

Article

Design of a Coffee Alternative by Brewing Roasted Seeds from Baobab (*Adansonia digitata*)

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Abstract

Background: The use of baobab seed beverages as coffee alternatives represents a novel approach to upcycling by-products. Baobab seed aqueous extract is caffeine-free and contains numerous compounds of nutritional interest. The composition and sensory characteristics of baobab seed beverage can be modulated by roasting and brewing conditions. **Objective:** This study aimed to assess the effect of using different fluidised bed roasting temperatures and microwave infusion on the nutritional and functional properties of the beverage. **Results:** Higher roasting temperatures increased solubility, melanoidin content, pH, titratable acidity, colour, phenolic content, and antioxidant activity, while the concentration of chlorogenic acid and caffeic acid decreased. Upon microwave infusion, antioxidant activity, phenolic content (gallic acid, coumaric acid, caffeic acid, and vanillic acid), protein content, and soluble fibre content increased. Chlorogenic acid was not present in microwave-infused samples, and the amount of caffeic acid decreased. The fat content remained similar across all samples. The major volatile components identified in the roasted seeds were furans and pyrazines. **Conclusions:** These findings highlight the potential of baobab seed beverages as coffee alternatives and the impact of roasting and brewing conditions on their nutritional and functional properties.

Keywords: Baobab seed; coffee alternative; brewing; functional beverage



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1. Introduction

In 2008, baobab fruit pulp (*Adansonia digitata*) was included in the European Union's list of novel food ingredients, and in 2009, the Food and Drug Administration recognised it as a food ingredient [1]. The fruit pulp constitutes 20% of the whole baobab fruit, while 80% is made up of a shell, fibres covering the seeds, and the seeds [2]. Baobab seeds are a byproduct of fruit processing. Studies have shown that they are rich in fatty acids, phenolic compounds, vitamin C, minerals, carbohydrates, and essential amino acids [3,4]. Locally, baobab seeds are roasted and eaten as a snack or ground into flour that is used as a flavouring agent or processed into a coffee alternative in homes or at a small scale [5,6]. Recent research has shown some interest in baobab seeds as a coffee alternative due to the lower amount of caffeine in the aqueous extract [3,5]. On the other hand, baobab seeds

are promising sources of functional compounds due to their nutritional profile. This is especially pertinent since consumers are becoming more aware of the importance of diet to health.

The roasting degree of the baobab brew—the so-called “baobab seed beverage” (BSB)—influences its quality, including pH, polyphenols, acidity, total soluble solids, colour, and volatile compounds [1,4]. After roasting, the powdered seeds are infused for ten minutes at 100 °C [4], requiring prolonged contact between solids and water. The extraction parameters are essential factors in the yield and quality of bioactive and volatile compounds, which affect the flavour profile and functional properties of hot beverages [7]. Interest in alternative brewing methods that minimise the sensory and functional compounds has generated popularity in the previous decade. One such technology, microwave-assisted extraction (MAE), has been gradually developed, and beneficial results have been reported [8]. Microwave-assisted extraction accelerates extraction and reduces infusion time during the extraction of target compounds in various matrices [9,10]. However, the roasting parameters for baobab seed brew are not yet fully standardised. Understanding roasting and subsequent infusion methods is an initial step towards improving processing techniques to produce ingredients for coffee alternatives effectively.

Therefore, this work aimed to investigate the comparison between conventional roasting and brewing and MAE on the physicochemical composition of BSB and show its potential as a source of natural ingredients, such as fibre, phenolic compounds, and volatile compounds. There were two hypotheses relating to (i) roasting parameters and (ii) brewing methods. The first hypothesis was that higher roasting temperatures would optimise colour and volatile compounds that mimic alternative coffee beverages. The second hypothesis was that regular hot water infusion gives similar outcomes to microwave extraction for the extraction of thermolabile components. The second hypothesis reinforces that the baobab beverage can be further exploited as a functional ingredient or an extract to fortify nutrient-limiting complementary foods crucial for human health in conventional settings.

2. Materials and Methods

2.1. Experimental Design

The baobab fruit pods were purchased from a local market in Zimbabwe. The baobab seeds were mechanically extracted from the pulp by blending and washing the seeds with warm water to remove the pulp, and subsequently, air dried in atmospheric conditions (25 °C, 60% RH). The seeds were then roasted in a pre-heated electric fluidised bed coffee roaster (Toper Optical Roaster, Izmir, Turkey) for 30 min at 100, 120, 150, and 180 °C and were provided with a fan with a constant speed of 2100 rpm (Figure 1). The fluidised bed roaster was programmed to cool the roasted seeds to 40 °C. The baobab seed roasted samples were then cryo-milled (Freezer mill 6875D, Spex Sample Prep LLC, Metuchen, NJ, USA) using 2 cycles, 2 min cooling, 5 min grinding, and 15 cycles per second. The baobab seed powder (BSP) fraction was sieved (250 µm pore size) and subsequently used in this study. BSP was subsequently frozen at −20 °C until further analysis. Based on the nutritional content results obtained from baobab seed roasted at different temperatures, the samples at 180 °C for 30 min were chosen for subsequent extraction. Demineralised water (produced by a local ion-exchange system, laboratory supply) was used for baobab infusions. Unless stated otherwise, Milli-Q water (Merck Millipore, Merck KGaA, Darmstadt, Germany, $\geq 18.2 \text{ M}\Omega\cdot\text{cm}$) was used throughout the study for all subsequent analyses.

