

This is a "Post-Print" accepted manuscript, which has been published in
"Ecology of Food and Nutrition".

Please cite this publication as follows:

Chadare, F.J., Gayet, D.P., Azokpota, P., Nout, M.J.R., Linnemann, A.R.,
Hounhouigan, A.R., Van Boekel, M.A.J.S. (2010) Three traditional fermented
baobab foods from Benin, *Mutchayan*, *Dikouanyouri*, and *Tayohounta*:
preparation, properties and consumption. *Ecology of Food and Nutrition* 49
(4), 279-297.

You can download the published version at:

<http://dx.doi.org/10.1080/03670244.2010.491051>

Title: Three traditional fermented baobab foods from Benin, *Mutchayan*, *Dikouanyouri* and *Tayohounta*: Preparation, properties and consumption

Running head: Three undocumented fermented baobab foods from Benin

Running title: Traditional fermented baobab foods from Benin

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ABSTRACT

Forest food resources contribute significantly to food supply in areas where they grow. Three fermented baobab foods were studied: *Dikouanyouri* (from seeds, pH = 6.5); *Tayohounta* (from seed kernels, pH = 7), and *Mutchayan* (from baobab pulp and sorghum, pH = 4.2). *Bacillus* spp. (8.5 and 9.5 Log cfu /g) and lactic acid bacteria (8.9 and 8.4 Log cfu /g,) dominate in *Dikouanyouri* and *Tayohounta*, respectively. In *Mutchayan*, lactic acid bacteria (8.1 Log cfu/g) and yeasts (7.2 Log cfu/ g) predominated. The arbitrary index of protein cleavage increases from 2.3% (unfermented products) to 13.7% in *Dikouanyouri* and 21.3% in *Tayohounta*, indicating significant protein degradation. *Mutchayan* is the most frequently consumed product.

Key words: Baobab foods, fermentation, *Bacillus*, lactic acid bacteria, yeasts, chemical composition, consumption.

INTRODUCTION

Forest food resources in general and local forest fruits in particular are important sources of food and income for rural populations. Indigenous fruits are essential for food security, health, social and economic welfare of rural communities (Akinnifesi *et al.*, 2004). Among them, baobab is one of the most important for local populations in Africa. It is widely distributed in many African countries and is used daily for food, as medicine and for other purposes (Sidibe and Williams, 2002; Wickens, 1982). Baobab fruits contain reniform seeds and a powdery pulp (Baum, 1995). The pulp is rich in vitamin C, minerals and other nutrients (Chadare *et al.*, 2009; Nour *et al.*, 1980; Osman, 2004) and also exhibits some

antioxidant activity (Besco *et al.*, 2007; Vertuani *et al.*, 2002). The seeds are rich in energy, essential fatty acids, and some minerals such as Ca and K (Glew *et al.*, 1997; Lockett *et al.*, 2000; Sidibe and Williams, 2002). Several indigenous foods are prepared from baobab fruits, either with the pulp or the seeds or the seed kernels (Chadare *et al.*, 2008). Studies related to baobab species have provided data on the ecology, genetics, ethnobotanical aspects, and the chemical composition of its parts (Assogbadjo *et al.*, 2006; Chadare *et al.*, 2009; Diop *et al.*, 2005) but no study specifically addressed the characterization of traditional baobab foods.

In Benin, local populations have broad knowledge on these food products, especially the *Ditamari* (also known as *Otamari*) ethnic group (Assogbadjo *et al.*, 2008). Thirty-five baobab foods were described based on indigenous knowledge of populations from Benin; many of these are prepared from baobab fruits, either with the pulp or the seeds or the seed kernels. Among them, some fermented baobab foods have been reported (Chadare *et al.*, 2008) but these have not yet been characterized. They are *Mutchayan* (fermented cereal paste with baobab pulp), *Dikouanyouri* (fermented baobab seeds) and *Tayohounta* (fermented baobab seed kernels). *Mutchayan* is made from cereals. In that respect it is similar to other fermented cereal doughs which have been researched for their production process, and their microbiological and physico-chemical characteristics, such as *Gowe*, a cooked product made from sorghum (Michodjehoun-Mestres *et al.*, 2005), *Mawè* (Hounhouigan *et al.*, 1993a) and *Ogi* (Agati *et al.*, 1998) made from maize.

