Premier article : Physico-chemical, microbiological characteristics and storability of baobab-milk nectar, a drink developed from the bottom of the pyramid in urban Benin

Par : F. J. CHADARE, Y. E. MADODE, F. B. I. TANDJI, F. O. M. EGUE, M. KOFFI, M L. D'ALMEIDA, T. K. FASSINOU, M. AFFONFERE et D. J. HOUNHOUIGAN

Pages (pp.) 01-09.

Bulletin de la Recherche Agronomique du Bénin (BRAB) - *Numéro Spécial Technologie Alimentaire & Sécurité Alimentaire (TA&SA) – Décembre 2017*

Le BRAB est en ligne (on line) sur les sites web http://www.slire.net & http://www.inrab.org

ISSN sur papier (on hard copy) : 1025-2355 et ISSN en ligne (on line) : 1840-7099

Bibliothèque Nationale (BN) du Bénin

Institut National des Recherches Agricoles du Bénin (INRAB)

Centre de Recherches Agricoles à vocation nationale basé à Agonkanmey (CRA-Agonkanmey)

Programme Information Scientifique et Biométrie (PIS-B)

01 BP 884 Recette Principale, Cotonou 01 - République du Bénin

Tél.: (229) 21 30 02 64 / 21 13 38 70 / 21 03 40 59 ; E-mail : brabinrab@yahoo.fr / craagonkanmey@yahoo.fr

Bulletin de la Recherche Agronomique du Bénin (BRAB)
Numéro spécial Technologie Alimentaire & Sécurité Alimentaire (TA&SA) – Décembre 2017
BRAB en ligne (on line) sur le site web http://www.slire.net
ISSN sur papier (on hard copy) : 1025-2355 et ISSN en ligne (on line) : 1840-7099

Physico-chemical, microbiological characteristics and storability of baobab-milk nectar, a drink developed from the bottom of the pyramid in urban Benin

F. J. CHADARE[1,2], Y. E. MADODE[2], F. B. I. TANDJI[2], F. O. M. EGUE[2], M. KOFFI[2], M L. D'ALMEIDA[2], T. K. FASSINOU[2], M. AFFONFERE[2] et D. J. HOUNHOUIGAN[2]

Abstract

Baobab (*Adansonia digitata*) is a key economic tree known for the high nutritional value of its parts and used on daily basis for food and medicinal purposes. The study documented the processing, the physicochemical, microbiological and storability of baobab-milk nectar, a baobab pulp based drink. Onsite production was followed with successive weighing followed by sampling and storage experiment for laboratory analyses. Basic physico-chemical characteristics and microbial load of baobab milk nectar were assessed fresh and after its storage at 30 °C for 1 day; 11 °C for 21 days and 4 °C for 30 days. The production of baobab nectar yielded 6.4 kg of nectar from 1 kg of baobab fruit pulp. From 3.38 right after production, the pH of the product reached after storage 3.28 at 30 °C for 1 day, 3.35 at 11 °C for 21 days and 3.30 at 4 °C for 30 days. Titratable acidity (mg citric acid/g of beverage) of the product during storage varied from 4.91 for freshly produced baobab-milk nectar, to 5.31 when stored at 30°C for 1 day; 5.24 when stored at 11 °C for 21 days; 5.68 when stored at 4 °C for 30 days, respectively. Color did not significantly ($p > 0.05$) vary during storage whatever the storage conditions. Total aerobic counts, yeasts and moulds and lactic acid bacteria (Log cfu/ml of beverage) were 4.53, 2.43 and 1.90 in the freshly produced baobab-milk nectar. These counts did not significantly change after storage. Baobab-milk nectar is an interesting product from natural resources and its conservation in distribution conditions (4-11 °C) allows keeping its pH low and its acidity close to the ones of freshly produced product towards storage. The values hinder most microbiological growth for the health benefit of its consumers. Pasteurized form of the product is suggested as another segment with a longer shelf life.

Key words: Acidity, Baobab-milk nectar, Storage, Benin

Caractéristiques physico-chimique et microbiologique du nectar de baobab, une boisson développée dans les zones urbaines du Bénin

RESUME

Le baobab (*Adansonia digitata*) est un arbre économique clé connu pour sa haute valeur nutritionnelle de ces différentes parties et est utilisé quotidiennement pour les buts médicinal et alimentaire. L'étude documente la transformation, la physicochimie, la microbiologie et la conservation du nectar de la pulpe de fruit de baobab. La production a été faite avec de pesées successives suivies d'un échantillonnage et des expériences de conservation pour les analyses de laboratoire. Les

[1] Dr. Ir. Flora Josiane CHADARE, École des Sciences et Techniques de Conservation et de Transformation des Produits Agricoles (ESTCTPA), Université Nationale d'Agriculture (UNA), 05 BP 1752, Lénine, Cotonou, E-mail: fchadare@gmail.com, Tél.: (+229) 66324157, République du Bénin

[2] Dr. Ir. Yann Eméric MADODE, Laboratoire de Sciences des Aliments (LSA), Université d'Abomey-Calavi (UAC), 03 BP 2819 Jericho, Cotonou, E-mail : yann.madode@gmail.com, Tél.: (+229) 66695705, République du Bénin

