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Influence of different traditional production processes on the antioxidant capacity and vitamin C content of baobab (*Adansonia digitata*) juice

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This study aims to assess the effect of traditional production processes of baobab juice on its antioxidant and vitamin C content. A survey was conducted in the North region of Cameroon and the 4 main processes identified were subsequently used to prepare juice samples at two different baobab pulp/sugar ratios per litre (42 g/62 g and 87.5 g/231 g) that were analysed. The main difference between the 4 processes reported by the 92 respondents was at the steeping step. Steeping was mostly done for 6 h in fresh water (84.3% of them); 3 h in warm or boiling water; or by boiling the pulp-water mixture for ± 5 min. A high pulp/water ratio and temperature led to a significant increase in the antioxidant properties of the juice. The juice with the highest pulp content produced using the last steeping approach had the highest quality (Total Antioxidant Capacity, 5.76 ± 0.26 g AAE/100 ml; Total Phenolic Content, 46.72 ± 0.61 mg GAE/100 ml; Radical Scavenging Activity: 33.08 ± 2.48% of DPPH· Inhibition/100 ml; Vitamin C, 38.51 ± 5.34 mg/100 ml). High temperature of water during the production of baobab juice is recommended to optimize health value of baobab juice.

Key words: *Adansonia digitata*, juice, production process, antioxidants, vitamin C.

INTRODUCTION

Baobab (*Adansonia digitata* L.) is an indigenous fruit tree belonging to the Malvaceae family and associated with Savannah dry lands of sub-Saharan Africa of which the North and Far North regions of Cameroon are part (Bremer et al., 2009; Muthai et al., 2017). This tree produces relatively large silvery green or brownish

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indehiscent fruits, with an outer hard shell, an inner soft whitish powdery pulp containing kidney-shaped dark brown seeds, all bound together by thin and light brown fibres (Sidibe and Williams, 2002). The baobab fruit is well known in Africa both for its medicinal properties and social uses (Cissé et al., 2013). Biochemical studies have shown that the pulp content is particularly rich in dietary fibres, carbohydrates, essential amino acids, minerals (Potassium, Calcium, Magnesium, Iron, Sodium, Zinc and Manganese) and extremely low in fat (Magdi, 2004; Soloviev et al., 2004; Chadare et al., 2009 and De Caluwé et al., 2010).This pulp has also been described as an excellent source of Vitamin C, since its ascorbic acid content (2.8-3 g/kg) was estimated to be six times higher than in orange (Sidibé et al., 1996; Donatien et al., 2011). Furthermore, the baobab pulp has a higher antioxidant capacity than commonly consumed fruits such as orange, strawberry, apple and kiwi fruits (Silvia et al., 2002; Latifou et al., 2012). Indeed, the Integral Antioxidant Capacity value of baobab fruit pulp is 10 times higher than that of orange pulp, with values of 11.1 mmol/g (Equivalents Trolox) and 0.3 mmol/g (Equivalents Trolox), respectively (Silvia et al., 2002). Epidemiological studies have shown a link between the intake of ascorbic acid and other antioxidant micronutrients to better health. This is associated to their capability in trapping the reactive oxygen species responsible for a broad-spectrum of damages to biological systems (degenerative diseases, cancer) (Elsayed, 2001).