There are many African fermented condiments which have been thoroughly investigated for their production techniques, and their microbiological and physico-chemical properties; usually they are made from plant seeds, just like *Tayohounta* and *Dikouanyouri*. Examples are *Afitin*, *Iru* and *Sonru* made by natural fermentation of decorticated African locust beans

(*Parkia biglobosa*) (Azokpota *et al.*, 2006); *Otiru* from African yam beans (*Sphenostylis sternocarpa*) (Jeff-Agboola, 2007); and *Dawadawa* from African locust beans or soya beans (Dakwa *et al.*, 2005; Odunfa, 1985). Fermented foods are of interest because of their desirable attributes such as attractive flavour, increased shelf life, ease of digestibility and health benefits. Detailed studies of these traditional fermented products revealed the predominance of, for example, specific microorganisms (generally *Bacillus* spp. in the seed-based products and lactic acid bacteria in the cereal-based products) and volatile components (Azokpota *et al.*, 2008). Such characterizations provide basic information that is needed before follow-up research can be done into nutritional optimisation, stabilization and valorisation .

The present study is the first that investigates *Mutchayan*, *Dikouanyouri* and *Tayohounta*, three traditional fermented baobab foods from North Benin. We (i) document the traditional processing techniques of *Mutchayan*, *Dikouanyouri* and *Tayohounta*; (ii) determine the microbiological and physico-chemical characteristics of these foods; (iii) assess the effect of fermentation on their physico-chemical composition; (iv) assess their consumption patterns in rural communities in Benin, and (v) formulate options for improvement of traditional processing techniques to the benefits of the users of these baobab foods.

MATERIALS AND METHODS

Survey

In July-August 2008 a survey was conducted among 150 consumers and processors of baobab fermented foods in Natitingou region (Tagaye, Kousoucoingou), Boukoumbe, and Korontière,

three localities in northern Benin. These localities were selected as the majority of the population belongs to the *Otamari* ethnic group (or *Ditamari*), which has demonstrated broad knowledge on baobab foods in general and fermented baobab foods in particular (Assogbadjo *et al.*, 2008). The study was based on discussions and interviews, after having obtained consent from the village chiefs and the persons involved. Volunteer informants, men and women of 25 to 60 years old, were randomly selected (50 per locality). All informants in Natitingou region and Boukoumbe belong to the *Otamari* ethnic group while 80% of the informants in Korontière belong to the *Namba* ethnic group; the others were *Otamari*. Individual interviews were conducted in the local language of the respondents with translation when necessary. Questions were related to traditional processing of baobab fermented foods and their consumption during periods of abundance and shortage, which are distinguished by the ample availability or shortage of raw material needed to prepare the baobab products. All the raw materials (pulp, seeds, kernels) from the baobab fruit can be freshly harvested and are thus readily available from January until May. Seeds and kernels can easily be obtained for a slightly longer period, i.e. until June. The period in which pulp is abundant corresponds to the period in which there is an ample supply of *Mutchayan*. When the seeds and seed kernels are abundant, *Tayohounta* and *Dikouanyouri* are easy to get. Preferences of the informants for the different foods were assessed through consumption frequencies of the fermented baobab foods.

Process and sampling

Baobab fruits were processed according to Figure 1. The fruit is broken and its content is ground using mortar and pestle. The ground mixture is then sieved to separate pulp, seeds and fibres. *Dikouanyouri*, *Mutchayan* and *Tayohounta* were prepared as shown in Figure 2. Three local processors of each of the three products were selected in the three above

mentioned localities. Village-style preparation of the 3 products was carried out in each of the 3 localities, with replications on the next day. Production was carried out during the rainy season. Since these samples were produced under local village conditions, the hygienic circumstances were uncontrolled. Unfermented and fermented samples were collected in sterile stomacher bags, packed in a thermocooler containing ice blocs, stored when necessary in a refrigerator at 4°C and transported to the laboratory for analyses.