MSc Fifamè Bérénice Inès TANDJI, LSA/UAC, 03 BP 2819 Jericho, Cotonou, E-mail : tandjib@yahoo.com, Tél.: (+229) 96388286, République du Bénin

BSc Fèmi Olivia Marilyne EGUE, LSA/UAC, 03 BP 2819 Jericho, Cotonou, E-mail : olivia.egue@yahoo.fr, Tél.: (+229) 67198932, République du Bénin

BSc Marlène KOFFI, LSA/UAC, 03 BP 2819 Jericho, Cotonou, E-mail : ingridyemi@gmail.com, Tél.: (+229) 62258058, République du Bénin

Marcelle Lyne D'ALMEIDA, LSA/UAC, 03 BP 2819 Jericho, Cotonou, E-mail : sewetess@yahoo.fr, Tél.: (+229) 21328480, République du Bénin

BSc Toyi Kévin FASSINOU, LSA/UAC, 03 BP 2819 Jericho, Cotonou, E-mail : faskeve@yahoo.fr, Tél.: (+229) 97787793 République du Bénin

BSc Marius AFFONFERE, LSA/UAC, 03 BP 2819 Jericho, Cotonou, E-mail: mariusaffonfere@gmail.com, Tél.: (+229) 66934608, République du Bénin

Prof. Dr. Ir. Djidjoho Joseph HOUNHOUIGAN, LSA/UAC, 03 BP 2819 Jericho, Cotonou, E-mail : joseph.hounhouigan@gmail.com, Tél.: (+229) 97141411, République du Bénin

caractéristiques physico-chimiques et la charge microbienne du nectar de baobab frais a été évaluée à la base et après sa conservation à 30°C pendant 1 jour; 11°C pendant 21 jours et 4°C pendant 30 jours. Le rendement de production du nectar de baobab était de 6,4 kg pour 1 kg de la pulpe du fruit de baobab. De 3,38 juste après la production, le pH du produit atteint après conservation 3,28 à 30 °C pendant 1 jour, 3,35 à 11 °C pendant 21 jours et 3,30 à 4 °C pendant 30 jours. L'acidité titrable (mg d'acide citrique /g de boisson) du produit lors de la conservation a varié de 4,91 pour le nectar de la pulpe de fruit de baobab frais à 5,3 quand c'est conservé à 30 °C pendant 1 jour; 5,24 quand c'est conservé à 11 °C pendant 21 jours; 5,68 quand c'est conservé à 4 °C pendant 30 jours respectivement. La couleur ne change pas significativement (p > 0,05) pendant la conservation quel que soit les conditions de conservation. Les germes aérobies totaux, les levures, les moisissures et les bactéries lactiques (Log cfu/ml de boisson) étaient 4,53, 2,43 et 1,90 dans le nectar de la pulpe de fruit de baobab frais. Ces charges ne changent pas significativement lors de la conservation. Le nectar de baobab est un produit intéressant provenant de ressources naturelles et sa conservation dans les conditions de distribution (4-11 °C) permet de garder son pH faible et son acidité reste la même aussi bien pour le produit frais que pour le produit après conservation. Ces conditions sont défavorables à la croissance microbienne ce qui est bénéfique sur la santé des consommateurs. La forme pasteurisée du produit est suggérée comme une autre alternative avec une longue durée de conservation.

Mots clés : Acidité, nectar de baobab, conservation, Benin

INTRODUCTION

Non-timber forest products (NTFP) are exploited throughout the world by populations for their daily needs and for their income (Dovie, 2003; Buchmann *et al.*, 2010). They represent an important part of forest production and contribute to nutrition of the population. They are often commercialized and represent a substantial part of local pharmacopoeia. In tropical countries, there is a high interest for edible forest fruit as part of the diet of rural populations (Ambé, 2001). The majority (80%) of populations from developing countries use, consume and take care of these resources (Wet *et al.*, 2010). Because of their importance, wild edible fruits tree species have been the focus of relatively comprehensive studies including distribution, ethnobotany, socioeconomics, conservation status, and genetics (Assogbadjo *et al.*, 2006; Fandohan *et al.*, 2011) the aim being to gather maximum information that can aid to inform policy makers of their potential contribution to alleviate poverty and to combat food insecurity. Unfortunately very few of those studies dealt with their derived products especially those addressing food and nutrition security. Among them, baobab (*Adansonia digitata* L.) is one of the most important one that provides a variety of food products from its leaves, fruit pulp, seeds and kernels, other parts of the tree, with the leaves and fruit pulp as the most utilized baobab share (Sidibé and Williams, 2002; Buchmann *et al.*, 2010). Its pulp have been acknowledge as novel food by the European Commission in 2008 and recognized as food ingredient in the US (The Commission of the European Communities, 2008). Baobab pulp is appreciated for its powdery nature and acid taste and its nutritional treasures since it is rich in Vitamin C, antioxidants, minerals in higher contents. Baobab fruit pulp contains up to 360 mg/100g dw Vitamin C, up to 3272 mg/100 g dw Potassium, up to 702 mg/100 g dw Calcium, up to 10.4 mg/100g dw iron and exhibit 30 times more antioxidant activity than kiwi (Chadare *et al.*, 2009). In west-African countries, derived-products of baobab pulp are gruel, nectar, sour dough, jam and jelly (Chadare *et al.*, 2008b; Kaboré *et al.*, 2011; Ndabikunze *et al.*, 2011).