The baobab fruit is exploited traditionally for the treatment of microbial diseases (dysentery, diarrhoea) in many African countries including Cameroon; it also has hepatoprotective effect (Hanafy et al., 2016), cardioprotective (Ghoneim et al., 2016), antidiabetic (Irondi et al., 2017) and is used by many communities as an alternative to imported Western drugs (FAO, 1993; Kamatou et al., 2011). This indigenous fruit contributes to nutrition and food security, health and income generation of local communities in Sub-Saharan Africa (Muthai et al., 2017), especially during times of seasonal food shortages or emergencies such as drought and floods (Saka et al., 2004). Baobab fruits may be processed into different products including juice, yoghurt, gruel, sour dough, oil and coffee-like drinks. It can also be dried as food reserve (Saka et al*.,* 2002). Despite its nutritional and health properties, it is not extensively studied (Russo et al., 2019) and remains underutilized in Cameroon. The small section of the population who knows about this indigenous fruit usually consumes it in the form of juice, a locally made beverage for home consumption or sold on the streets. This leads to a variety of production processes involved, each with a different impact on health value of the final juice. Eulalia and Agnieszka (2009) showed that the antioxidant capacity of a product is influenced by the technological processes involved. This study is therefore aimed at identifying the traditional processes of baobab juice production in Cameroon and their impact on its antioxidant and vitamin C contents.

MATERIALS AND METHODS

Survey on the consumption and production of baobab juice

A survey was carried out in June 2016 in the Benoue Subdivision (North Region of Cameroon) where baobab trees are naturally found and its juice is highly consumed by the population. A total of 92 persons (either sellers or consumers) randomly selected took part in the study. The only inclusive criterion was the consumption or the production of baobab juice. A questionnaire was used to collect information on the level of consumption and motivations, ingredients and production process used for preparation, factors determining its quality, and preservation methods.

The influence of traditional production processes on baobab juice antioxidants and vitamin C

Baobab fruits were harvested in the Benoue Subdivision and immediately transported to the laboratory. Sugar (from sugarcane) was purchased from a local market. The baobab fruit pods were broken and lightly pounded in a traditional mortar to separate the pulp from the seeds. Samples for juice were then prepared following the main traditional production processes identified from the survey and their antioxidant content directly assessed by determining their Total Antioxidant Capacity (TAC), Total Phenolic Content (TPC), Radical Scavenging Activity (RSA) and Vitamin C (ascorbic acid) content. All the analyses were performed in triplicate.

Determination of total antioxidant capacity

One millilitre of juice was diluted in 3 ml of distilled water; then 0.3 ml of the diluted juice was mixed with 3 ml of phosphomolybdenum reagent solution and put in a boiling Water Bath at 95°C for 90 min. The mixture was cooled to room temperature (25°C) and the absorbance was measured at 695 nm against the reagent blank. Ascorbic acid was used as standard at a concentration of 5 mg/mL. The TAC of the samples was calculated using Equation 1 and results expressed in mg Ascorbic Acid Equivalent (AAE)/100 mL of juice (Prieto et al., 1999).

$$\text{TAC} = (\text{DO Sample}/\text{DO Standard}) \times \text{Concentration of Standard} \quad (1)$$

Determination of total phenolic content

The Total Phenolic Content (TPC) was determined using the Folin-Ciocalteu method (Medina, 2011). 0.5 ml of diluted juice (1 ml of juice in 3 ml of distilled water) was mixed with 4.3 ml of distilled water and 0.2 ml of Folin-Ciocalteu reagent. The mixture was homogenized and incubated for 5 min at room temperature. Into each tube, 0.5 ml of 20% sodium carbonate was added, followed by 4.5 ml of distilled water. The tubes were homogenized and incubated in the dark for 1 h at room temperature and the absorbance was read at 725 nm. A standard curve was plotted using different concentrations of gallic acid which was used as standard (0, 25, 50, 100, 150, 200, 250, 300, 350, 400, 450 and 500 mg/L). The TPC was expressed as mg Gallic Acid Equivalent

Table 1. Consumption of baobab juice.

Parameter	Incidence (%)	Parameter	Incidence (%)
Reasons for consumption		**Frequency of consumption (No of times/month)**	
Taste	37.8	[1-4]	**50.8**
Health	**38.9**	[5-10]	12.8
Pleasure	36.7	[11-20]	16.4
No alcohol	20.0	[21-30]	20
Other	1.1		

(GAE)/100 ml of juice.