Microbiological analyses

Ten grams of each sample were taken aseptically and transferred into 90 ml sterile peptone physiological saline solution (1g peptone, 8.5g NaCl and 1000 mL distilled water, pH = 7.0) and homogenized with a Stomacher Lab Blender (Type 400, Seward Medical, London, UK) for 1 min to obtain dilution 10^{-1} . Dilution 10^{-2} was obtained by adding 1mL of dilution 10^{-1} to 9 mL sterile physiological saline solution to get the required dilution level, and so on. For the drop plate method (Herigstad *et al.*, 2001), the volume of the inoculum was 30 μ l on 1/2 petri dish for the Nutrient Agar or 1/4 petri dish for Malt Yeast Glucose Peptone and Man Rogosa Sharpe media, respectively.

Total aerobic mesophilic bacteria were enumerated on Nutrient Agar (Remel 454182, Bie and Berntsen, Rodovre, Denmark) (37°C, 48 h), and reported as Total Mesophilic Count. Bacterial spores (mainly *Bacillus* spp.) were enumerated on Nutrient Agar (Remel 454182, Bie and Berntsen, Rodovre, Denmark) (37°C, 48 h), from a 10^{-1} dilution that had been heated for 10 min at 80 °C to kill vegetative cells, and were reported as Total Spores. Lactic acid bacteria were enumerated on de Man Rogosa and Sharpe Agar (MRSA, CM 361, Oxoid, Hampshire, England) containing 0.1% (w/v) natamycin (Delvocid, Gist-Brocades, Delft, The

Netherlands) after incubation at 30°C for 3-4 days. Colonies were confirmed by oxidase and catalase tests, and confirmed counts were reported as lactic acid bacteria (Nout, 1991). Yeasts were counted after incubation at 25°C for 3-5 days on a mixture -MYGP- of Malt Extract (3g), yeast extract (3g), Glucose (10g), Peptone (5g) and Agar (20g) supplemented with chloramphenicol and chlortetracycline (Jespersen *et al.*, 1994) and reported as yeasts. Per 30 µl seeding, numbers of colonies ranging between 9 - 90 were considered for calculation (Herigstad *et al.*, 2001). The number of microorganisms was calculated as follows:

$$X = \frac{A.V}{I} \quad \text{with:}$$

X=number of microorganisms per g

A= number of counted colonies on ½ or ¼ petri dish

V = 1/dilution factor

I = Inoculum volume (mL)

Physico-chemical analyses

The pH and titratable acidity were measured immediately on the wet samples according to Nout *et al.* (1989). The dry matter, crude fat, and ash contents of the samples were determined, using AOAC methods 27.005, 27.006, and 27.009, respectively (A.O.A.C., 1984). Protein contents were measured using Dumas method (Jung, 2003). Free amino nitrogen was measured using formol titration (Han *et al.*, 1999). Protein was converted and expressed as total nitrogen using the factor of 6.25.

Statistical analyses

For microbiological and physical-chemical data, analysis of variance (ANOVA) was performed and least significant difference (Student–Newman–Keuls test) was determined, using SAS v8 (SAS Institute Inc.). For consumption data, analysis of variance was performed to assess the effect of the principal factors (product, area, consumption period, consumption frequency, gender) and the effect of their interactions. Prior to ANOVA, consumption data were transformed using a logarithmic function $\ln(x+1)$ in order to have normality of the data and equality of population variance. Significance was accepted at $P < 0.05$.