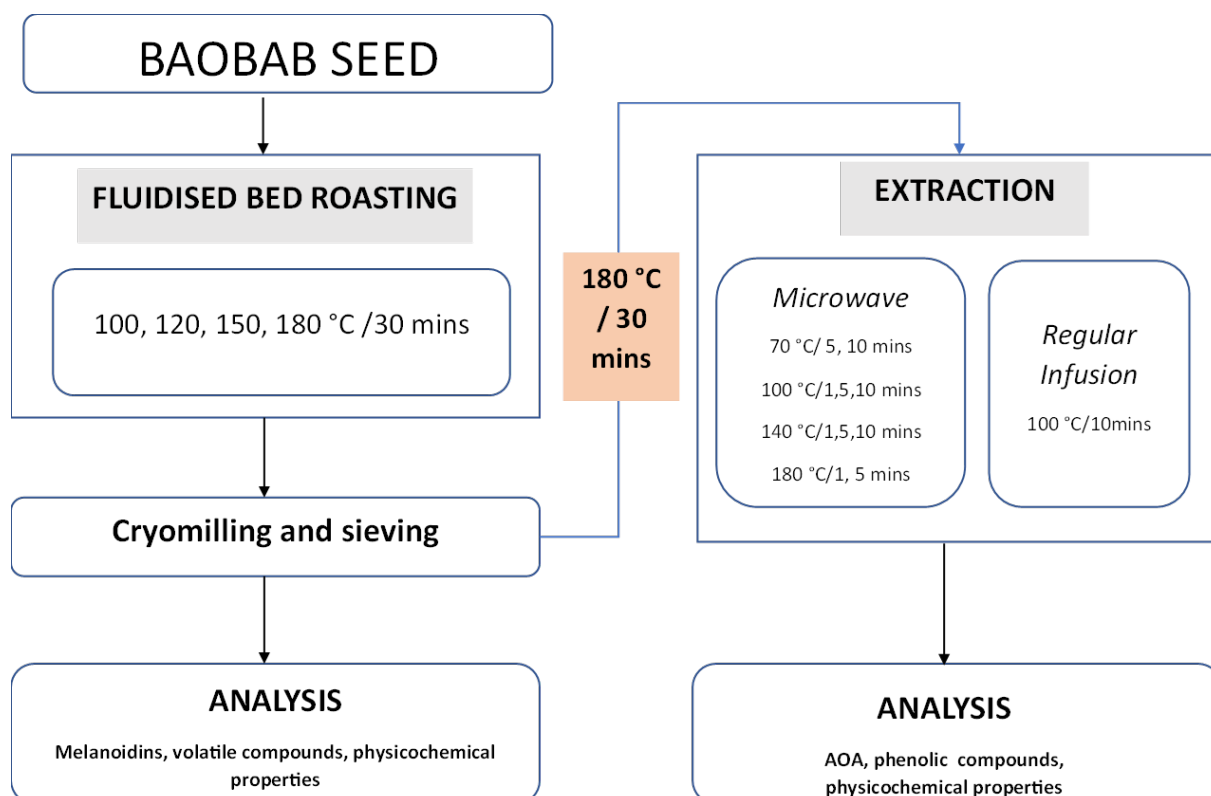


Figure 1. Sample preparation and experimental plan for baobab seed roasting and infusion. Note that the sample roasted at 180 °C for 30 min was the one used for microwave extraction or regular infusion.

2.1.1. Microwave-Assisted Extraction (MAE)

MAE was performed using a microwave reactor (Monowave400, Anton Paar). The suspension in the reactor was continuously stirred with a magnetic bar that minimised the heterogeneous microwave heating. The BSP samples were extracted using water as a solvent at a solid-to-solvent ratio of 1:12 (*w/v*). The magnetic stirring was adjusted to 100% (~400 rpm). The microwave power was adjusted to attain 70, 100, 140, and 180 °C, and the temperature was maintained for 1, 5, and 10 min, depending on the temperature setting, as illustrated in Figure 1. After extraction, the reactors were cooled down to room temperature. The samples were then centrifuged (4000 rpm, 10 min, 4 °C), and the supernatant, or baobab seed beverage (BSB), was frozen at −20 °C for further analysis.

2.1.2. Regular Infusion

Regular infusion was prepared using demineralised water as a solvent at a ratio of 1:12 (*w/w*) of BSP at 100 °C for 10 min. The extracted samples were centrifuged (4000 rpm, 10 min, 4 °C), and the supernatant (BSB) was stored at −20 °C until further analyses.