As a result of its high natural vitamin C content, baobab fruit pulp has a well-documented antioxidant capability (Besco *et al.*, 2007). Antioxidants could help prevent oxidative stress related diseases such as cancer, aging, inflammation and cardiovascular diseases as they may eliminate free radicals which contribute to these chronic diseases (Blomhoff R *et al.*, 2010; Kaboré *et al.*, 2011). In Benin, baobab pulp is processed into sour dough, a variety of gruels (in combination with various cereals), ice nectar, natural refreshing drinks such as pasteurized and non-pasteurized nectar (Chadare *et al.*, 2008b; Buchmann *et al.*, 2010). Scientific work relative to baobab fruit documented their morphology, local perceptions, food uses, process technologies, nutritional value and some reviews on those various aspects (Osman, 2004; Assogbadjo *et al.*, 2008; Chadare *et al.*, 2008a; Chadare *et al.*, 2009). However, research studying details of processing or effects of processing treatments on the quality of its derived-products are scarce. Baobab nectar pasteurization tests in Senegal, showed that pasteurization reduces acidity, vitamin C content, reducing sugar content while pH and brix value stayed stable (Cisse *et al.*, 2009). Moreover, after ten minutes, the microbial load stretches to zero at a pasteurization temperature of 90 °C to 95 °C. However, the storage ability of the concerned nectars was not fully investigated. Recently, a new type of baobab-milk nectar emerged on urban markets in

Benin: it is unpasteurized baobab nectar obtained from baobab pulp and milk powder and served cold. Similar cold products from Benin mainly cereal based such as mawè, Gowe, akpan, were documented for their microbiological physico-chemical properties (Hounhouigan *et al.*, 1993; Michodjehoun-Mestres *et al.*, 2005; Sacca *et al.*, 2012) So far, baobab-milk nectar has not been characterized for its physico-chemical, microbial, organoleptic properties hence its quality. Moreover, the effects of processing and storage conditions on these properties remain unknown. For the safety of consumers of baobab-milk nectar, such kind of studies needs to be performed.

This study aimed to investigate the quality of baobab-milk nectar by assessing its physico-chemical and microbiological characteristics, and its storability in different conditions.

MATERIAL AND METHODS

Elaboration of the technological diagram of baobab-milk nectar

To elaborate technological diagram of the product under investigation, data was collected using production follow up and interactive discussion with the producers. Practical onsite production of baobab milk nectar was followed three times on different production days at production sites at Cotonou. During each production, mass balance was tracked using successive weighing. Temperatures were measured using a thermometer. The duration of each process unit was recorded.

Physico-chemical and microbiological analysis of baobab-milk nectar

Samples collection

After each production follow up, the end product was sampled. Freshly collected samples of baobab milk-nectar were stored on ice in an isolated bag during transport from processing unit to the laboratory. Samples were investigated as described in the experimental design.

Experimental design

One batch of baobab-milk nectar sample was analyzed for various parameters as soon as it reached the laboratory (T0) while the others were stored in different storage conditions as follows: 30°C for 24h (T1); 11°C for 21 days (T2); 4°C for 30 days (T3). Storage conditions were chosen in accordance with common distribution temperatures and based on the fact that low temperatures are supposed to favor a longer preservation than higher ones.

Physico-chemical analyses

Physico-chemical Analyses were performed on fresh and stored samples of baobab-milk nectar.

pH and titratable acidity: pH and titratable acidity were assessed as described by Nout et al. (Nout *et al.*, 1989). However, titratable acidity was expressed in citric acid per gram of product considering 1 ml of NaOH 0.1 N is equivalent to 6.4 mg of citric acid.

Color measurement: The color of baobab milk nectar was determined using the CR410 Konica Minolta colorimeter and L^*, a^*, b^* measuring system. The lightness, L^* represents the darkest black at $L^* = 0$, and the brightest white at $L^* = 100$. The color channels, a^* and b^*, will represent true neutral gray values at $a^* = 0$ and $b^* = 0$. The red/green opponent colors are represented along the a^* axis, with green at negative a^* values and red at positive a^* values. The yellow/blue opponent colors are represented along the b^* axis, with blue at negative b^* values and yellow at positive b^* values.

Brix value: Total soluble matter was expressed in terms of brix value which was measured using a refractometer (Erma).

All analyses were performed in duplicate.

Microbiological counts

Total aerobic counts, yeasts and moulds, lactic acid bacteria counts were measured. Ten grams of each sample were sampled aseptically and transferred into 90 ml sterile peptone physiological saline solution (1 g peptone, 8.5 g NaCl, and 1000 ml distilled water, pH = 7.0) and homogenized with a Stomacher Lab Blender (Type 400, Seward Medical, London, UK) for one minute to obtain dilution 10^{-1}. Dilution 10^{-2} was obtained by adding 1 ml of dilution 10^{-1} to 9 ml sterile physiological saline solution to get the required dilution level, and so on.