RESULTS

Traditional manufacturing processes of *Dikouanyouri*, *Tayohounta* and *Mutchayan*

The flow diagram for *Dikouanyouri*, *Mutchayan* and *Tayohounta* processing is shown in Figure 2. *Dikouanyouri* is usually made from baobab seeds. Ample water is added to the seeds during cooking until they get sufficiently soft (on average this takes 5-8 hours). Approximately 12 volumes of water are needed for 1 volume of seeds, after which the remaining water is discarded. The cooked seeds are put in a pot and fermented for about 3 days at ambient temperature, i.e. ranging from 24 to 31°C (Assogbadjo, 2006; Natta, 2003). The fermented seeds are then pounded to pulp using mortar and pestle, and some potash (0.25 L / kg cooked seeds) is added to the resulting mash. The mixture is further fermented overnight at ambient temperature (24 - 31°C). The obtained product, *Dikouanyouri*, is usually sun dried for 5-7 days to extend its shelf life. The dried product intended for domestic consumption is usually stored in a closed vessel or wrapped in leaves and kept near

the traditional clay stoves until it is finished after 2-3 weeks. It is used as flavouring agent in sauces.

Tayohounta is made from kernels obtained by decortication of baobab seeds. These are roasted (5-10 min), cooked (about 20-30 min), drained off, put in a pot and covered with leaves. Usually leaves of *Annona senegalensis* are used and these are in contact with the product. The type of leaves used may affect product quality but this aspect was not studied by us. The kernels are left for about 2 days to ferment at ambient temperature (24 - 31°C) (Assogbadjo, 2006; Natta, 2003), followed by hand kneading for a few minutes to shape the product into balls with a diameter of 5-10 cm. These balls are usually sun dried to increase their shelf life; the dried balls that are intended for domestic consumption are mostly stored wrapped in leaves and kept near the traditional clay stoves until they are finished within 2-3 weeks. They are used as flavouring agent in sauce.

For the preparation of *Mutchayan*, diluted baobab pulp/baobab pulp drink is required. This can be obtained by soaking the content of a baobab fruit in water or by diluting baobab pulp in water (1 volume of pulp for 1.5 volume of water), see Figure 1. The second important ingredient for *Mutchayan* is cereal (maize, millet or sorghum) flour. In order to obtain the flour, cereal grains are sun dried and cleaned. Dry grains are then finely milled in a cereal mill to obtain the flour, which is sometimes further sieved. The cereal flour is used to prepare a cooked paste by boiling a mixture of flour and water to obtain a porridge, which is then thickened with additional flour. The paste is mixed with baobab pulp drink (Figure 1); 450 mL of baobab pulp drink (made from about 110g of baobab pulp) for 450 g of sorghum paste (Figure 2). The mixture of the paste and the pulp drink is put in a jar, covered and fermented for at least 1 day. The obtained fermented paste (*Mutchayan*) is either diluted in

water and used as a drink or consumed as a main dish with sauces. It can be preserved as such in its vessel for 1 week without spoilage.

Microbiological characteristics of *Mutchayan*, *Dikouanyouri* and *Tayohounta*

Table 1 shows that *Dikouanyouri* and *Tayohounta* contain very high levels of mesophilic aerobic bacteria (9.5 Log cfu/g). Sporeformers (8.5 and 9.1 Log cfu/g respectively) and lactic acid bacteria (8.9 and 8.4 Log cfu/ g respectively) were detected in large numbers. Especially for the sporeformers, this indicates that also high levels of vegetative cells of *Bacillus* spp. may be expected (i.e. the difference between the total count and the sporeformers and lactic acid bacteria). Possibly, other microorganisms will be present in these products; this will require further investigation. *Mutchayan* contained mainly lactic acid bacteria (7.6 Log cfu/ g) with a substantial amount of yeasts (7.2 Log cfu/ g) (Table 1). Counts in unfermented products were below the minimum level to be considered of relevance.

