAP value was $2.55 \times 10^3 \pm 240$ mg TEAC/100 g FW with levels ranging from $2.37 \times 10^3 \pm 8.52$ to $2.88 \times 10^3 \pm 16.0$ mg TEAC/100 g FW. While at 15 °C, the mean FRAP value was $2.45 \times 10^3 \pm 352$ mg TEAC/100 g FW and levels ranged from $1.85 \times 10^3 \pm 37.3$ to $2.63 \times 10^3 \pm 21.1$ mg TEAC/100 g FW. Finally at 30 °C, the mean FRAP value was $2.61 \times 10^3 \pm 196$ mg TEAC/100 g FW and levels ranged from $2.27 \times 10^3 \pm 35.4$ to $2.74 \times 10^3 \pm 22.5$ mg TEAC/100 g FW.

The mean values of DPPH radical scavenging activity in the juice were 44.2 ± 4.87, 44.1 ± 4.80 and $46.0 \pm 2.38\%$ DPPH at 6, 15 and 30 °C respectively. Antioxidant activity was fluctuating during storage and consistent with TPC in most cases (Table 4). Evolution of antioxidant activity during storage by ABTS assay was similar to those observed using FRAP and DPPH assays. For instance, at 6 °C, the mean ABTS value was $1.30 \times 10^3 \pm 205$ mg TEAC/ 100 g FW and values ranged from $1.09 \times 10^3 \pm 17.8$ to $1.52 \times 10^3 \pm 18.7$ mg TEAC/100 g FW. While at 15 °C, the mean ABTS value was $1.30 \times 10^3 \pm 186$ mg TEAC/100 g FW and values ranged from $1.00 \times 10^3 \pm 12.2$ to $1.50 \times 10^3 \pm 1.87$ mg TEAC/100 g FW. Finally at 30 °C, the mean ABTS value was $1.35 \times 10^3 \pm 148$ mg TEAC/100 g FW and values ranged from $1.11 \times 10^3 \pm 121$ to $1.50 \times 10^3 \pm 1.87$ mg TEAC/100 g FW.

The overall mean ABTS antioxidant activity values between all storage temperatures were not significantly different ($p > 0.05$).

From this study, all antioxidant activity assays considered showed few systematic fluctuations of antioxidant activity in baobab juice. It seems pasteurisation stabilised antioxidant activity of baobab juice. The significantly higher levels of antioxidant activity was consistent with higher levels of vitamin C or total phenol content. The reduction in antioxidant activity observed during storage could be attributed to reduction in vitamin C and heat sensitive phenolic compounds and the formation of melanoidins with pro-oxidant properties (Rufián-Henares and Morales, 2007). Fluctuation in antioxidant activity of juice may be attributed to several factors including the tendency of polyphenols to undergo polymerisation reactions whereby the resulting oligomers possess larger surface area available for charge delocalisation and formation of melanoidins which lead to an increase in antioxidant activity. Baobab contains higher levels of (-)-epicatechin and procyanidin B_2 (Table 2), which may likely undergo polymerisation during storage under ideal conditions of temperature and pH leading to higher antioxidant activity. Higher antioxidant activity observed during storage could also be attributed to increased ability of partially oxidised phenolic compounds to donate an electron or hydrogen atom from the aromatic hydroxyl group to a probe ($[Fe\,(III)\,(TPTZ)_2]^{3+}$, $ABTS^{\cdot+}$ or $DPPH^{\cdot}$) in the FRAP, ABTS and DPPH assays (Apak et al., 2016; Huang et al., 2005). When the degree of polymerisation of monomeric or oligomeric flavan-3-ols exceeds a certain critical value the increased molecular complexity and steric hindrances reduce the availability of hydroxyl groups in reaction with the reagents (Piljac-Žegarac et al., 2009) thereby reducing antioxidant activity.

Non-enzymic browning of juice during thermal pasteurisation or storage is likely due to ascorbic acid browning (Fennema, 1996). Baobab fruit pulp and resultant juice is acidic ($pH < 3.01$) and contains high levels of ascorbic acid and amino acids (Chadare et al., 2009). Protein accounts for 3.2% of the baobab fruit pulp, with tyrosine, glutamic acid and aspartic acid being the predominant amino acids (Osman, 2004), all of which can influence ascorbic acid browning at elevated temperatures and the melanoidins formed (Davies and Wedzicha, 1994) could account for higher antioxidant activity observed in stored juice. The increase in antioxidant activity towards the end of storage coincided with the formation of brown pigments which were clearly visible at 30 °C. Processing conditions that optimise the preservation of total phenols and AA will inevitably preserve the antioxidant capacity of baobab juice. Although some degradation of natural antioxidants was likely at high temperatures, the effect on overall antioxidant capacity was not as noticeable due to the presence of phenolic compounds and the antioxidant activity of the melanoidins.