Total aerobic counts were enumerated on Plate Count Agar (OXOID CM 463, Basingstoke, England) after incubation at 30°C for three days (norm ISO 4833). Yeasts and moulds were counted after incubation at 25°C for five days on Sabouraud supplemented with chloramphenicol agar Biokar (Abtek Biologicals Ltd, Liverpool) (norm ISO 21527-2 : 2008). Lactic acid bacteria were enumerated on de Man Rogosa and Sharpe medium after incubation at 30°C for three days (norm ISO 15214: 1998).

After enumeration, micro-organisms number per gram of sample was calculated. For analysis, two replications were made and the average value was reported. The number of Colony Forming Unit (CFU) were expressed in log 10 CFU/g of sample.

Data processing

Descriptive statistics was used to calculate means and standard errors of each treatment. An analysis of variance (ANOVA) was done using statistica 7.1 software to assess the effect of storage conditions on nectar quality, and the microbial counts. Following the ANOVA, the Dunett test was considered to compare the three storage conditions to the nectar just after production and to check for significant differences.

RESULTS AND DISCUSSION

Elaboration of the processing diagram of baobab-milk nectar

Ingredients: Baobab-milk nectar is made from baobab fruit pulp, milk powder, water and sometimes sugar. It is flavored with *Cymbopogon citrat*us leaves and other banana aroma (Figure 1).

Process: From about 1 kg (0.91 kg ± 0.4) of baobab pulp and 0.4 kg ± 0.2 of milk powder, 6.10 kg ± 2.6 of baobab-milk nectar is produced. Basically, a mixture of water at ambient temperature (28.13 °C ± 1.8 when freshly collected from tap and 26.9 °C later) and hot water flavored with *Cymbopogon citratus* leaves is added. The temperature of the later is 73.75 °C ± 19.4 at the beginning of the process and it cools down to to 34.6 °C ± 4.2 at the end of the process. Mixing steps are interspersed with sieving steps. At starting, only the baobab pulp is mixed, homogenized with water and hot water flavored with leaves of *C. citratus* and the mix is sieved. Additional water and hot flavored water is added to further clean and wastes are discarded. Wastes are mainly made of baobab fruit fibers. To the sieved mash (32.37 °C ± 1.8), milk powder is added and mixed with the baobab mash with further addition of water and hot flavored water. After a sieving process, sugar and drops of banana aroma are added. The end product which is at a temperature of 28.9 °C ± 1.2 is packed in plastic bottle, adequately sealed and preserved in cold conditions for distribution.

Processing diagram of baobab-milk nectar is similar with the one described in previous studies since ingredients such as sugar, aroma, and cold water are use. Moreover, they involve numerous sieving steps to remove fibers. The main difference noticed were the use of milk powder and natural aroma as ingredient for the current process in the one hand, and in the other hand the pasteurization step described by Cissé *et al.* (Cisse *et al.*, 2009). The lack of thermal processing in the present diagram is critical for the sanitary quality of the end product especially during storage. The product investigated in the present study is stored in cold conditions suggestions a slowing down of microbial growth and degradation reactions.