Physico-chemical characteristics of *Mutchayan*, *Dikouanyouri* and *Tayohounta*: impact of fermentation

Dikouanyouri

As shown in Table 2, the unfermented product contained 13 g / 100g dm crude lipids, 4.7 g / 100g dm ash, 342.5 mmol total nitrogen / 100g dm and 7.9 mmol / 100g dm free amino nitrogen. After fermentation, crude lipids and ash significantly increased to 17.2 g / 100g dm, and 10.9 g / 100g dm, respectively ($p < 0.05$). Protein degradation allowed an increase of free amino nitrogen to 39.7 mmol/100g dm. The ratio amino nitrogen / total nitrogen (i.e., an

arbitrary index of protein cleavage) rose from 2.3% in the non fermented product to 13.7% in the fermented *Dikouanyouri*.

Tayohounta

Non-fermented *Tayohounta* had a dry matter content of 49.7 g/100g, a crude lipid content of 36.9 g/100g dm a pH of 6.4; a total nitrogen content of 476.6 mmol N/100g dm and free amino nitrogen content of 11 mmol / 100 g dm. After fermentation, the pH increased to 7; crude lipids and the content of free amino nitrogen rose significantly ($p < 0.05$) to 42.5 g/100g dm and 105.7 mmol / 100g dm, respectively. The ratio amino nitrogen / total nitrogen increased from 2.3% in the non fermented to 21.3 % in the fermented food (table 2).

Mutchayan

The impact of fermentation in *Mutchayan* is mild. Both the unfermented and the fermented product had a pH of 4.2 and a dry matter content of about 22.5 g/ 100g. The lipid content increased from 0.7 in the unfermented to 1.6 g/100g dm in the fermented product and the titratable acidity decreased significantly from 23.2 to 17.5 mmol NaOH / 100 g dm ($p < 0.05$). No free amino nitrogen was present: not in the unfermented or in the fermented product (Table 2).

Use and consumption patterns of *Mutchayan*, *Dikouanyouri* and *Tayohounta*

The *Otamari* ethnic group is one of the largest consumers of baobab products in Benin. *Otamari* people have demonstrated profound knowledge of baobab foods, and the tree is highly important for their daily life. Baobab is used for foods, medicines and as a worship in this community (Assogbadjo *et al.*, 2008). *Mutchayan* is the most widely consumed baobab

pulp product in *Otamari* area (Table 3). It can be consumed as a thick and nutritious drink at all times of the day. Most farmers take some *Mutchayan* beverage in their bottle when they go to their fields and drink it when they are thirsty or feel tired. It is also the beverage that is offered to visitors during the period when pulp is available. In addition, it is used as a local cure against cough, and appreciated as appetizer, prior to a meal. *Dikouanyouri* is used as a flavouring agent in sauce consumed with cooked paste of cereals (maize, sorghum, millet) during lunch and dinner. *Tayohounta* is a flavouring agent too, used in most sauces and stews consumed during lunch and dinner. All members of the population that eat in a family context (i.e. everybody except infants) may consume *Tayohounta*.

Table 3 shows the consumption frequencies of *Mutchayan*, *Dikouanyouri* and *Tayohounta* in the municipalities of Natitingou, Boukoumbe and Korontière in northern Benin. The quantity consumed was not specified for each product. However, because *Tayohounta* and *Dikouanyouri* are condiments, they are likely to be consumed in much lower quantities than *Mutchayan*. After a logarithmic transformation of the data in Table 3, ANOVA showed that the factor “consumption frequency” ($p=0.01$) and the interaction “consumption frequency” * “Product” ($p<0.001$) are significant. The consumption frequency thus depends on the product. A high number of respondents mentioned a consumption frequency of 6-7 times per week for *Mutchayan* (namely 90% of informants in Natitingou region, 40% in Boukoumbe and 18% in Korontière during the pulp abundance period and 54% in Natitingou, 22% in Boukoumbe and 16% in Korontiere during the pulp shortage period), while only a few mentioned this frequency for *Dikouanyouri* (namely 26% of the informants in Natitingou region, 18% in Boukoumbe and 4% in Korontière during seed abundance period, and 3% of the informants in Natitingou region and none of them in the 2 other regions during seed