Determination of radical scavenging activity

The Radical Scavenging Activity (RSA) was evaluated using the 1.1-diphenyl-2-picryl hydrazyl (DPPH) radical. The analysis was carried out as described by Plaza et al. (2006). 7.8 ml of methanolic solution of DPPH (0.03 g/L) was added to 0.2 ml of diluted juice (1 ml of juice in 3 ml of HCl 1 %). The tubes were homogenized and incubated in the dark for 30 min at room temperature, followed by the reading of the absorbance at 515 nm. The DPPH solution was also read at the same wavelength using the reagent blank (HCl 1% + 0.03 g/L of DPPH). The RSA was calculated using the formula of Yen and Duh (1994) (Eq 2):

$$RSA\ \% = AA\ \% = [(A_{DPPH} - A_{Sam}) / A_{DPPH}] \times 100 \qquad (2)$$

Where, A_{DPPH} = the absorbance of the DPPH control and A_{Sam} = the absorbance of the sample.

The RSA was expressed as antioxidant activity (AA %), which is the percentage of inhibition with respect to the control.

Determination of vitamin C content

The vitamin C content was determined by the titrimetric method (Peter et al., 2010). 50 µl of diluted fruit juice (10 times with distilled water) was put in a tube and 450 µl of glacial acetic acid 90% was added. The mixture was homogenized and a dye solution of dichloro-2.6-phenolindophenol (2.6-DCPIP) was added until a permanent light pink colour was obtained. The titre (*T*) was recorded. The titration was repeated and the sample replaced with 50 µl of water (for the control) (*B*1) and 50 µl of ascorbic acid, 0.2 mg Ascorbic acid/20 ml glacial acetic acid 90% (for the standard solution) (*st*).

The vitamin C content of the sample was calculated using the following Equation 3:

$$\text{Vitamin C (mg/100 ml)} = [(T - B1)/ (st - B1)] \times \text{Dilution factor} \qquad (3)$$

Statistical analysis

The data collected during the survey were edited, coded and registered into a Microsoft Excel spreadsheet where they were treated, and the mean standard deviation of the values obtained was calculated. The software SPSS 20.0 for Windows was used to perform Analysis of Variance (ANOVA) and the post-hoc Turkey test to assess the significant differences observed (p<0.05) between these values.

RESULTS AND DISCUSSION

Survey

Socio-demographic variables

Among the 92 participants who took part in the survey, 66.3% were females. Most of these respondents were from the Far North (40.7%) and North (37.4%) regions of Cameroon, which are the only regions where baobab trees are naturally found due to the climate (Savannah drylands of sub-Saharan Africa) (Muthai et al., 2017). The other few respondents originated from the West (12.1%), Centre (6.6%), Adamawa (2.2%) and East (1.1%) regions of Cameroon. Baobab is rooted in the culture of the North and Far North regions of Cameroon, where the juice is often used as the main entertainment beverage in traditional ceremonies. This juice (the main product from baobab pulp) is recognised for its nutritional and health value and it is a source of income for these communities (Muthai et al., 2017).

Consumption of baobab juice

The data collected on baobab juice consumption (Table 1) shows that the surveyed population consume it for its healthy value (38.9%), its taste (37.8%), for pleasure (36.7%) and also because it is a non-alcoholic beverage (20%). Half of them consume the juice at a frequency of 4 times maximum/month (50.8%). Up to 20 % of respondents consume this juice, 21 to 30 times/month which means almost every day.

Production of baobab juice

Frequency of production: 52.4% of the respondents prepare baobab juice, maximum 4 times /month. The number of people was almost the same in the 3 other

Table 2. Parameters associated to baobab juice production.