2.2. Sample Analysis of Roasted Baobab Seed Powder

2.2.1. Physico-Chemical Properties

Moisture Content and Solubility

The moisture content on the BSP was determined according to AOAC analysis methods [4]. The solubility analysis was determined according to Doğan et al. (2019) with a few modifications [11]. A mixture of 1 g BSP and 10 mL of water was heated in plastic centrifuge tubes with continuous shaking at 100 °C for 30 min. After heating, the tubes were centrifuged (4700 rpm, 5 min, 4 °C). The supernatant was poured into an aluminium

tray and dried using an oven to constant weight at 105 °C overnight. After drying, Equation (1) was used to calculate the solubility of the samples.

$$\text{Solubility (\%)} = (W_1 - W_2) \times 100W_1 \quad (1)$$

where W_1 is the weight of the sample before drying, and W_2 is the weight of the sample after drying.

Colour

Colour measurements were obtained in the CieLab system (L^* , a^* , b^*) using a Hunter ColorFlex LAB instrument (Escolab, Gelderland, The Netherlands), previously calibrated with black, green, and white standard tiles. The L^* coordinate represents brightness, with values ranging from 0 (black) to 100 (white). The a^* coordinate varies from -100 (green) to $+100$ (red), while the b^* coordinate extends from -100 (blue/purple) to $+100$ (yellow). Colour differences were calculated as $\Delta E^* = [(\Delta L^2) + (\Delta a^2) + (\Delta b^2)]^{1/2}$. The sample exhibiting the highest brightness was selected as the reference. Transparency was assessed by determining the percentage of light transmittance at 660 nm with a Cary 50 Bio UV–Vis spectrophotometer (Agilent, Santa Clara, CA, USA), using distilled water as the blank. The transmittance percentage (%T) was calculated using the following formula: $\%T = \text{antilog}^*(2 - \text{Absorbance})$.

Titrateable Acidity (TA) and pH

The pH was measured by mixing 1 g of ground samples with 10 mL of hot water at 100 °C for 30 min with continuous shaking using a water bath shaker. The samples were cooled to room temperature, and pH was measured using a pH metre (pH1002, VWR phenomenal). The TA was determined using the method by Kipkorir, Patrick and Simon [12] with modifications. Briefly, 5 g of ground samples were mixed with 35 mL of 80% ethanol. The mixture was kept under continuous rotation overnight for ~12 h using a laboratory rotator at a constant speed of 20 rpm. Then, 10 mL of the supernatant was filtered through a 0.45 µm CA nylon filter. The filtrate was diluted to 100 mL with water and titrated with 0.1 N NaOH, with three drops of phenolphthalein indicator.

2.2.2. Melanoidins

The melanoidin components were quantified spectrophotometrically according to the method of Pérez-Hernández et al. [13] without modifications.

2.2.3. Volatile Compounds (VOC)

The VOC headspace of the baobab seed powder was measured according to the method described by Peña-Correa et al. [14] without modification. Approximately 2 g of baobab seed powder was transferred to 10 mL headspace glass vials. Gas chromatography–mass spectrometry (GC–MS) analysis was carried out using a Thermo Scientific™ Trace GC Ultra system coupled with a Thermo Scientific™ TriPlus RSH™ autosampler (Thermo Fisher Scientific, Waltham, MA, USA). For sample extraction, a 75 µm Carboxen/Polydimethylsiloxane solid-phase microextraction (SPME) fibre was inserted into the vials and exposed to the headspace for 10 min. The fibre was then placed in the GC injection port to release the adsorbed volatile compounds. The temperature programme consisted of an initial ramp from 40 °C to 200 °C at 10 °C/min, followed by an isothermal hold at 200 °C for 5 min. Helium was employed as the carrier gas at a constant flow rate of 2.0 mL/min. Separation of volatile compounds was achieved on a Stabilwax®-DA column (30 m × 0.25 mm; Restek Corporation, Bellefonte, PA, USA). The mass spectrometer was operated with a scan range of 33–250 m/z , and both instrument control and data acquisition

were performed with Xcalibur 3.0 software (Thermo Fisher Scientific, Waltham, MA, USA). Finally, the retention time and the analyte peak area of each identified VOC were processed with Chromeleon 7.2 software.

2.3. Sample Analysis of Baobab Seed Beverage

2.3.1. Antioxidant Activity (AOA) and Total Phenolic Compounds (TPC)

The TPC and AOA followed the same protocol as Vilas-Franquesa and colleagues [15] with slight modifications for the DPPH assay. The dilution factor employed in the AOA analysis of BSB was 1:24 with milli-Q water (*v/v*) before analysis.

2.3.2. Quantification of Specific Phenolics Through LC-MS/MS

BSB diluted 1:24 with milli-Q water was used for LC-MS quantification of specific phenolic compounds (gallic acid, *p*-coumaric acid, caffeic acid, and vanillin) following the method previously used in spent coffee ground by Montemurro and colleagues (2024).

Ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC–MS/MS) was carried out on a Nexera UPLC system equipped with an LCMS-8050 triple quadrupole mass spectrometer (Shimadzu Corporation, Kyoto, Japan). The UPLC platform included a SIL-30AC autosampler, LC-20ADXR solvent delivery unit, DGU-20ASR degasser, CTO-20AC column oven, and FCV-20AH2 valve module.