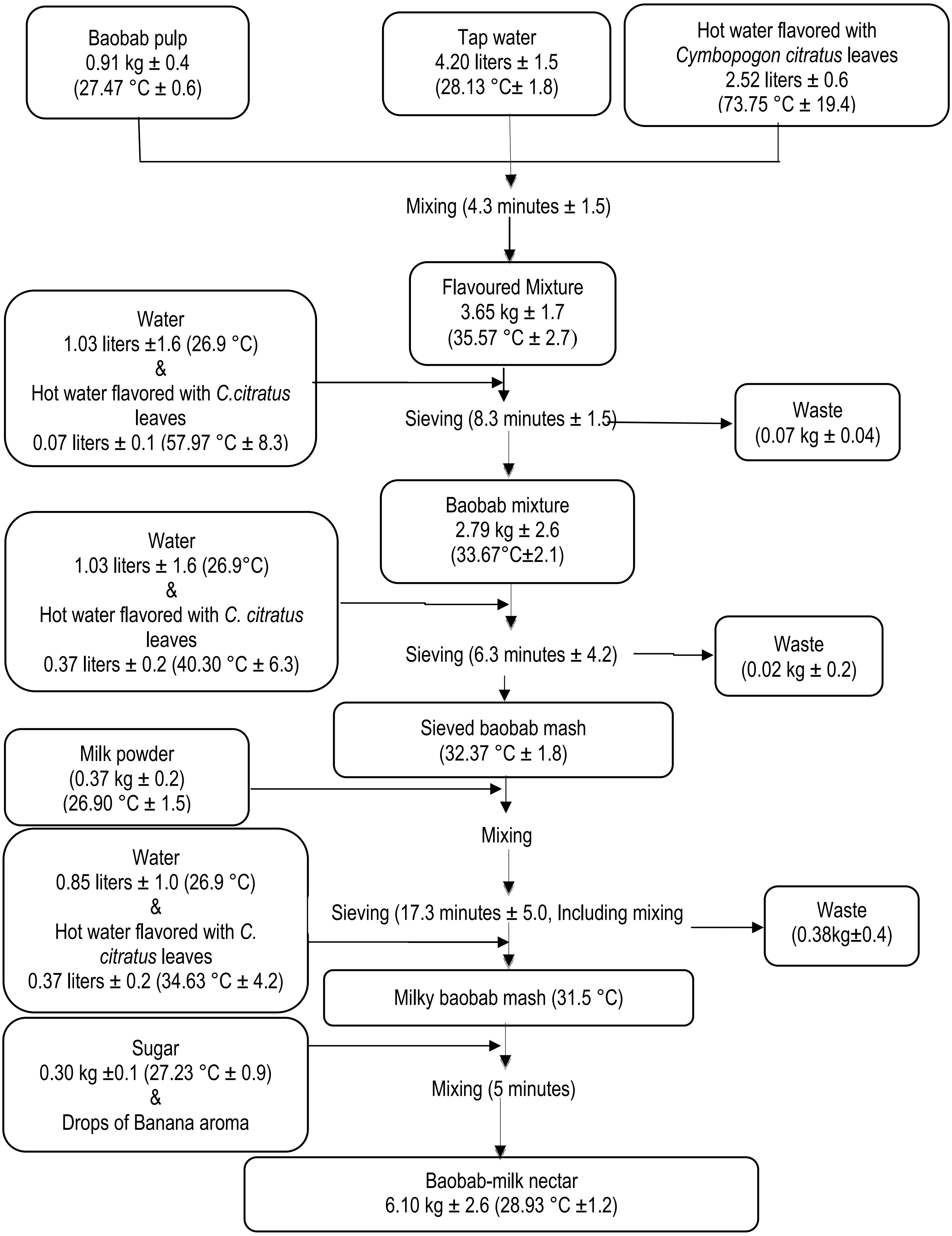

Figure 1. Processing diagram of Baobab-milk nectar

Effects of storage on physico-chemical characteristics of baobab-milk nectar

pH and Titratable acidity.

Freshly collected baobab nectar (T0), nectar stored at 30 °C for one day (T1), 11 °C for 21 days (T2) and 4 °C for 30 days (T3) showed a pH value of 3.37 ± 0.09, 3.28 ± 0.09, 3.37 ± 0,09 and 3,32 ± 0.04 respectively (Table 1). The pH value of the fresh product is very similar to the one reported by other authors who found that a traditional unpasteurized nectar prepared with 1 volume of baobab fruit content (mixture of pulp, fibers, seeds) for 3 volumes of water (fruit content/water ratio 1/3) showed a pH of 3.1 to 3.3 (Cisse *et al.*, 2009). Similar pH values of 3.3 to 3.4 were found for fresh and pasteurized orange juices (Cortés *et al.*, 2008) and in nectar of parkia pulp (pH=3) (Ouattara, 2011). The product under investigation in the present study contains some milk which apparently did not induce a significant pH variation. More diluted nectar prepared with fruit content/water ratio of 1/4 and 1/5 had the same pH as the nectar prepared with a ratio of 1/3. This implies that dilution did not affect the pH of baobab nectar conferring a buffering capacity to the product. In the present study, storage at 11°C for 21 days and 4°C for 30 days had no significant effect on pH. However, storage at 30°C for one day induced a significant decrease in pH. Rodrigo *et al.* (Rodrigo *et al.*, 2003) reported no significant ($p > 0.05$) variation in pH values of different pasteurized orange and orange-Carrot juice during their storage at 4 °C and 10 °C. In contrary, Del Caro *et al.* (Del Caro *et al.*, 2004) observed an increase in pH values during storage of citrus juices at 4°C. Titratable acidity increased significantly during storage at 30 °C, one day and 4 °C for 30 days from 4.91 mg/g to 5.31 mg/g and 5.71 mg/g respectively. These results suggested a beginning of fermentation probably induced by the long duration of storage at 4 °C or due to the high temperature for one day of storage time. The decrease of pH during storage at 30 °C, one day is confirmed by the increase of titratable acidity. High temperature or long duration storage was in favor of the beginning of a fermentation process that would induce alcohol and acid formation which will reduce pH and logically increase acidity. In other studies, degradation in terms of ethanol production of baobab nectar mildly pasteurized was observed in product stored at 4 °C for 11 days suggesting a more severe pasteurization for that specific drink (Cisse *et al.*, 2009). Whatever the treatment, pH is conformed to the one of yoghourt in accordance to specifications of Spain (pH < 4,6), Netherlands, Mexico and Japan (pH < 4. 5) (Loumani, 2012).