Parameter	Incidence (%)	Parameter	Incidence (%)	Parameter	Incidence (%)
Frequency of production (times/month)		**Reasons for production**		**Ingredients for juice preparation**	
[1-4]	**52.4**	Consumption	**45.8**	Baobab's pulp	100.0
[5-10]	17.5	Commercial	12.0	Sugar	100.0
[11-20]	15.8	Both	42.2	Water	100.0
[21-30]	14.3			Extra ingredients	81.9
Source of baobab fruits		**Extra ingredients used**		**Parameters that affect the quality of juice**	
Market	**78.3**	Chemicals aroma	**84.74**	Quantity of water	**89.5**
Harvest	17.4	Chemicals instant drink (powder)	42.37	Quantity of pulp	25.6
Directly from Producers	4.3	Colouring	30.50	Temperature	18.6
		Other fruit juice	5.08.	Duration	9.3
		Natrum	3.38		
		Milk	1.69		
Volume of water (L) for 175 g of pulp (One plate)		**Quantity of sugar (g) used for the preparation of 1L of Juice**		**Steeping of pulp**	
[0.5-1]	2.8	<50	4.2	In fresh water (25°C)	**84.3**
[1-2]	14	[50-100]	28.0	In lukewarm water (40°C)	11.4
[2-3]	**42.3**	[100-150]	**42.2**	In hot water (100°C)	2.9
[3-4]	18.3	[150-200]	8.4	Boiling the mixture of pulp and fresh water	1.4
[4-5]	8.5	[200-250]	4.2		
[5-6]	9.9	[250-300]	8.4		
[6-8]	4.2	[300-500]	4.2		

classes: [5-10]; [11-20] and [21-30] times of juice production/month (Table 2).

Reasons for production: From the people interviewed, 45.8% prepare baobab juice only for home consumption, 12% for sale and 42.2% for both reasons.

Source of Baobab fruits: Most of respondents (78.3%) buy baobab fruits from the markets, 17.4% harvest it by themselves and 4.3% buy directly from producers. This tree is mostly found in bushes in mountainous areas, while some people have it in their land. It is difficult to climb because of the large size of the tree, which reaches, 18-25 m tall (Chadare et al., 2009), the reason why most of them buy it.

Main ingredients for Baobab juice*:* Baobab pulp, water and sugar were the main ingredients used by all respondents (100 %) in the formulation of baobab juice.

Extra ingredients for Baobab juice: 81.9% of the people add extra ingredients (chemical aroma, 84.74%; chemical instant drink, 42.37%; colouring agents, 30.50%; other fruit juices 5.08%; natron, 3.38% and milk, 1.69%) for commercial and/or sensory reasons. They add other sweeteners to reduce the quantity of sugar used, colouring agents to diversify the colour of the juice and other ingredients to modify the taste. These food additives were said to reduce the cost of production, attract customers and increase profits.

Volume of Water for one plate of pulp: The volume of water generally used for 175 g (one plate) of the baobab fruit pulp varied from 0.5 to 8 L. For this quantity of pulp, most people (42.3%) used between 2-3 L of water.

Quantity of sugar used/litre of juice: The quantity of sugar used for the preparation of 1 L of baobab juice varied from 31.25 g to 500 g, but the most widely used quantity ranged from 100-150 g (42.2%) followed by the range, 50-100 g (28%). The pulp/water ratio and the quantity of sugar used for 1 L of juice tended to change depending on the aim of production. Those who prepare it for their own consumption focused on the health value and the taste. They therefore apply a high pulp/water ratio to have a thick juice, and little or much sugar according to their preference. Sellers rather apply a low pulp/water ratio, less sugar and additional sweeteners, in