For chromatographic separation of phenolic compounds, 5 µL of sample was injected into an Acquity Premier BEH C18 column (1.7 µm, 2.1 × 100 mm) fitted with an Acquity UPLC BEH C18 VanGuard pre-column (130 Å, 1.7 µm, 2.1 × 5 mm; Waters Chromatography B.V., Etten-Leur, The Netherlands). The mobile phase consisted of 0.1% formic acid in water (solvent A) and acetonitrile containing 0.1% formic acid (solvent B). The elution gradient (time [min]/%B) was programmed as follows: 0.0/5, 2.0/35, 7.5/95, 9.5/95, and 9.6/5. The flow rate was maintained at 0.3 mL/min and the column oven temperature at 40 °C. Each run lasted 13.5 min, during which MS data were acquired.

Multiple reaction monitoring (MRM) conditions were optimised by flow injection analysis of phenolic standards (20 ppm in H₂O/ACN, 1:1 *v/v*), using LabSolutions support software (Shimadzu Corporation, Kyoto, Japan). The mass spectrometer was operated in negative ionisation mode. Turbo ion-spray voltage was set at 4.0 kV, with the electrospray ionisation probe heated to 300 °C, the desolvation line at 250 °C, and the heat block at 400 °C. The collision-induced dissociation (CID) gas pressure was adjusted to 4 kPa, while the drying gas, nebulising gas, and heating gas were delivered at 10, 3, and 10 mL/min, respectively.

Due to sample limitations, experimental determination using signal-to-noise ratios was not feasible. Based on the Codex Alimentarius CAC/GL 74-2010, we used statistically derived estimates [16]. The calibration curves, LOD, LOQ, and matrix effect values were evaluated (see Supplementary Material). LOD and LOQ were calculated using the standard deviation of the intercept from the calibration curve. Data was processed with LabSolutions Insight software (Shimadzu Corporation, Kyoto, Japan). A 5-point calibration curve was constructed from 0.1 to 2.5 ppm for gallic acid ($R^2 = 0.999$), from 1.6 to 4.3 ppm for *p*-coumaric acid ($R^2 = 0.998$) and caffeic acid ($R^2 = 0.994$), and from 10 to 500 ppb for vanillin ($R^2 = 0.999$).

2.3.3. Physicochemical Properties

The beverage was dehydrated in a freeze-dryer to obtain a powder. The protein content ($N \times 5.71$) was determined by the Dumas combustion method using an analyser (EA 1112 NC, Thermo Fisher Scientific Inc., Waltham, MA, USA) following the manufacturer's protocol. Cellulose and D-methionine were used to prepare the control and calibration curves, respectively. The quantification of free amino groups (NH₂) was determined by the

O-phthalaldehyde (OPA) assay according to Nielsen et al. [17], after diluting the samples with MilliQ water 1:3 (*v/v*). Soxhlet petroleum ether extraction system was used to extract fat from 5 g of sample according to the official method [11]. The extracted fat was then expressed as the mass percentage of the original sample.

2.3.4. Ethanolic Precipitable Matter (EPM)

The EPM was quantified after enzymatic digestion as described by Vilas-Franquesa et al. [18] with slight modifications. Briefly, 12.5 μ L of α -amylase was added to 10 g of BSB and incubated at 100 °C for 30 min under gentle stirring. The mixture was then cooled to 60 °C, after which 25 μ L of protease was introduced and incubated for 30 min with continuous agitation. Following protease treatment, the pH was adjusted to 4.1–4.8, when necessary, using 5% NaOH. Subsequently, 50 μ L of amyloglucosidase was added, and the sample was incubated for another 30 min at 60 °C with agitation. Precipitation was carried out by adding preheated ethanol (95%, 60 °C) at a 4:1 (*v/v*) ratio, and the mixture was left to stand at room temperature for 60 min. The precipitate was collected by centrifugation at 3000 rpm for 10 min, separating the EPM from the ethanol-soluble matter (ESM). The EPM fraction was freeze-dried and quantified gravimetrically. All enzymes were obtained from Neogen Europe Ltd. (Lansing, MI, USA) as part of the Total Dietary Fibre Assay Kit (AACC Method 32-05.01).

2.4. Statistical Analysis

All results are given as means $\pm$ standard deviation of three fully independent determinations of each parameter. Significant differences between means were determined by one-way ANOVA followed by the Tukey post hoc test when significant. Differences were considered significant at $p < 0.05$. The Statistical Package for Social Sciences (SPSS v23.0) software was used for all analyses. Experiments were conducted in triplicate.

3. Results and Discussion

3.1. Roasted Baobab Seeds

3.1.1. Physico-Chemical Properties

Table 1 presents a decrease in moisture content, L^* , and pH and an increase in TA, solubility, and clarity with increasing roasting temperatures of the baobab seeds.

Table 1. The physical properties of roasted baobab seeds.