scarcity period). For consumption frequencies of 2-3 and 4-5 times per week, more respondents indicated *Mutchayan* followed by *Dikouanyouri* and *Tayohounta*. For consumption frequencies rarely and 1 time per week, *Dikouanyouri* is indicated by a high number of respondents followed by *Tayohounta*, while a low number of respondents indicated *Mutchayan*. All the 3 products are consumed more frequently when the required raw materials are readily available. However, when the raw materials are in short supply, the number of respondents who consume *Mutchayan* 6-7 times per week is almost 2 times higher, than those who consume *Tayohounta* and 15 times higher than those consume *Dikouanyouri*. In short, *Mutchayan* is the most popular and most frequently consumed fermented baobab food while *Dikouanyouri* is the least frequently consumed one.

DISCUSSION AND CONCLUSION

Dikouanyouri is made from whole baobab seeds, while *Tayohounta*, like several other African fermented condiments, is made with dehulled/decorticated seeds, thus resulting in a product with other sensorial and nutritional characteristics. Moreover, dehulling improves the nutritional value for most macronutrients and micronutrients except for the potassium content. A comparison between the nutritional value of seeds and seeds kernels is presented in a review by Chadare *et al.* (2009). Dehulling also makes it easier to extract oil from the seeds, for food and medicinal purposes. Finally, seed kernels get a better price on the market.

Mutchayan is primarily made from cereals and in that aspect it is similar to fermented cereal doughs such as *Gowe*, *Mawè* and *Ogi* made from sorghum or maize (Agati *et al.*, 1998; Hounhouigan *et al.*, 1993b; Michodjehoun-Mestres *et al.*, 2005). However, *Mutchayan* is the only fermented cereal food enriched with baobab pulp juice. This addition increases the

ascorbic acid content, which may play an important role as enhancer of mineral uptake (Hemalatha *et al.*, 2005). The consumption of approximately 300 g of *Mutchayan* (which is very common for adults) can provide 10.7 to 11.3 mg of vitamin C (all vitamin C is supposed to come from baobab pulp), which corresponds to 12-14% of the daily recommended intake (RDI) for pregnant women; 11.9-12.6% of RDI for an adult man and 14.3-15.1% of RDI for an adult woman. The consumption of 100 g *Mutchayan* by a child (4-8 years) can provide 14-15% of his daily recommended intake of vitamin C.

The dominant microflora in *Tayohounta* and *Dikouanyouri* are bacteria, especially sporeforming bacilli (*Bacillus* spp.) and lactic acid bacteria. The predominance of *Bacillus* spp. was to be expected as it the case for most fermented seed products. In such seed products the fermentation is proteolytic; the bacilli are strong producers of proteolytic enzymes. Their predominance in these fermentations may be due to their ability to survive cooking and to initiate fermentation of both nitrogenous and carbohydrate products (Omafuvbe *et al.*, 1999). Abundance of *Bacillus* spp. was reported in fermented plant seeds such as *Afitin*, *Sonru* (Azokpota *et al.*, 2006) and *Iru* (Azokpota *et al.*, 2006; Sanni *et al.*, 2000) made from African locust bean (*Parkia biglobosa*); *Ogiri* made from melon seeds (*Citrullus* spp.) or castor seeds (*Ricinus communis*); *Ugba* (Sanni *et al.*, 2000) made from African oil bean seeds (*Pentaclethra macrophylla Benth*); *Dawadawa* (Dakwa *et al.*, 2005) made from soya bean (*Glycine max*), and *Kpaye* (Omafuvbe *et al.*, 1999) made from African mesquite seeds (*Prosopis africana*) seeds. Further research into the identities of the microbiota of fermented baobab seed products is necessary, and ongoing for *Tayohounta*.