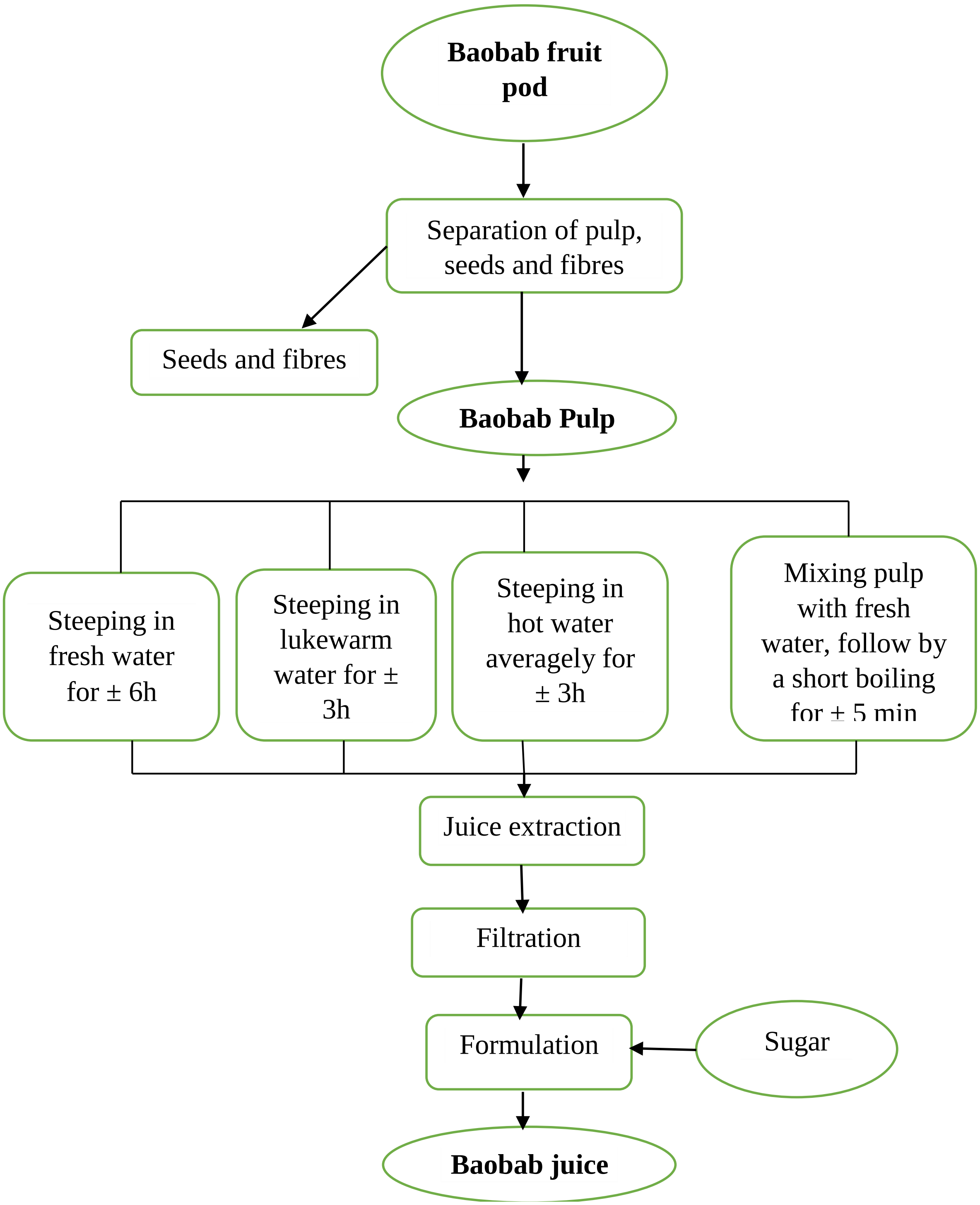

Figure 1. Diagram of the traditional production processes of baobab juice.

order to have more benefits.

Production processes: Figure 1 gives an overview of the 4 traditional production processes identified. They all include the following steps: The separation of baobab pulp from seeds and fibres, steeping with water, extraction and filtration of the juice and addition of sugar. It is at this last step that the other extra ingredients previously cited are added by the producers who use

Table 3. Preservation of baobab juice.