Parameter	Moisture Content (%)	ΔE	L^*	Solubility (%)	Clarity (% Transmittance at 660 nm)	pH	TA (g/L)	Melanoidin Concentration (g/100 g)
FB1	2.54 ± 0.07^a	24.94	47.52 ± 0.02^a	24.28 ± 0.39^a	29.70 ± 0.01^a	6.15 ± 0.02^a	0.011 ± 0.005^a	9.91 ± 0.01^a
FB2	1.56 ± 0.07^b	20.79	43.23 ± 0.01^b	32.77 ± 0.40^b	25.58 ± 0.02^b	6.13 ± 0.02^a	0.026 ± 0.005^b	$9.99 \pm 0.01^{b,c}$
FB3	0.78 ± 0.06^c	11.36	33.61 ± 0.02^c	33.41 ± 0.46^c	$24.58 \pm 0.01^{b,d}$	5.97 ± 0.01^b	0.041 ± 0.005^c	$10.04 \pm 0.01^{b,d,e}$
FB4	0.44 ± 0.07^d	3.56	25.61 ± 0.02^d	34.93 ± 0.17^d	15.51 ± 0.01^c	5.22 ± 0.07^c	0.109 ± 0.005^d	10.09 ± 0.05^e
Traditional pan-roasted	0.68 ± 0.07^e	16.73	38.44 ± 0.02^e	29.22 ± 0.28^e	22.81 ± 0.01^d	5.77 ± 0.03^d	0.079 ± 0.005^e	$9.99 \pm 0.04^{c,d}$
Control	1.65 ± 0.06^b	-	22.86 ± 0.01^f	25.28 ± 0.37^f	18.12 ± 0.01^e	5.16 ± 0.05^e	0.150 ± 0.02^f	10.20 ± 0.06^f

Different superscripts highlight differences in each measured variable at $p < 0.05$.

Values are listed as a mean of the measurements and standard deviation. Different superscripts in the same column denote a statistically significant difference ($p < 0.05$). Fluidised bed roasting FB1: 100 °C, FB2: 120 °C, FB3: 150 °C, and FB4: 180 °C, each roasting condition for 30 min.

Moisture content decreased below 2.5% for all samples. A decreasing trend from 2.4% to 0.44% with increased roasting temperature was observed, resulting in dehydration of the seeds during the roasting process. Solubility increased with increasing roasting temperature, while the moisture content decreased. All samples had moisture content below 5%, which enables flowability, increases porosity, and reduces density [19,20]. Colour differences between samples were perceptually insignificant except in FB4, where a steep decrease in L^* and clarity in the same direction was observed. Consequently, the increased solubility improved the clarity of the samples.

The tolerance criteria of a standard observer not perceiving the colour difference between two samples are accepted at $\Delta E < 3.5$ [21]. The lower L^* correlated to higher temperatures is primarily related to non-enzymatic reactions, notably the Maillard reaction and sugar caramelisation, and to a certain extent by reactions such as metal ion complexes (mainly Fe and Cu) with phenolic compounds or ascorbic acid degradation [22]. The Maillard reaction, a primary non-enzymatic browning process, becomes prominent when constituents like reducing sugars and amines—comprising amino acids, peptides, or proteins—interact during thermal food processing [23]. Consequently, thermally processed foods typically contain diverse Maillard reaction by-products, which serve as valuable indicators of the duration and intensity of the thermal treatment [24]. These results may suggest a reduced nutritional quality (due to intense heat treatment), which may affect protein digestibility and require further investigation. However, the calculated (ΔE) unperceivable difference between FB4 and instant coffee has the potential for acceptability by consumers.

The pH dropped from 6.16 to 5.22. An inverse correlation was observed for pH ($R = -0.98$; $p = 0.01$) with titratable acidity and roasting temperature. Low pH is explained by the release of organic acids from the breakdown of aliphatic acid compounds and the release of protons into solution, acidic caramelisation by-products (such as pyruvic acid), or hydrolytic activity of amylolytic enzymes during roasting at higher temperatures [25,26]. The pH and TA trend falls within the range of a previous study [4]. A relatively lower pH and higher TA positively impact the quality of the beverage, i.e., its shelf life and sensory perception.

3.1.2. Melanoidin Content

Table 1 shows a positive association between melanoidin content and the roasting temperature. These results corroborate those reported by Sacchetti and coauthors [27] where the melanoidin formation of cocoa beans increased with the increase in roasting temperature. The control resulted in the highest concentration of melanoidin content since mild instant coffee is generally roasted up to 200 °C, according to other published information [28]. Melanoidins are high molecular weight compounds responsible for the characteristic brown colour formation and other sensory properties: taste, flavour, and texture [29] and their concentration can be modulated by MAE (Lopes et al., 2020) [23]. The relation between melanoidin content and colour is directly proportional. The increase in melanoidin concentration decreased the lightness value and ΔE values of the samples. This illustrates the importance of Maillard reactions associated with the characteristic brown colour formation in a typical coffee-like beverage.

3.1.3. Volatile Compounds

A total of 65 and 135 volatile compounds were tentatively identified in roasted baobab seeds and instant coffee, respectively. The volatiles are classified into chemical groups: furans, pyrroles, pyridines, pyrazines, ketones, hydrocarbons, aldehydes, and others (Table 2).

Table 2. The major volatile compounds identified in roasted baobab seed powder from FB4 * and traditional roasted samples.