3.10 *Pearson's correlation*

The antioxidant activity of fruits and vegetables is mostly ascribed to AA and polyphenols. The antioxidant activity of AA is caused by the ease of its loss of electrons and subsequent stabilisation by the π-electron system (Sanmartin et al., 2000) while antioxidant activity of polyphenols is mainly because of their redox properties which enable them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (Kaur and Kapoor, 2001).These compounds may act independently or in combination in exerting antioxidant properties (Frankel and Meyer, 2000). For this reason, overall correlation analysis was conducted to explore the relationship between antioxidant activity and variables (vitamin C (AA + DHA), procyanidin B_2, (-)-epicatechin and total phenol content). Results (Tab. 5) show that antioxidant activity (FRAP, DPPH and ABTS) was significantly correlated ($p \leq 0.05$) with TPC, vitamin C and procyanidin B_2. The correlation coefficients of FRAP, DPPH and ABTS with TPC were 0.876, 0.575, and 0.530, respectively, while the correlation coefficients of FRAP, DPPH and ABTS with vitamin C were 0.259, 0.351 and 0.366 respectively. Not surprising, vitamin C and TPC were weakly correlated ($r = 0.011$, $p > 0.05$). The correlations between (-)-epicatechin and antioxidant activity was weaker compared to procyanidin B_2. This suggests that procyanidin B_2 contributed more to the overall antioxidant activity of baobab juice than (-)-epicatechin. Significantly high ($p \leq 0.01$) positive correlations between assays was observed and confirmed presence of more antioxidant compounds. Significant ($p \leq 0.05$) correlation between antioxidant activity, vitamin C and procyanidins (B_1 and B_2) in other plant foods has been reported by several authors (Guendez et al., 2005; Martínez-Flores et al., 2015; Thaipong et al., 2006; Tuberoso et al., 2010). Thus baobab fruit pulp is a potential source of antioxidant compounds necessary for the development of novel functional foods.

4. Conclusions and recommendations

Baobab fruit pulp is rich source of vitamin C, procyanidin B_2, (-)-epicatechin and organic acids which may have a wide range of application in the food and pharmaceutical industry. Uncontrolled thermal treatment and storage temperature significantly affects the level bioactive compounds and antioxidant activity of the juice. Prolonged domestic batch thermal pasteurisation (60 °C, 30 min; 60 °C, 60 min) significantly reduce vitamin C content in baobab juice. The use of conventional thermal pasteurisation (72 °C, 15 s) is recommended to retain bioactive compounds and improve antioxidant activity of baobab juice. Flavan-3-ols fluctuate during storage reasonably indicative of a series of transformation reactions taking place. Refrigeration (6 °C) preserved bioactive

compounds over a period of 60 days. Consumption of quality baobab juice may improve nutritional status and health benefits for the people of Malawi. Further research is required to investigate availability of other phenolic compounds including conjugated hydroxycinammates in the fresh pulp and metabolic profile of baobab juice during storage.

References

Agbenorhevi, J., Marshall, L.J., 2012. Investigation into the Total Phenols and Antioxidant Activity during Storage of Fruit Smoothies. J. Food Sci. Eng. 2, 72–79.

Ahmad, Mohammad Shamsherand Siddiqui, M.W., 2015. Factors Affecting Postharvest Quality of Fresh Fruits. Springer International Publishing.

AOAC, 2005. Official Methods of Analysis, 18 th. ed. Association of Official Analytical Chemists.Inc, Arlington, AV.

Apak, R., Özyürek, M., Güçlü, K., Çapanoılu, E., 2016a. Antioxidant activity/capacity measurement. 1. Classification, physicochemical principles, mechanisms, and electron transfer (ET)-based assays. J. Agric. Food Chem. 64, 997–1027.

Apak, R., Özyürek, M., Güçlü, K., Çapanoılu, E., 2016b. Antioxidant Activity/Capacity Measurement. 2. Hydrogen Atom Transfer (HAT)-Based, Mixed-Mode (Electron Transfer (ET)/HAT), and Lipid Peroxidation Assays. J. Agric. Food Chem. 64, 1028–1045.

Aron, P.M., Kennedy, J.A., 2008. Flavan-3-ols: Nature, occurrence and biological activity. Mol. Nutr. Food Res. 52, 79–104.

Atkins, P.W., 2010. Physical Chemistry ., 9th ed. Oxford, UK: Oxford University Press.

Awuah, G.B., Ramaswamy, H.S., Economides, A., 2007. Thermal processing and quality: Principles and overview. Chem. Eng. Process. 46, 584–602.

Benzie, I., Strain, J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal. Biochem. 76, 70–76.

Besco, E., Braccioli, E., Vertuani, S., Ziosi, P., Brazzo, F., Bruni, R., Sacchetti, G., Manfredini, S., 2007. The use of photochemiluminescence for the measurement of the integral antioxidant capacity of baobab products. Food Chem. 102, 1352–1356.

Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. LWT - Food Sci. Technol. 28, 25–30.

Brewer, M., Begum, S., 2003. Effect of microwave power level and time on ascorbic acid content , peroxidase activity and colour of selected vegetables. J. Food Process. Preserv. 27, 411– 426.

Burdurlu, H.S., Karadeniz, F., 2003. Effect of storage on nonenzymatic browning of apple juice concentrates. Food Chem. 80, 91– 97.

Burdurlu, H.S., Koca, N., Karadeniz, F., 2006. Degradation of vitamin C in citrus juice concentrates during storage. J. Food Eng. 74, 211–216.

Chadare, F., Linnemann, A., Hounhouigan, J., Nout, M., Van Boekel, M., 2009. Baobab food products: a review on their composition and nutritional value. Crit. Rev. Food Sci. Nutr. 49, 254–74.

Chandrasekara, N., Shahidi, F., 2011. Effect of roasting on phenolic content and antioxidant activities of whole cashew nuts, kernels, and testa. J. Agric. Food Chem. 59, 5006–14.

Chebrolu, K.K., Jayaprakasha, G.K., Yoo, J.L., Patil, B.S., 2012. An improved sample preparation method for quantification of ascorbic acid and dehydroascorbic acid by HPLC. LWT - Food Sci. Technol. 47, 443–449.