Brix value

Brix value was on average 19 for samples stored at 30 °C for one day (T1), 19.3 for the ones stored at 11 °C for 21 days (T2) and 18.8 for samples stored at 4 °C for 30 days (T3). No significant changes were observed between Brix values of samples in various conditions ($P > 0.05$). In fact, apparent but not significant decrease of brix value during storage at 4 °C for 30 days can be explained by the fact that fermentative lactic bacteria convert almost glucose in excess quantitatively in alcohol and lactic acid. It was the case for baobab nectar stored at 4 °C for 11 days which lost 35% of its total sugar content (Cisse *et al.*, 2009). Sugar metabolism leads notably to lactic acid production and to decrease of pH that was sought for in food products fabrication (Loumani, 2012).

Color change

Color of baobab-milk nectar did not significantly change during preservation, irrespective of the treatment. The lightness (L value) remains between 57 and 58, the redness (positive a* value) was between 8.9 and 9 and the yellowness (positive b* value) between 19.8 and 20.3 (Table 1). It was shown is other studies that browning is sometimes limited in juice without thermal processing; as such pasteurized orange juice presents greater yellow tendency (b^*) and less red tendency (a^*) than untreated orange juice during storage in refrigeration at 2 °C and 10 °C; however, there was a significant increase in this parameter from the fourth week of storage in all the juices stored at 10 °C, while in the ones stored at 2 °C the browning index values are maintained during more time (Cortés *et al.*, 2008). In the present study, the longest storage duration experimented was 4 weeks (at 4 °C) which is in accordance with what is observed at 2 °C as just described.

Bulletin de la Recherche Agronomique du Bénin (BRAB)
Numéro spécial Technologie Alimentaire & Sécurité Alimentaire (TA&SA) – Décembre 2017
BRAB en ligne (on line) sur le site web http://www.slire.net
ISSN sur papier (on hard copy) : 1025-2355 et ISSN en ligne (on line) : 1840-7099

Table 1. Physico-chemical characteristics of baobab-milk nectar according to treatments

Treatments	pH	Titratable Acidity	Brix Value	L*	a*	b*	ΔE
T0 (Freshly produced)	3.37±0.09	4.91±0.10	19.0±1.28	58.36±3.95	9.22±0.86	20.25±0.94	41.46±1.58
T1 (Stored at 30 °C, one day)	3.28±0.09**	5.31±0.13**	19.25±1.14	56.95±1.59	9.39±0.81	20.44±0.73	41.99±1.51
T2 (Stored at 11 °C, 21 days)	3.37±0.09	5.21±0.10	19.25±1.14	57.14±1.91	9.05±0.57	19.84±0.78	41.52±1.64
T3 (Stored at 4 °C, 30 days)	3.32±0.04	5.71±0.19**	18.75±0.45	57 .07±1.34	8.88±0.78	19.98±1.41	41.46±1.34

L = lightness; a = redness; b = yellowness

Values in the same columns with different signs (*) are significantly different (p<0.05)

Effects of storage on microbiological counts of baobab-milk nectar

The load of total aerobic count apparently increased during preservation from 4.53Logcfu/g in the control sample to 6.33Logcfu/g for samples stored at 30 °C for 24 hours; decreased for those stored at 11 °C for 21 days (T2) from 6.33Logcfu/g to 5.86Logcfu/g and increased from 5.86Log cfu/g to 6.95Logcfu/g for samples stored at 4 °C for 30 days (T3). Nevertheless, load of total aerobic counts was the lowest in samples stored at 11 °C for 21 days. Yeast and moulds load apparently increased during preservation from 2.43Logcfu/g in fresh nectar to 2.77, 3.13, and 2.50Logcfu/g in the sample stored at 30 °C for 1 day, 11 °C for 21 days and 4 °C for 30 days respectively. The load of lactic acid bacteria were 1.90Logcfu/g, 2.93Logcfu/g, 1.43Logcfu/g and 2.30Logcfu/g in freshly collected sample, sample stored at 30 °C for 1 day, 11 °C for 21 days and 4 °C for 30 days respectively (Table 2). Changes in microbial load were not significant whatever the storage duration (p > 0.05, Table 2).

Table 2. Microbiological counts of baobab-milk nectar according to treatments

Treatments	Microbial load (log10CFU/g)		
	Total aerobic counts	Yeasts and moulds	Lactic acid bacteria
T0 (Freshly produced)	4.53 ± 0.48	2.43 ± 0.22	1.90 ± 0.45
T1 (Stored at 30°C, 1 day)	6.33 ± 0.37	2.77 ± 0.41	2.93 ± 1.42
T2 (Stored at 11°C, 21 days)	5.86 ± 0.89	3.13 ± 0.52	1.43 ± 0.34
T3 (Stored at 4°C, 30 days)	6.95 ± 0.65	2.50 ± 1.00	2.30 ± 0.00
Analysis of variance	Prob = 0.138	Prob = 0.737	Prob = 0.609