FB4	Traditionally Roasted	Control
(2-Aziridinylethyl)amine	(2-Aziridinylethyl)amine	3-Trifluoroacetoxypentadecane
Acetone	Acetone	Pyrazine
Acetic acid	1-Decyne	Pyrazine, methyl-
1-Decyne	Pyrazine, methyl-	Pyrazine, 2,5-dimethyl
Pyrazine, methyl-	2-Propanone, 1-hydroxy-	Pyrazine, 2-ethyl-3-methyl
Pyrazine, 2, 6-dimethyl	Pyrimidine, 4,6-dimethyl-	3(2H)-Furanone, dihydro-2-methyl
Pyrazine, 2-ethyl-6-methyl	Ammonium acetate	2-Propanone, 1-hydroxy-
Pyrazine, 2-ethyl-3-methyl	Furfural	Disulfide
Pyrimidine, 4,6-dimethyl-	2(1H)-Pyridinone	Furfural
2(1H)-Pyridinone	Propanoic acid	Furan, 3-methyl
Propanoic acid	2-Furanmethanol	2(1H)-Pyridinone
Furfural	2-Furancarboxaldehyde	Propanoic acid
2-Furanmethanol		Paromomycin
2-Furancarboxaldehyde		2-Furanmethanol

* FB4: Fluidised bed roasting: 180 °C for 30 min.

The aroma formation of baobab seeds was modified during fluidised bed roasting. New volatiles: Acetic acid, 2,6-dimethyl pyrazine, and pyrazine 2-ethyl-6-methyl were identified and profiled. Though acetic acid is mildly potent, 2, 6-dimethyl pyrazine and pyrazine 2-ethyl-6-methyl have been previously reported and are very potent coffee odorants [30]. Hydroxydisulfide was only found in the control and diminished with both roasting methods. A low-threshold odour, 3 (2H) Furanone, was profiled in the control sample, which diminished with roasting. Furans and pyrazines represented the major chemical group in roasted baobab seeds with increased roasting temperature. Similar observations were reported by Kim and Lee [31], where significant thermal reactions at roasting temperatures above 170 °C resulted in pyrazine formation. Furans and pyrazines are generated through the Maillard reaction, which is responsible for generating the characteristic roasted flavour of coffee: burnt, caramel-like, and meaty aromas [14,32]. The volatile aroma compounds are essential for quality and sensorial attributes in the BSB.

3.2. Baobab Seed Beverage

3.2.1. Physico-Chemical Properties

Table 3 presents the physicochemical properties of BSB obtained through regular infusion.

Table 3. Nutritional composition of baobab seed beverage extracted by regular infusion after roasting at different temperatures.

Roasting Temperature (°C)	Fat (g/100 g)	EPM (%)	Protein (g/100 g)	DH (%)
100 °C	13.906 ± 0.106	1.443 ± 0.140 ^b	0.329 ± 0.044	0.287 ± 0.001 ^b
120 °C	13.541 ± 0.330	1.866 ± 0.133 ^a	0.282 ± 0.049	0.294 ± 0.000 ^a
150 °C	14.073 ± 0.052	2.156 ± 0.182 ^a	0.207 ± 0.028	0.293 ± 0.000 ^a
180 °C	13.385 ± 0.240	2.875 ± 0.194 ^a	0.230 ± 0.0366	0.292 ± 0.001 ^a

Different superscripts highlight differences in each measured variable at $p < 0.05$. Values in each column (small letter) bearing different superscripted numbers are statistically different ($p < 0.05$) for each roasting temperature. EPM—Ethanolic precipitable matter, DH—Degree of (protein hydrolysis). Data expressed as mean ± standard deviation.

It was noted that the BSB was characterised by increasing amounts of free amino acids and EMP after 100 °C; there was no significant difference from 120 to 180 °C, while protein

content decreased. The fat content was highest at 150 °C. The EMP fraction was used as a proxy for soluble fibre. The protein and fat content of the roasted seed was reported in other studies as 21.5–32.87 and 30.97–31, respectively, g/100 g [33,34]. The fat content of the baobab infusion was considerably higher, while the protein content was lower when compared to previous research on the avocado seed drink [35]. Lipids act as carriers for flavour. Roasted coffee oil added to instant coffee improves flavour and prevents granules from fragmenting [36]. Furthermore, lipids can be a source of vitamin E and enhance the AOA of the infused beverage; therefore, the highest content at 150 °C can be used to indicate the correlation with the AOA (Figure 2). The soluble fibre was similar to that found in the studies of Diaz Robia and Saura-Calixto in espresso and filtered powdered coffee extracts [37]. These amounts of oligosaccharides would be necessary to determine the nutritional significance. Given their health and economic advantages, further in vivo tests would be useful to determine their fate in the human body.