Chen, Y., Yu, L., Rupasinghe, H., 2013. Effect of thermal and non-thermal pasteurisation on the microbial inactivation and phenolic degradation in fruit juice: a mini-review. J. Sci. Food Agric. 93, 981–986.

Coe, S.A., Clegg, M., Armengol, M., Ryan, L., 2013. The polyphenol-rich baobab fruit (Adansonia digitata L.) reduces starch digestion and glycemic response in humans. Nutr. Res. 33, 888–96.

Davies, C.G.A., Wedzicha, B.L., 1994. Ascorbic acid browning: the incorporation of C_1 from ascorbic acid into melanoidins. Food Chem. 49, 165–167.

Du, G., Li, M., Ma, F., Liang, D., 2009. Antioxidant capacity and the relationship with polyphenol and Vitamin C in Actinidia fruits. Food Chem. 113, 557–562.

Fennema, O., 1996. Food Chemistry, 3rd ed. London, UK: Taylor & Francis, CRC Press.

Frankel, E.N., Meyer, A.S., 2000. The problems of using one-dimensional methods to evaluate multifunctional food and biological antioxidants. J. Sci. Food Agric. 80, 1925–1941.

Gamboa-Santos, J., Megías-Pérez, R., Soria, A.C., Olano, A., Montilla, A., Villamiel, M., 2014. Impact of processing conditions on the kinetic of vitamin C degradation and 2-furoylmethyl amino acid formation in dried strawberries. Food Chem. 153, 164–70.

Gosetti, F., Mazzucco, E., Gianotti, V., Polati, S., Gennaro, M.C., 2007. High performance liquid chromatography/tandem mass spectrometry determination of biogenic amines in typical Piedmont cheeses. J. Chromatogr. A 1149, 151–157.

Govardhan Singh, R.S., Negi, P.S., Radha, C., 2013. Phenolic composition, antioxidant and antimicrobial activities of free and bound phenolic extracts of Moringa oleifera seed flour. J. Funct. Foods 5, 1883–1891.

Guendez, R., Kallithraka, S., Makris, D.P., Kefalas, P., 2005. Determination of low molecular weight polyphenolic constituents in grape (Vitis vinifera sp.) seed extracts: Correlation with antiradical activity. Food Chem. 89, 1–9.

Harris, D.C., 2007. Quantitative Chemical Analysis, 7th ed, New York: WH Freeman and Co.

Huang, D., Ou, B., Prior, R., 2005. The chemistry behind antioxidant capacity assays. J. Agric. Food Chem. 53, 1841–1856.

Ibrahima, C., Didier, M., Max, R., Pascal, D., Benjamin, Y., Renaud, B., 2013. Biochemical and nutritional properties of baobab pulp from endemic species of Madagascar and the African mainland. African J. Agric. 8, 6046–6054.

Igual, M., García-Martínez, E., Camacho, M.M., Martínez-Navarrete, N., 2010. Effect of thermal treatment and storage on the stability of organic acids and the functional value of grapefruit juice. Food Chem. 118, 291–299.

Kaboré, D., Sawadogo-Lingani, H., Diawara, B., Compaoré, C., Dicko, M., Jakobsen, M., 2011. A review of baobab (Adansonia digitata) products: Effect of processing techniques, medicinal properties and uses. African J. Food Sci. 5, 833–844.

Kamatou, G.P.P., Vermaak, I., Viljoen, A. M., 2011. An updated review of Adansonia digitata: A commercially important African tree. South African J. Bot. 77, 908–919.

Karadeniz, F., 2004. Main organic acid distribution of authentic citrus juices in Turkey. Turkish J. Agric. For. 28, 267–271.

Kaur, C., Kapoor, H.C., 2001. Antioxidants in fruits and vegetables - The millennium's health. Int. J. Food Sci. Technol. 36, 703–725.

Keenan, D.F., Brunton, N., Gormley, R., Butler, F., 2011. Effects of thermal and high hydrostatic pressure processing and storage on the content of polyphenols and some quality attributes of fruit smoothies. J. Agric. Food Chem. 59, 601–7.

Klimczak, I., Małecka, M., Szlachta, M., Gliszczyńska-Świgło, A., 2007. Effect of storage on the content of polyphenols, vitamin C and the antioxidant activity of orange juices. J. Food Compos. Anal. 20, 313–322.

Kris-Etherton, P.M., Lefevre, M., Beecher, G.R., Gross, M.D., Keen, C.L., Etherton, T.D., 2004. Bioactive compounds in nutrition and health-research methodologies for establishing biological function: The Antioxidant and Anti-inflammatory Effects of Flavonoids on Atherosclerosis. Annu. Rev. Nutr. 24, 511–538.

Lamien-Meda, A., Lamien, C.E., Compaoré, M.M.Y., Meda, R.N.T., Kiendrebeogo, M., Zeba, B., Millogo, J.F., Nacoulma, O.G., 2008. Polyphenol Content and Antioxidant Activity of Fourteen Wild Edible Fruits from Burkina Faso. Molecules 13, 581–594.

Li, Z., Zeng, J., Tong, Z., Qi, Y., Gu, L., 2015. Hydrogenolytic depolymerization of procyanidin polymers from hi-tannin sorghum bran. Food Chem. 188, 337–342.