The non thermal processing suggests a risk of microorganism growth during storage even if this can be slowed down by storage at in the cold such as refrigeration and freezing temperatures. In fact, it is expected that a thermal processing would reduce microbial load. Despite its absence, the microbial load remained rather low compared with the ones recommended by Codex Alimentarius for yoghourt (10^7 bacteria/g) which is served cold and most of the time unpasteurized as the baobab milk nectar. This may be explained by the rather acidic medium that baobab-milk nectar constitutes. Total aerobic counts load in initial product is similar with the one found in other studies in baobab nectar (Cisse *et al.*, 2009). Loads of total aerobic counts and lactic acid bacteria in baobab milk nectar are lower than the ones found by Cissé *et al.* (Cisse *et al.*, 2009) which are $5.6.10^5$ and $1.6.10^2$ in the nectar of baobab, respectively. This difference can be due to several factors such as fruit stage of maturity at harvest and pulp storage conditions (Chadare *et al.*, 2009). The presence of yeast and moulds in baobab-milk nectar can be explained by the fact that they may have grown in the raw materials (milk and baobab pulp) if exposed to humidity. Yeast and moulds can grow at temperature range from 0°C to 60°C with a water activity between 0.7 and 0.8 (Alborch *et al.*, 1995). On local market and in local processing unit, it is sometimes hard to fully preserve raw material against humidity as mentioned for pulp of *Parkia biglobosa* used to prepare some nectar (Ouattara, 2011). This suggests that the practices of good handling of raw material and good manufacturing practices would help to prevent presence of some microorganisms.

The acceptable microbial load in baobab-milk nectar which is in favor with a safe preservation of baobab may be explained by the very low pH of such a drink favored by an increase of titratable acidity. In fact at pH lower than 4.5, very limited pathogenic microorganisms can grow except the acidophilic ones (Davidson and Critzer, 2012). As such, distribution temperatures, if not too high may keep for a short period the product with low microbial load if there is no cross contamination during production, packaging and distribution. Products stored at 11°C for 21 days in the present experimentation showed good storage performance. However, this does not always guarantee a good organoleptic characteristic for the product.

Storage of traditional unpasteurized baobab nectar in Senegal showed that while stored at 4 °C browning and off flavors are detected within 4 days. This was not the case for baobab-milk nectar investigated in the present study which showed a stability of samples color whatever the treatment meaning that no browning occurred during preservation in the different conditions. Lactic acid bacteria were predominant in the product stored at 30 °C for one day whose pH was 3.28. This temperature is in accordance with temperature margin (25 °C-45 °C) which allows lactic acid bacteria growth (Barthe, 2009). It is suggested that the high vitamin C and antioxidant content of baobab pulp may have a role to play in the extension of shelf-life for food and beverages (Kaboré *et al.*, 2011). However, care should be taken of the metabolic and sensorial changes that may occur in the foods.

CONCLUSION

Storage in different conditions of baobab-milk nectar, new product derived from baobab pulp in Benin is investigated. Investigations reveal that physico-chemical characteristics of sample stored at 11 °C during 21 days are more or less close to the ones of fresh sample. Moreover, whatever the treatment, no significant difference is noticed in terms of microbiological aerobic counts and color. This investigation requires a nutritional characterization of baobab milk nectar for its better valorization and pasteurization tests to create another segment for the product which will have a longer shelf-life.

ACKNOWLEDGEMENTS

Authors wish to thank International Foundation for Science for its financial support for the research and the baobab-milk nectar producers for their collaboration.

REFERENCES

Alborch, L., M.R. Bragulat, M.L. Abarca, F.J. Cabañes, 1995: Temperature and incubation time and rice by Fusarium sporotrichioides isolates under diverse culture conditions.International biosynthesis of fumonisin B1 on maize grain as a function of different water activities. Letters in Applied Microbiology , 20, 247-250.

Ambé, G.A., 2001: Les fruits sauvages comestibles des savanes guinéennes de Côte-d'Ivoire : état de la connaissance par une population locale, les Malinké. . Biotechnologie. Agronomie. Société et Environnement 5, 43-58.

Assogbadjo, A.E., R. Glèlè Kakaï, F.J. Chadare, L. Thomson, T. Kyndt, B. Sinsin, P. Van Damme, 2008: Folk Classification, Perception, and Preferences of Baobab Products in West Africa: Consequences for Species Conservation and Improvement. Economic Botany 62, 74-84.

Assogbadjo, A.E., T. Kyndt, B. Sinsin, G. Gheysen, P.Van Damme, 2006: Patterns of Genetic and Morphometric Diversity in Baobab (*Adansonia digitata*) Populations Across Different Climatic Zones of Benin (West Africa). Annals of Botany 97, 819-830.

Barthe C., 2009: Lignes directrices et normes pour l'interprétation des résultats analytiques en microbiologie alimentaire, Centre québécois d'inspection des aliments et de santé animale, Gouvernement du Québec, Québec

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

Blomhoff R, M., C. B . Halvorsen , K. Holte, S. Bohn, S. Dragland, L. Sampson, C. Willey, H. Senoo, Y. Umezono, C. Sanada, I. Barikmo, N. Berhe, W. Willett, K. Phillips, D. Jacobs, 2010: The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. Nutrition Journal 9(3), 2-11.

Buchmann, C., S. Prehsler, A. Hartl, C.R. Vogl, 2010: The importance of baobab (AdansoniadigitataL.) in rural West African subsistence–suggestion of a cautionary approach to international market export of baobab fruits. Ecology of Food and Nutrition 49, 145-172.