Parameter	Incidence (%)	Parameter	Incidence (%)
Storage methods		Duration of preservation	
Freezing	51.1	Less than one week	75.6
Refrigeration	35.9	Between one-four weeks	13.3
Traditional methods	13.0	Between one-three months	5.6
		More than three months	5.6

them. The way steeping is conducted was the key step differentiating the processes.

Steeping of pulp: Most of the respondents (84.3%) used the method which consisted of steeping the pulp in fresh water for an average of 6 h, while 11.4% steeped the pulp in warm water and 2.9% in hot water for about 3 h; and 1.4 % put the pulp in fresh water followed by boiling for a short time, ± 5 min (Table 2).

In order of importance, respondents estimated that the volume of water (89.5%), quantity of pulp (25.6%), steeping temperature (18.6%) and duration of steeping (9.3%) determine the quality of the baobab juice (Table 2). As reported by Charles et al. (2007), the pulp/water ratio influences the texture of juice. It determines whether the juice will be dense or light. The temperature affects the colour, odour, flavour and taste of the juice.

Preservation of baobab juice

Methods of preservation: Freezing was the most commonly used preservation method (51.1%), followed by refrigeration (35.9%) (Table 3). 13% of the surveyed population use other traditional preservation methods like steeping the bottle of juice in clay pots containing water (limited efficiency). This was due to poverty or electricity problems.

Duration of preservation: These different methods of preservation were usually needed to preserve juice for less than one week (75.6% of respondents). Generally, the consumers prepare juice only when they need to consume it, they do not have to preserve it for long. For traders, they sell it every day, so they do not also keep it for long.

Preparation of Baobab juice samples using different traditional processes

Eight different juice samples were prepared following the 4 traditional processes identified in the survey. In fact, for each process, baobab juice with two different pulp / sugar ratios (42 g/62 g, Process 1-4 and 87.5 g/231 g, Process 1'-4') per litre was prepared. These ratios were chosen to simulate those used when preparing this juice for sale and home consumption, respectively. As a pilot study, the first three processes were those where the pulp was steeped in fresh water at 25°C (Process 1), warm water at 40°C (Process 2) and hot water at 100°C (Process 3) all for 15 min. For process 4, the pulp was mixed with fresh water and directly boiled for 2 min. These durations were the shortest reported from the survey. The temperature profile for this steeping step, under the 8 conditions tested is presented in Figure 2. It was not affected by the change of pulp/water ratio. All the extracted juice samples were filtered using a sieve of 0.5 mm diameter before addition of sugar.

Antioxidants and vitamin C contents of baobab juice

The results obtained from the biochemical analysis of the juice samples are presented in Table 4.

Baobab pulp is known to be a rich source of antioxidants and vitamin C (Donatien et al., 2011). As expected, an increase in the pulp/water ratio of baobab juice led to an increase in the values obtained for the different parameters analysed. However, this rise was not statistically significant in the case of vitamin C.

The Total Antioxidant Capacity (TAC) of the 8 samples varied between 0.65 g AAE/100 ml and 5.76 g AAE/100 ml. For the ratio of pulp/sugar/water of 42 g/62 g/1 L, there was a significant difference ($p < 0.05$) between Process 1 and the 3 others which had the same TAC. For a high ratio of pulp/sugar/water (87.5 g/231 g/1 L), there was no statistical difference ($p > 0.05$) among processes (1'&2'; 2'&3' and 3'&4'). The use of boiling water for the steeping step (Process 3) or boiling of water and pulp mixture for a short time (Process 4) significantly ($p < 0.05$) enhanced the TAC of the juice. In each Process, the TAC significantly ($p < 0.05$) increased with the amount of pulp.