Martínez-Flores, H.E., Garnica-Romo, M.G., Bermúdez-Aguirre, D., Pokhrel, P.R., Barbosa-Cánovas, G. V., 2015. Physico-chemical parameters, bioactive compounds and microbial quality of thermo-sonicated carrot juice during storage. Food Chem. 172, 650–656.

Masola, S.N., Mosha, R.D., Wambura, P.N., 2009. Assessment of antimicrobial activity of crude extracts of stem and root barks from Adansonia digitata (Bombacaceae) (African baobab). African J. Biotechnol. 8, 5076–5083.

Mulaudzi, R.B., Ndhlala, A.R., Kulkarni, M.G., Van Staden, J., 2012. Pharmacological properties and protein binding capacity of phenolic extracts of some Venda medicinal plants used against cough and fever. J. Ethnopharmacol. 143, 185–193.

Ndabikunze, B.K., Masambu, B.N., Tiisekwa, B.M., 2010. Vitamin C and mineral contents, acceptability and shelf life of juice prepared from four indigenous fruits of the Miombo woodlands of Tanzania. J. Food, Agric. Environ. 8, 91–96.

Nhukarume, L., Chikwambi, Z., Muchuweti, M., Chipurura, B., 2010. Phenolic content and antioxidant capacities of Parinari curatelifolia, Strychnos spinosa and Adansonia digitata. J. Food Biochem. 34, 207–221.

Nicoli, M., Anese, M., Parpinel, M., 1999. Influence of processing on the antioxidant properties of fruit and

vegetables. Trends Food Sci. Technol. 10, 94–100.

Osman, M., 2004. Chemical and nutrient analysis of baobab (Adansonia digitata) fruit and seed protein solubility. Plant Foods Hum. Nutr. 59, 29–33.

Ozdal, T., Capanoglu, E., Altay, F., 2013. A review on protein-phenolic interactions and associated changes. Food Res. Int. 51, 954–970.

Parada, J., Aguilera, J., 2007. Food microstructure affects the bioavailability of several nutrients. J. Food Sci. 72, 21–32.

Pérez-Jiménez, J., Neveu, V., Vos, F., Scalbert, A., 2010. Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. Eur. J. Clin. Nutr. 64 Suppl 3, S112-20.

Piljac-Zegarac, J., Belscak, A., Piljac, A., 2009. Antioxidant capacity and polyphenolic content of blueberry (Vaccinium corymbosum L.) leaf infusions. J. Med. Food 12, 608–614.

Piljac-Žegarac, J., Valek, L., Martinez, S., Belščak, A., 2009. Fluctuations in the phenolic content and antioxidant capacity of dark fruit juices in refrigerated storage. Food Chem. 113, 394–400.

Pimpão, R.C., Dew, T., Oliveira, P.B., Williamson, G., Ferreira, R.B., Santos, C.N., 2013. Analysis of phenolic compounds in Portuguese wild and commercial berries after multienzyme hydrolysis. J. Agric. Food Chem. 61, 4053–4062.

Piras, C., Scano, P., Locci, E., Sanna, R., Cesare Marincola, F., 2014. Analysing the effects of frozen storage and processing on the metabolite profile of raw mullet roes using ^{1}H NMR spectroscopy. Food Chem. 159, 71–79.

Prior, R.L., 2004. Absorption and metabolism of anthocyanins: potential health effects. In: Meskin, M., Bidlack, W.R., Davies, A.J., Lewis, D.S., Randolph, R.K. (Eds), Phytochemicals. Mechanisms of Action. CRC Press, Boca Raton, FL, pp. 1-19.

Prior, R.L., Gu, L., 2005. Occurrence and biological significance of proanthocyanidins in the American diet. Phytochemistry 66, 2264–2280.

Rawson, A., Patras, A., Tiwari, B., 2011. Effect of thermal and non thermal processing technologies on the bioactive content of exotic fruits and their products: Review of recent advances. Food Res. Int. 44, 1875–1887.

Re, R., Pellegrini, N., Proteggente, A., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26, 1231–1237.

Remini, H., Mertz, C., Belbahi, A., Achir, N., Dornier, M., Madani, K., 2015. Degradation kinetic modelling of

ascorbic acid and colour intensity in pasteurised blood orange juice during storage. Food Chem. 173, 665–673.

Rio, D. Del, Rodriguez-mateos, A., Spencer, J.P.E., Tognolini, M., Borges, G., Crozier, A., 2013. Dietary (poly) phenolics in human health: structures, bioavailability, and evidence of protective effects against chronic diseases. Antioxidants Redox Signal. 18, 1882–1892.

Rössle, C., Gormley, T.R., Brunton, N., Butler, F., 2011. Quality and antioxidant properties of fresh-cut apple wedges from 10 cultivars during modified atmosphere packaging storage. Food Sci. Technol. Int. 17, 267–76.

Rufián-Henares, J.A., Morales, F.J., 2007. Functional properties of melanoidins: In vitro antioxidant, antimicrobial and antihypertensive activities. Food Res. Int. 40, 995–1002.

Saka, J., Rapp, I., Akinnifesi, F., Ndolo, V., Mhango, J., 2007. Physicochemical and organoleptic characteristics of Uapaca kirkiana, Strychnos cocculoides, Adansonia digitata and Mangiferia indica fruit products. Int. J. Food Sci. Technol. 42, 836–841.