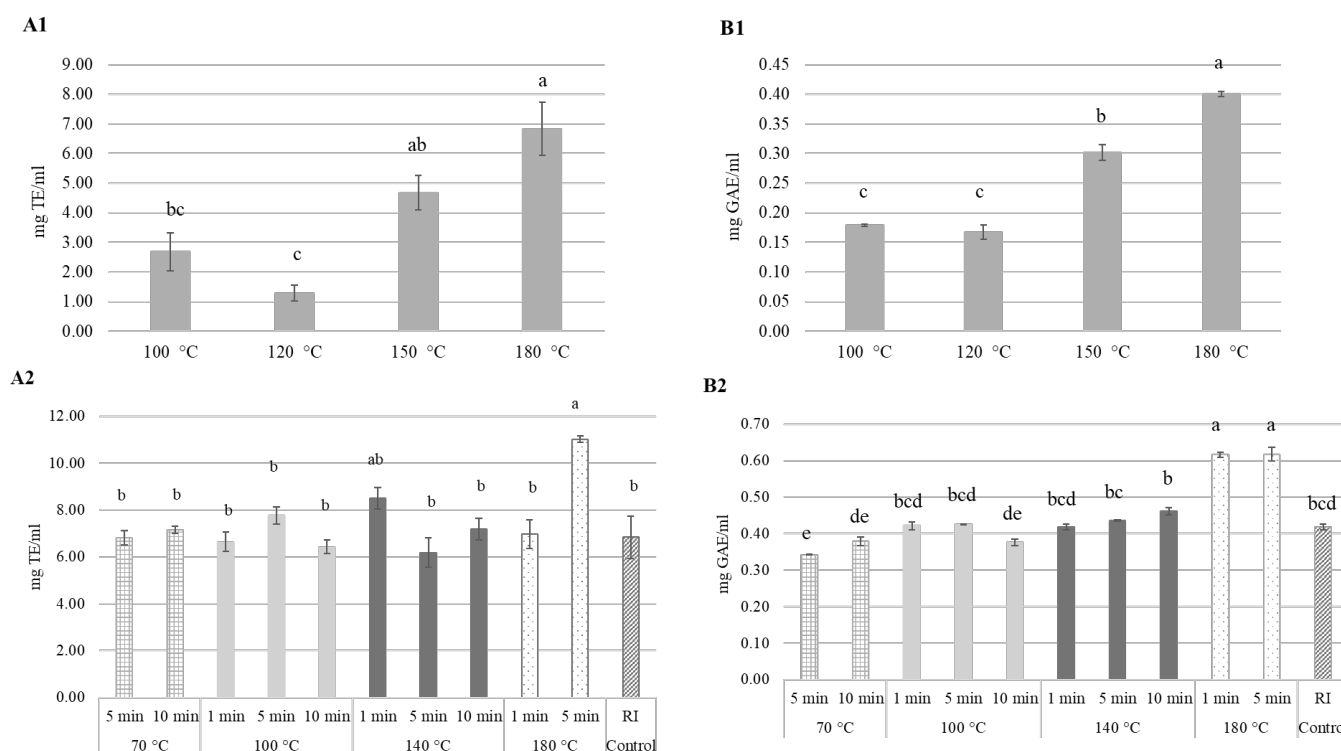


Figure 2. Total antioxidant activity and phenolic concentration in the infused samples using different roasting temperatures (A1,B1) and different microwave conditions (A2,B2). Values bearing different superscripted letters in the same graph are statistically different ($p < 0.05$).

Table 4 shows the physicochemical properties of BSB by MAE.

There was a significant decrease in amino acids and fat, while EPM and crude protein significantly increased at 180 °C. At each temperature point, the extraction time did not affect the nutritional attributes, except for fat, where each increase in time resulted in a significant decrease. The fat content was higher, while the protein content was lower in the microwave than in regular infusions, as shown in Table 3. It was interesting to note that at 140 °C, the protein content was the highest. However, it is paralleled by a significantly lower amino acid content, with a 3.4% difference. The assumption is that amino acids occur in different concentrations, and their subsequent partial conversions are dependent, among other factors, on the temperature applied during MAE. Determining the specific amino acids is therefore crucial to understanding the observed decrease at 140 °C and its impact on the final quality. The amino acid content indicates the quality and degree of processing.

In some recent studies, it was concluded that amino acid content is an important parameter for defining quality in dried tea because the amino acids are also responsible for the aroma of tea components [38].

Table 4. Nutritional composition of baobab seed beverage extracted by microwaving after roasting at 180 °C.

Extraction Temperature (°C)	Extraction Time (min)	Fat (g/100 g)	EPM (%)	Protein (g/100 g)	DH (%)
70 °C	5 min	16.87 ± 0.025 ^a	0.961 ± 0.050 ^b	0.169 ± 0.005 ^b	0.294 ± 0.001 ^a
	10 min	16.579 ± 0.015 ^a	0.936 ± 0.127 ^b	0.169 ± 0.000 ^b	
100 °C	1 min	16.607 ± 0.445 ^a	2.131 ± 0.289 ^a	0.191 ± 0.013 ^b	
	5 min	14.448 ± 0.298 ^{bc}	1.930 ± 0.289 ^{ab}	0.173 ± 0.001 ^b	0.296 ± 0.001 ^a
	10 min	15.549 ± 0.657 ^{ab}	1.987 ± 0.326 ^{ab}	0.181 ± 0.029 ^b	
140 °C	1 min	13.498 ± 0.091 ^{cde}	2.041 ± 0.136 ^{ab}	0.169 ± 0.009 ^b	
	5 min	14.123 ± 0.318 ^{bcd}	1.98 ± 0.234 ^{ab}	0.184 ± 0.004 ^b	0.294 ± 0.001 ^a
	10 min	13.665 ± 0.281 ^{cde}	2.610 ± 0.076 ^a	0.197 ± 0.004 ^b	
180 °C	1 min	12.234 ± 0.314 ^e	2.716 ± 0.052 ^a	0.330 ± 0.002 ^a	
	5 min	12.525 ± 0.250 ^{de}	2.943 ± 0.188 ^a	0.393 ± 0.055 ^a	0.284 ± 0.001 ^b
Control	RI	13.385 ± 0.240 ^{cde}	2.875 ± 0.194 ^a	0.230 ± 0.0366 ^b	0.292 ± 0.001 ^a

Different superscripts highlight differences in each measured variable at $p < 0.05$. Data expressed as mean ± standard deviation. Values in each column bearing different superscripted letters are statistically different ($p < 0.05$) for each roasting temperature. EPM—Ethanol precipitable matter, DH—Degree of (protein) Hydrolysis. The DH was measured at 5 min for all temperature points.