The Total Phenolic Content (TPC) of the juice ranged from 22.97-46.72 mg GAE/100 ml. Irrespective to the ratio, the temperature did not show any effect on TPC ($p > 0.05$). In each process, increase of pulp amount was associated with a significant increase in TPC ($p < 0.05$). Process 4' had the highest content of total phenolic

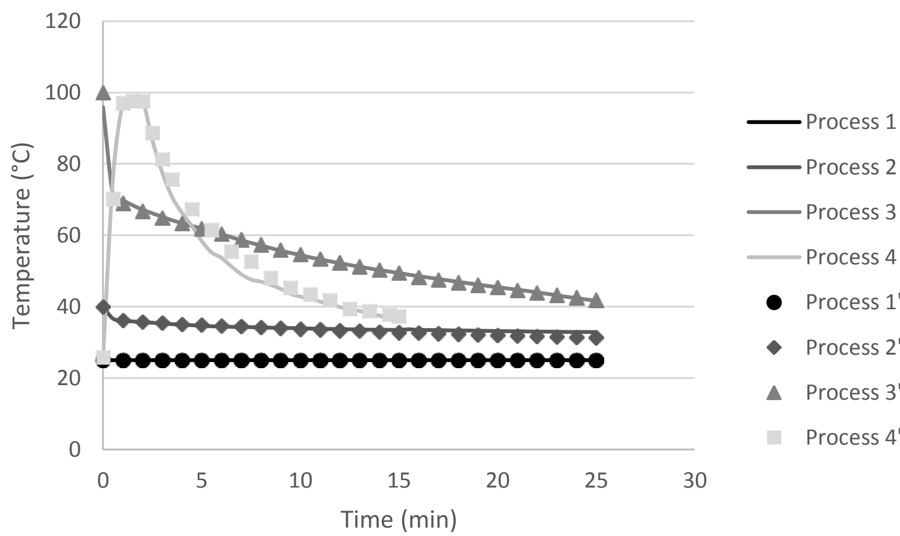

Figure 2. Temperature profiles during the steeping step. Processes 1, 2, 3 & 4= Pulp / sugar ratio of 42 g / 62 g per litre of juice; Processes 1’, 2’, 3’ & 4’= pulp / sugar ratio of 87.5 g /231 g per litre of juice.

Table 4. Biochemical characteristics of baobab juice samples produced.

Samples*		**TAC (g AAE /100 ml)**	**TPC (mg GAE/100 ml)**	**DPPH (% of Inhibition)/100 ml**	**Vitamin C (mg/100 ml)**
Process 1 (Fresh water :	1	0.65±0.05[a]	24.05±0.70[a]	10.85±0.70[a]	19.25±1.48[a]
25°C, 15 min)	1’	4.06±0.17[c]	39.67±1.61[bc]	28.83±2.33[b]	22.22±0.00[ab]
Process 2 (Lukewarm water :	2	1.85±0.02[b]	22.97±0.44[a]	8.69±1.66[a]	22.96±0.74[ab]
40°C, 15 min)	2’	4.45±0.19[cd]	37.14±0.45[bc]	25.94±3.29[b]	25.92±0.74[ab]
Process 3 (Boiling water :	3	2.06±0.16[b]	26.16±1.13[a]	14.57±1.00[a]	24.44±1.28[ab]
100°C, 15 min)	3’	5.24±0.43[de]	41.95±0.42[cd]	28.40±1.74[b]	31.11±0.00[bc]
Process 4 (Boil the mixture	4	2.51±0.12[b]	34.12±3.13[b]	25.79±1.58[b]	30.37±0.74[bc]
of pulp and water for 2 min)	4’	**5.76±0.26[e]**	**46.72±0.61[d]**	**33.08±2.48[b]**	**38.51±5.34[c]**

*1, 2, 3, 4= Pulp / sugar ratio of 42 g / 62 g per litre of juice; **1’, 2’, 3’, 4’=** pulp / sugar ratio of 87.5 g /231 g per litre of juice