Saka J.D.K, Mwendo-Phiri, E., Akinnifesi, F.K., 2002. Community processing and nutritive value of some miombo indigenous fruits in central and southern Malawi. In: Akinnifesi, F.K., AGumya, A. (Eds.), Proceedings of 14th Southern African Regional Review and Planning Workshop, 3 – 7 September 2001, Harare, Zimbabwe, International Centre for Research in Agroforestry. ICRAF, Harare, pp. 164–169.

Sanmartin, M., Davey, M.W., Montagu, M. Van, Inze, D., Kanellis, A., Smirnoff, N., Benzie, I.J.J., Strain, J.J., Favell, D., Fletcher, J., 2000. Plant L-ascorbic acid : chemistry, function, metabolism, bioavailability and effects of processing. J. Sci. Food Agric. 860, 825–860.

Scherer, R., Rybka, A., Ballus, C., Meinhart, A., Filho, J., Godoy, H., 2012. Validation of a HPLC method for simultaneous determination of main organic acids in fruits and juices. Food Chem. 135, 150–154.

Shahat, A. A., 2006. Procyanidins from Adansonia digitata. Pharm. Biol. 44, 445–450.

Shahidi, F., Zhong, Y., 2015. Measurement of antioxidant activity. J. Funct. Foods 18, 757–781.

Singleton, V., Rudolf, O., Lamuela-Raventos, R., 1999. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol. 299, 152–178.

Sun, Y., Qiao, L., Shen, Y., Jiang, P., Chen, J., Ye, X., 2013. Phytochemical Profile and Antioxidant Activity of Physiological Drop of Citrus Fruits. J. Food Sci. 78, 37–42.

Tembo, D.T.W., 2008. Optimisation of Vitamin C Extraction and Physicochemical Properties of Adansonia digitata, Parinari curatellifolia, Strychnos cocculoides and Ziziphus mauritiana fruits of Malawi. MSc

Thesis, University of Malawi, Chancellor College, Zomba.

Thaipong, K., Boonprakob, U., Crosby, K., Cisneros-Zevallos, L., Hawkins Byrne, D., 2006. Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. J. Food Compos. Anal. 19, 669–675.

Thiong'o, M.K., Kingori, S., Jaenicke, H., 2000. The taste of the wild: Variation in the nutritional quality of marula fruits and opportunities for domestication. Acta Hortic. 575, 237–244.

Tuberoso, C.I.G., Rosa, A., Bifulco, E., Melis, M.P., Atzeri, A., Pirisi, F.M., Dessì, M.A., 2010. Chemical composition and antioxidant activities of Myrtus communis L. berries extracts. Food Chem. 123, 1242–1251.

Vermaak, I., Kamatou, G.P.P., Komane-Mofokeng, B., Viljoen, A.M., Beckett, K., 2011. African seed oils of commercial importance - Cosmetic applications. South African J. Bot. 77, 920–933.

Zhang, M., Chen, H., Li, J., Pei, Y., Liang, Y., 2010. Antioxidant properties of tartary buckwheat extracts as affected by different thermal processing methods. LWT - Food Sci. Technol. 43, 181–185.

Figure 1. Flow chart showing steps during preparation of baobab juice in Malawi.

Figure 2. HPLC-DAD chromatogram of organic acids (A) and bioactive compounds (B) identified in fresh baobab pulp. Peaks: 1, tartaric acid; 2, malic acid; 3, ascorbic acid; 4, citric acid. EGCG; (-)-epigallocatechin-3-O-gallate.

Figure 3. Percent retention of ascorbic acid (AA) identified by HPLC in baobab pulp and juice after heat treatment using microwave, plate heat exchanger and water bath. Each bar represents means of three independent samples ± SD. Bars with different letters are significantly different ($p \leq 0.05$).

Figure 4. Changes in vitamin C concentration in conventional thermal pasteurised juice (72 °C, 15 s) stored at 6, 15 and 30 °C for 60 days. Each bar represents means of three independent samples ± SD. Bars with different letters are significantly different ($p \leq 0.05$).