3.2.2. Antioxidant Activity, Total and Individual Phenolics

The AOA and TPC increased with increasing roasting temperatures (Figure 2), while the extraction time of MAE was positively correlated to AOA.

Longer MAE time resulted in higher AOA in the BSB, across all temperatures. The highest AOA and TPC were obtained with a roasting temperature of 180 °C. These findings can be attributed to the release of bioactive compounds, specifically phenolic compounds, resulting from enhanced solubility, as shown in Table 1. The high AOA value can also be influenced by the higher recovery of melanoidins at higher roasting temperatures (Table 1). A higher antioxidant activity was also previously reported by Marcolino et al. (2023) upon increasing the roasting temperature [24].

Microwave extraction of BSB at 180 °C yielded significantly higher AOA compared to regular infusion (Figure 2). The effect of the extraction time on the recovery of phenolic compounds and higher antioxidant activity has already been shown in spent coffee grounds, especially during the first 10 min [39]. Nevertheless, this trend was not observed in the gallic acid concentration, as gallic acid recovery increased with increasing MAE temperature but decreased with high roasting temperatures. Recent articles show that a higher extraction temperature is linked to an increase in gallic acid content [40], because of the wide distribution of gallic acid in higher molecular weight phenolic molecules. The effect of higher MAE temperature on the concentration of phenolic compounds can also be observed when analysing individual phenolic compounds (the recovery of caffeic and *p*-coumaric acid was below the LOQ, thus not shown). Notably, these compounds were only detected in trace amounts after RI, rather than in any MAE setup.

These results could open windows of opportunity for the sequential step recovery of different phenolic compounds from baobab seeds. Gallic acid and vanillin increased with temperature and time (Table 5). The peak for both compounds was achieved at 180 °C and 5 min of extraction, indicating a positive correlation with AOA and demonstrating relative heat stability during roasting and infusion. In other studies, higher temperature

and time lead to greater recovery of arabinogalactans, which can contribute to AOA [23]. In addition, the recovery of phenolic compounds through high MAE temperatures has also been reported in other matrices [23].

Table 5. Gallic acid and vanillin in the baobab seed beverage produced by different treatments.

Regular infusion	Infusion time (min)	Roasting temperature (°C)	Gallic acid (ppm)	Vanillin (ppm)
	10	100	9.260 ^a ± 1.231	<LOD
	10	120	3.489 ^b ± 2.471	<LOD
	10	150	8.963 ^a ± 0.742	<LOD
	10	180	3.579 ^b ± 0.130	<LOD
Microwave	Extraction time (min)	Extraction temperature (°C)		
	5	70	3.096 ^b ± 0.180	1.104 ^c ± 0.010
	10	70	3.028 ^b ± 0.201	1.115 ^c ± 0.112
	1	100	3.425 ^b ± 0.071	1.232 ^{bc} ± 0.055
	5	100	3.510 ^b ± 0.113	1.191 ^{bc} ± 0.064
	10	100	3.405 ^b ± 0.027	1.148 ^{bc} ± 0.028
	1	140	4.007 ^b ± 0.275	1.258 ^{bc} ± 0.006
	5	140	4.289 ^b ± 0.370	1.326 ^b ± 0.038
	10	140	4.597 ^b ± 0.119	1.262 ^{bc} ± 0.043
	1	180	5.095 ^b ± 0.360	1.317 ^b ± 0.075
	5	180	5.486 ^b ± 0.043	1.557 ^a ± 0.001

Data expressed as mean ± standard deviation. Values in each column bearing different superscripted letters are statistically different ($p < 0.05$) for each compound. Microwave-assisted extraction samples were roasted at 180 °C for 30 min before extraction.

4. Conclusions

This study shows the first comprehensive demonstration of a caffeine-free coffee alternative derived from upcycled baobab. It investigated the effect of roasting temperatures and MAE infusion on the physical-chemical properties of a baobab seed beverage. The increased roasting temperature significantly increased titratable acidity, solubility, and clarity, and decreased colour parameters (L^* , b^* , and a^*), alongside the formation of coffee-characteristic furans and pyrazines. Microwave-assisted extraction (MAE) further enhanced total phenolic content and AOA. Apart from the nutritional value and functional properties of roasted baobab seed, findings indicate its use in food for mulations. Furthermore, co-formulation with other natural flavours in the BSB could enhance the baobab seed's value as a sustainable, high-value coffee substitute. Further analysis of the extraction of functional compounds from the seed and the spent ground could present an interesting approach to the standardised production of a healthy beverage product.

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