Means in the same row with different superscript letters are significantly different (p < 0.05), **AAE**= Equivalent Ascorbic Acid; **GAE**= Equivalent Gallic Acid

compounds (46.72 ± 0.61 GAE/100 ml). The TPC of our samples was much lower than the values (260.80 ± 0.27 mg GAE/100 ml), reported by Tembo (2016) for baobab juice samples from Malawi made with fresh water (pulp/water ratio of 100 g/1 L), and by Konan et al. (2015) with commercialised baobab juice (CBJ) in Ivory Coast (50.1 ± 15.5 mg GAE/ml). However, the use of Process 4 (boiling pulp-water mixture for 2 min) led to a significantly (p < 0.05) high TPC of the final products. Balunkeswar et al. (2015) showed that thermal processing increased the total antioxidant activity of some vegetables. It may cause complex physical and chemical reactions affecting the phenolic composition, such as release of phenolic compounds from their bound forms, degradation of polyphenols and the breakdown and transformation of phenolic compounds (Lo Scalzo et al., 2004; Chen et al., 2013).

The radical scavenging activity (RSA) of the juice ranged from 8.69-33.08% of Inhibition/100mL. The DPPH scavenging activity from the different processes and the two ratios was not temperature-dependent (p > 0.05). An increasing amount of baobab pulp had a positive effect on DPPH inhibition (p < 0.05), except in Process 4. The highest percentage of inhibition obtained was 33.08 ± 2.48% (Process 4’) which was lower than the DPPH inhibition value (80.94 ± 0.72%) of commercial baobab juice (CBJ) in Malawi. The higher DPPH values observed in CBJ could be attributed to high TPC and sugar metabolites (Tembo, 2016).

Vitamin C content

The Vitamin C content was from 19.25 mg/100 ml (with fresh water) to 38.51 mg/100 ml (boiled mixture of pulp and water for 2 min). The vitamin C was also highest in the sample that was prepared using Process 4, as for TAC, TPC and DPPH. Since, vitamin C is known to be a thermosensitive compound (Ranu and Uma, 2012), its highest content in samples produced by boiling (using Process 3 and 4) suggests that the short duration at high temperature during the steeping step (Figure 2) helps to enhance its extraction and also that of the bioactive compounds studied. The vitamin C content of these samples (19.25-38.51 mg/100 ml) was higher than the one obtained with traditional baobab juices in Senegal (12-14 mg/100 ml) made by steeping pulp in fresh water for 5 to 480 min at the ratio of 1/3 (w/v) and to the values obtained with commercialised baobab juice in Malawi (5.09 ± 0.39 mg/100 mL) (Cissé et al., 2009; Tembo, 2016). Besides the differences in the production processes, the difference in values may be also be associated to the variation of baobab pulp composition from one region to another, as noticed by Tembo (2016) when comparing the TPC of pulp samples from Malawi (1866.81 ± 1.61 mg /100 g FW) to those from Burkina Faso (3518-4058 mg GAE/100 g by Lamien-Meda et al., 2008) and Madagascar (1085 mg GAE /100 g, by Cissé et al., 2013),

Conclusion

This study reveals that, both men and women produce and consume baobab juice in the North and Far North regions of Cameroon. The main ingredients used are baobab pulp, water and sugar, and many people add extra ingredients for commercial and/or sensory reasons. The pulp/water ratio and the quantity of sugar used for 1 L of juice varies depending on the aim of production (consumption or for sale). Four main traditional processes of baobab juice production (steeping the pulp in fresh water for 6 h, in warm water or in hot water for about 3 h; and boiling the pulp-water mixture for a short time, ± 5 min) in Cameroon were identified, the main difference being the temperature of water during the steeping step. In order of importance, the volume of water, quantity of pulp, steeping temperature and duration of steeping determine the quality of the baobab juice prepared. These production methods differently affect the final antioxidants and vitamin C contents of the juice. The highest TAC, TPC, DPPH and vitamin C was in the sample that was prepared using a short boiling step.
The process involving a short boiling step or steeping with hot water shall be recommended to optimize their extraction from the pulp.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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