Figure 5. Degradation reaction kinetics of vitamin C in conventional thermal pasteurised juice (72 °C, 15 s) stored at 6, 15 and 30 °C for 60 days.
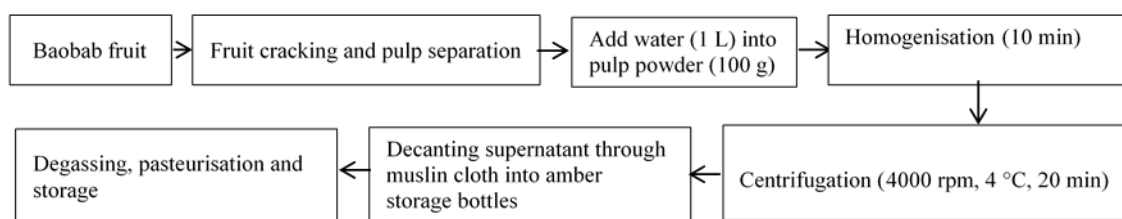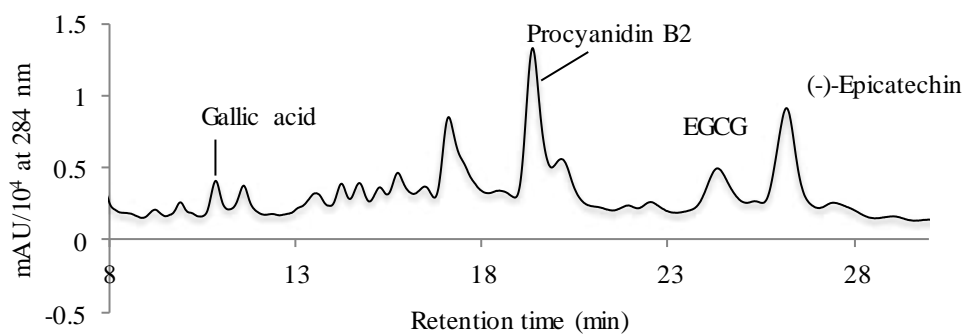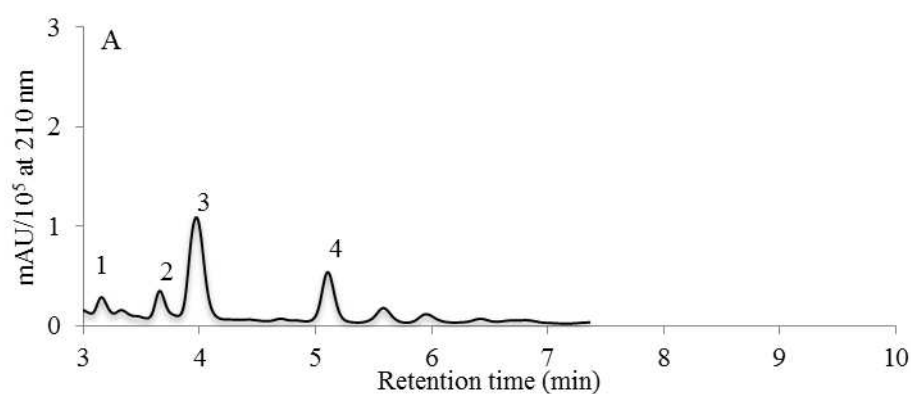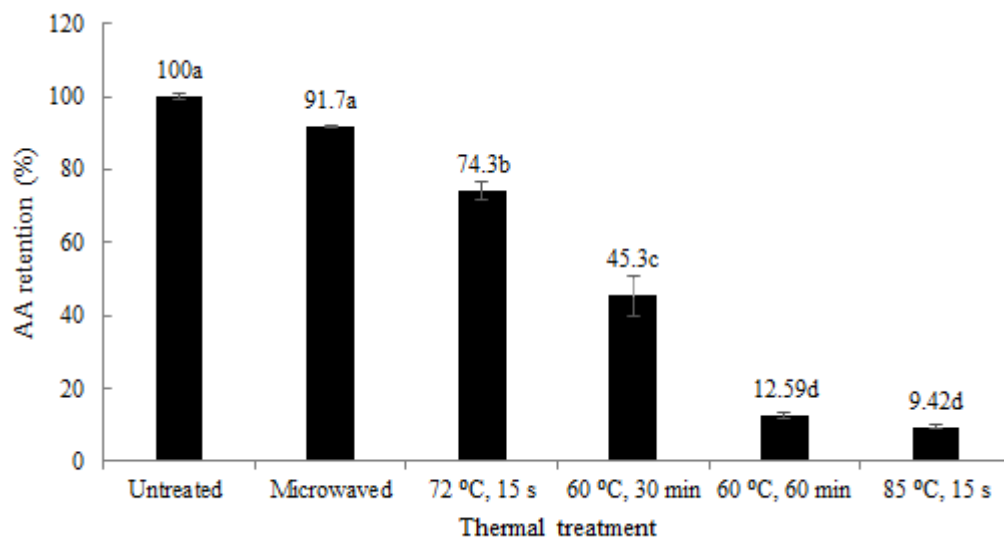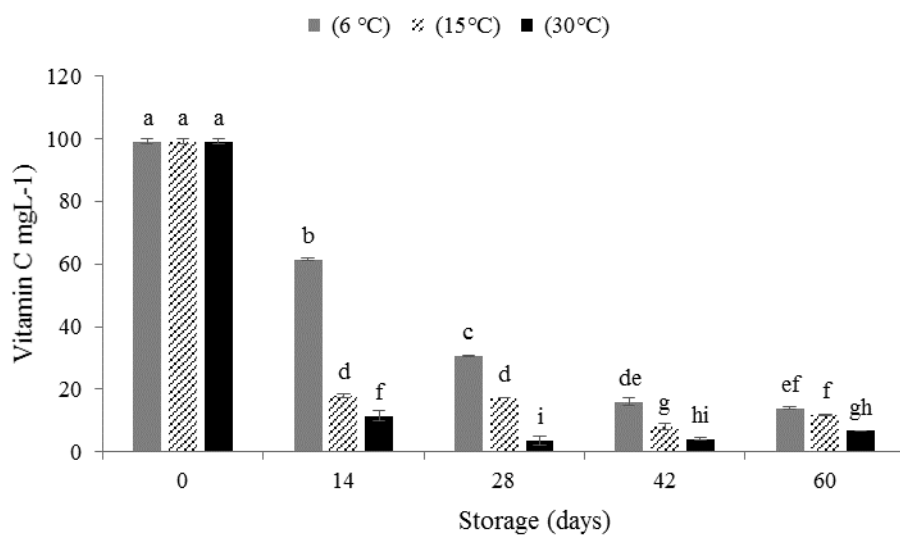
Fig 1.