Chadare, F.J., J.D Hounhouigan, A.R. Linnemann, M.J.R Nout, M.A.J.S. Van B, 2008a: Indigenous Knowledge and Processing of Adansonia Digitata L. Food Products in Benin. Ecology of Food and Nutrition 47, 338 - 362.

Chadare, F.J., J.D. Hounhouigan, A.R. Linnemann, M.J.R. Nout, M.A.J.S. van Boekel, 2008b. Indigenous knowledge and processing of *Adansonia digitata* L. food products in Benin Ecology of Food and Nutrition 47, 338-362.

Chadare, F.J., A.R. Linnemann, J.D. Hounhouigan, M.J.R. Nout, M.A.J.S. van Boekel, 2009: Baobab food products: a review on their composition and nutritional value. Critical Reviews in Food Science and Nutrition 49, 254-274.

Cisse, M., M. Sakho, M. Dornier, C.M. Diop, M. Reynes, O. Sock, 2009: Caractérisation du fruit du baobab et étude de sa transformation en nectar. Fruits 64, 19-34.

Cortés, C., M.J. Esteve, A. Frígola, 2008: Color of orange juice treated by High Intensity Pulsed Electric Fields during refrigerated storage and comparison with pasteurized juice. Food Control 19, 151-158.

Davidson, P.M., Critzer, F.M., 2012: Interventions to Inhibit or Inactivate Bacterial Pathogens in Foods. Springer New York, New York, NY., 189-202

Del Caro, A., A. Piga, V. Vacca, M. Agabbio, 2004: Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage. Food Chem. 84.

Dovie, D.B.K., 2003: Rural economy and livelihoods from the non-timber forest products trade. Compromising sustainability in southern Africa? International Journal of Sustainable Development and World Ecology 10, 247-262.

Fandohan, B., A.E. Assogbadjo, R. Glèlè Kakaï, T. Kyndt, B. Sinsin, 2011: Quantitative morphological descriptors confirm traditionnaly classified morphotypes of Tamarindus indica L. fruits. Genetic Resources and Crop Evolution 58, 299-309.

Hounhouigan, D.J., M.J.R. Nout, C.M. Nago, J.H. Houben, F.M. Rombouts, 1993: Composition and microbiological and physical attributes of mawè, a fermented maize dough from Benin. International Journal of Food Scienceand Technology 28, 513-517.

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

Loumani, A., 2012 : Étude microbiologique et hygiénique du yaourt fabriqué et commercialisé dans l'ouest Algérien. Mémoire pour l'obtention de diplôme de Magister. Université d'Oran. 97 pages + annexes.

Michodjehoun-Mestres, L., D.J. Hounhouigan, J. Dossou, C. Mestres, 2005: Physical, chemical and microbiological changes during natural fermentation of "gowe", a sprouted or non sprouted sorghum beverage from West-Africa. African journal of biotechnology 4, 487-496.

Ndabikunze, B., B. Masambu, B.Tiisekwa, A. Issa-Zacharia, 2011: The production of jam from indigenous fruits using baobab (Adansonia digitata L.) Powder as a substitute for commercial pectin. African Journal of Food Science 5, 168-177

Nout, M.J.R., F.M. Rombouts, A. Havelaar, 1989: Effect of accelerated natural lactic fermentation of infant good ingredients on some pathogenic microorganisms. International Journal of Food Microbiology 8, 351-361.

Osman, M.A., 2004: Chemical and nutrient analysis of baobab (*Adansonia digitata*) fruit and seed protein solubility. Plant Foods for Human Nutrition 59, 29-33.

Ouattara, K., 2011: Essai de production de nectar de pulpe de nere (Parkia biglobossa) et sa caractérisation. Agroalimentaire. Université Polytechnique de Bobo-Dioulasso, Burkina Faso, p. 65.

Rodrigo, D., J.I. Arranz, S. Koch, A. Frıgola, M.C. Rodrigo,E.M., 2003. Physicochemical characteristics and quality of refrigerated spanish orange–carrot juices and influence of storage conditions. J. Food Sci. 68, 2111-2116.

Sacca, C., L. Adinsi, V. Anihouvi, N.Akissoe, G. Dalode, C. Mestres, A.Jacobs, N. Dlamini, D., P., J.D. Hounhouigan, 2012.: Production, consumption, and quality attributes of Akpan – a yoghurt-like cereal product from West Africa Food Chain 2, 207-220.

Sidibé, M., Williams, J.T., 2002: Baobab- Adansonia digitata L., Fruits for the future University of Southampton (United Kingdom), 100 p.

The Commission of the European Communities, 2008: Commission decision of 27 June 2008 authorising the placing on the market of Baobab dried fruit pulp as a novel food ingredient under Regulation (EC) No 258/97 of the European Parliament and of the Council. Official Journal of the European Union 38- 39.

Wet, H., M.N. Nkwanyana, V.S.F. Van, 2010: Medicinal plants used for the treatment of diarrhoea in northern Maputaland, KwaZulu- Natal Province, South Africa. J. Ethnopharmacol. 130, 284-289.