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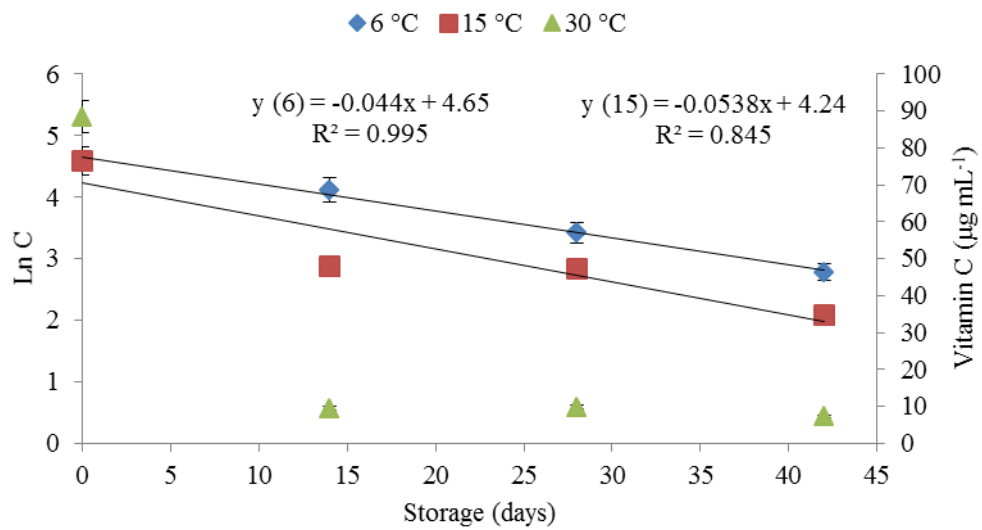
Fig. 5

Table 1. Validation of chromatographic parameters using standard compounds and samples. .

Standard compound	Retention time (min)	LOD (mg/mL)	LOQ (mg/mL)	Wavelength (nm)	Linearity (R²)	Recovery (%)
Ascorbic acid	3.97 ± 0.00	0.011	0.037	254	1.000	97.0
Citric acid	5.01 ± 0.01	0.058	0.193	210	0.999	114
Malic acid	3.58 ± 0.01	0.087	0.290	210	0.998	93.0
Tartaric acid	3.15 ± 0.01	0.024	0.080	210	0.999	109
(-)-Epicatechin	25.8 ± 0.03	0.009	0.030	284	0.999	96.1
Procyanidin B2	19.3 ± 0.29	0.068	0.227	284	1.000	100
EGCG	24.3 ± 0.03	0.002	0.007	284	1.000	106
Gallic acid	11.1 ± 0.01	0.037	0.123	284	1.000	106
HMF	11.5 ± 0.01	0.016	0.053	284	0.999	102

Abbreviations: Limit of Detection (LOD); Limit of Quantification (LOQ); (-)-Epigallocatechin-3-O-gallate (EGCG); 5-hydroxymethylfurfural (HMF). Values are means of at least three independent samples ± SD (n>3).

	Retention time (min)	LOD (mg/mL)	LOQ (mg/mL)	Wavelength (nm)	Linearity (R²)	Recovery (%)

Table 2. HPLC quantification of organic acids and selected bioactive compounds (mg/100 g FW fresh weight) in the fresh baobab pulp.

Vitamin C	Concentration	Phenolic compounds	Concentration
Vitamin C (AA + DHA)	466 ± 2.50	(-)-Epicatechin	43.0 ± 3.08
Ascorbic acid	352 ± 7.41	Procyanidin B$_2$	533 ± 22.6
Dehydroascorbic acid	115 ± 1.10	(-)-Epigallocatechin-3-O-gallate	9.98 ± 0.08
		Gallic acid	68.5 ± 12.4
Organic acids	**Concentration**		
Citric acid	3300 ± 0.90	**Others**	**Concentration**
Malic acid	2360 ± 28.8	5-Hydroxymethylfurfural	1.1 ± 0.0
Tartaric acid	174 ± 5.50		

Abbreviations: AA= Ascorbic acid; DHA = Dehydroascorbic acid; FW = Fresh weight. Values are means of three independent samples ± SD.

Table 3. Vitamin C (ascorbic acid (AA) and dehydroascorbic acid (DHA)) and organic acid concentration of pasteurised juice (72 °C, 15 s).

Storage Temperature (°C)	Time(Days)	Vitamin C (AA+DHA)(mg L^{-1})	Ascorbic acid (mg L^{-1})	Citric acid (mg L^{-1})	Malic acid (mg L^{-1})
Untreated (control)		97.1 ± 0.89	87.1 ± 8.47	3300 ± 18.5	2360 ± 28.8
6	0	99.12 ± 0.95^a	75.80 ± 0.13^a	3340 ± 21.2^{abc}	2410 ± 43.1^{ab}
	14	61.44 ± 0.77^b	22.42 ± 1.08^b	3250 ± 3.09^{abc}	1920 ± 10.7b^{cde}
	28	30.55 ± 1.57^c	6.57 ± 0.00^c	3020 ± 168^{bcd}	1840 ± 45.8^{de}
	42	16.08 ± 0.77^{de}	3.45 ± 0.04^{efg}	3230 ± 73.0^{abc}	2060 ± 349^{abcd}
	60	14.04 ± 0.03^{ef}	2.15 ± 0.06^{gh}	3360 ± 31.8^{abc}	2370 ± 34.9^{abc}
15	14	17.82 ± 0.53^d	5.46 ± 0.47^{cd}	3260 ± 6.43^{abc}	1940 ± 20.4b^{cde}
	28	17.28 ± 0.15^d	3.53 ± 0.72^{efg}	2990 ± 283^{bcd}	1800 ± 128de
	42	8.12 ± 1.20^g	3.29 ± 0.77^{fg}	2760 ± 191^d	1510 ± 318^e
	60	11.95 ± 0.22^f	2.42 ± 0.11^{gh}	3380 ± 15.3^{bc}	2310 ± 22.6^{abcd}
30	14	11.56 ± 1.47^f	4.71 ± 0.42^{de}	3510 ± 27.9^a	2230 ± 39.8^{abcd}
	28	3.71 ± 1.36^i	1.81 ± 0.20^h	2970 ± 213^{cd}	1890 ± 68.4^{cde}
	42	4.13 ± 0.40^{hi}	4.76 ± 0.26^{de}	3030 ± 176^{bcd}	1820 ± 387de
	60	6.73 ± 0.14^{gh}	4.63 ± 0.22^{def}	3440 ± 36.9^a	2480 ± 21.0^a

Abbreviations: AA = Ascorbic acid; DHA = Dehydroascorbicc acid;

Values are means of three independent samples ± SD; Mean values within a column with different superscript letters indicate significant differences (Tukey's test, $p \leq 0.05$).

Table 4. Procyanidin B_2, (-)-epicatechin, total phenol content and antioxidant activity of pasteurised juice (72 °C, 15 s).

Storage Temperature (°C)	Time (Days)	Procyanidin B_2 (mg/100 g)	(-)-Epicatechin (mg/100 g)	TPC (mg GAE/100 g)	FRAP (mg TEAC/100 g)	DPPH (% DPPH)	ABTS (mg TEAC/100 g)
Untreated (control)		533 ± 22.6	43.0 ± 3.1	1560 ± 1.60	2810 ± 92.8	50.9 ± 0.43	1520 ±17.1
6	0	466 ± 56.5[f]	47.7 ± 8.5[d]	1080 ± 3.67[f]	2740 ± 13.5[b]	49.3 ± 0.16[a]	1500 ± 1.87[a]
	14	1060 ± 16.1[cde]	89.6 ± 3.5[b]	1100 ± 0.80[f]	2400 ± 7.05[de]	42.7 ± 0.60[de]	1290 ± 13.1[bc]
	28	1300 ± 1.8[b]	73.7 ± 2.4[c]	1310 ± 1.60[a]	2880 ± 16.0[a]	49.1 ± 0.22[a]	1520 ± 18.7[a]
	42	1720 ± 81.7[a]	98.6 ± 4.9[b]	1020 ± 5.26[g]	2380 ± 11.9[de]	41.9 ± 0.14[e]	1100 ± 9.70d[e]
	60	1020 ± 9.3[de]	58.1 ± 4.7[d]	1160 ± 4.81[de]	2370 ± 8.52[e]	38.1 ± 0.72[f]	1090 ± 17.8[de]
15	14	1120 ± 7.7[cd]	100 ± 0.9[b]	1260 ± 4.01[b]	2630 ± 21.1[c]	41.7 ± 0.47[e]	1380 ± 5.60[ab]
	28	1140 ± 4.5[cd]	91.4 ± 1.8[b]	1170 ± 16.8[d]	2450 ± 9.77[d]	46.0 ± 1.55[bc]	1270 ± 7.46[bcd]
	42	1180 ± 107[bc]	22.9 ± 0.7[e]	723 ± 16.10[h]	1850 ± 37.3[g]	37.0 ± 0.76[f]	1000 ± 12.2[e]
	60	1370 ± 0.6[g]	97.1 ± 3.1[b]	1210 ± 1.60[c]	2610 ± 15.3[c]	46.6 ± 0.33[b]	1350 ± 13.5[ab]
30	14	9560 ± 77.5[e]	156 ± 7.4[a]	1160 ± 4.81[d]	2660 ± 3.91[c]	43.3 ± 0.19[de]	1110 ± 121[cde]
	28	1020 ± 0.9[de]	88.3 ± 9.4[b]	1270 ± 5.78[b]	2740 ± 22.5[b]	46.9 ± 0.30[b]	1320 ± 61.7[b]
	42	9290 ± 21.2[e]	61.4 ± 2.1[cd]	1020 ± 10.24[g]	2270 ± 35.4[f]	44.2 ± 0.25[cd]	1420 ± 61.9[ab]
	60	1630 ± 44.5[a]	56.3 ± 0.3[d]	1140 ± 9.25[e]	2640 ± 39.1[c]	46.5 ± 0.51[b]	1410 ± 155[ab]

Abbreviations: Total phenol content (TPC); Ferric reducing antioxidant power (FRAP); 2, 2–Diphenyl-1-picrylhydrazyl (DPPH); 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS). Values are means of three independent samples ± SD. Mean values within a column with different superscript letters indicate significant differences (Tukeys's test, $p \leq 0.05$)

Table 5. Pearson's correlation coefficients (r) for antioxidant variables and antioxidant activity

	TPC	PRO	EC	FRAP	DPPH	ABTS
Vit C	0.011	-0.325*	-0.173	0.259	0.351*	0.366*
TPC		0.212*	0.060	0.876**	0.575**	0.530**
PRO			0.271*	0.309*	0.252*	0.198*
EC				0.053	0.009	0.055
FRAP					0.786**	0.637**
DPPH						0.781**

Abbreviations: Vitamin C (Vit C); Total phenol content (TPC); Procyanidin B_2 (PRO); (-)-Epicatechin (EC); Ferric reducing antioxidant power (FRAP); 2, 2–Diphenyl-1-picrylhydrazyl (DPPH).