In *Dikouanyouri* and *Tayohounta* also significant numbers of lactic acid bacteria were found, namely 8.9 Log cfu/g and 8.4 Log cfu/g, respectively. The origin of the lactic acid bacteria in

these products is presumably the vessels, utensils and other sources of post-cooking contamination during the preparation process. Lactic acid bacteria were also observed in *Otiru* made from African yam bean (*Sphenostylis stenocarpa* Harms), which also contained *Lactobacillus jensenii*, but in lower numbers (3.8×10^3 cfu/g \approx 3.6 Log cfu/g after 72h of fermentation) (Jeff-Agboola, 2007). The microflora of *Mutchayan* was dominated by lactic acid bacteria but the product contained also substantial amount of yeasts. This was to be expected in such a product due to its acidic pH of 4.2, which is favourable for the development of such microorganisms. Lactic acid bacteria and yeasts were also reported as dominant microflora in *Gowe*, a fermented dough made from non malted and malted sorghum (Michodjehoun-Mestres *et al.*, 2005), and in *Mawè*, a fermented maize dough (Hounhouigan *et al.*, 1993b). Lactic acid bacteria also dominate in *Ogi* (Agati *et al.*, 1998).

For the present study fresh *Dikouanyouri* and *Tayohounta* were used, which have a relatively high moisture content making them highly susceptible to spoilage. In order to increase their shelf life, these products are usually sun dried by the local population. The drying may induce changes in the microbiological successions which needs to be considered in further studies.

The dry matter content of *Dikouanyouri* is quite stable and increases slightly from 36.6 g/100g dm to 37.5 g / 100g. This could be due to limited evaporation of water during the fermentation process (4 days in total), which occurred in covered vessels. A more important increase was reported by Azokpota *et al.* (2006) during the fermentation of African locust bean. In contrast, a significant decrease in dry matter content was observed in *Tayohounta*, namely from 49.7 to 46.1 g / 100 g ($p < 0.05$). This was also reported in the fermentation of *Prosopis africana* seeds for *Kpaye* production and the fermentation of African locust bean for *Daddawa* production (Omafuvbe *et al.*, 1999; Omafuvbe *et al.*, 2000) and can be related

to uptake of water during fermentation periods in conditions where covering with leaves favours exchange with the environment.

With respect to the pH of *Dikouanyouri* and *Tayohounta* (6.5 and 7.1, respectively), similar or slightly higher values are encountered in foods fermented by *Bacillus* spp.: 8.2 to 8.3 in *Dawadawa* from roasted and boiled soya beans (Dakwa *et al.*, 2005); 7.9 in *Iru* from African locust beans; 7.6 in *Ugba* from African oil bean seeds and 8.0 in *Ogiri* from castor seeds (Sanni *et al.*, 2000). Such pH values are typical for proteolytic fermentation, also referred to as “alkaline fermentation” (Steinkraus, 1995). Whereas the pH of *Dikouanyouri* remained stable after the fermentation, there was an increase in pH (from 6.4 to 7.1) and of titratable acidity (from 11.6 to 17.5 mmol NaOH/ 100g dm) in *Tayohounta*. The simultaneous rise in pH and acidity observed in *Tayohounta* has been reported for fermentation of legume seeds (Dakwa *et al.*, 2005). This may be due to the high buffering capacity of legume beans and microbial proteolytic activity leading to ammonia release, which is characteristic for most vegetable protein fermentations (Hesseltine, 1965). For such a proteolytic type of fermentation, protein degradation is to be expected. Proteolysis is the enzymatic degradation of proteins leading to formation of water-soluble peptides and amino acids, thereby improving the bioavailability of proteins for human metabolism (Odunfa, 1985). The increase of the ratio free amino nitrogen / total nitrogen from 2.3% in the unfermented products to 13.7% in fermented *Dikouanyouri* and 21.3% in fermented *Tayohounta* is an expression of protein cleavage. The phenomenon is less pronounced in *Dikouanyouri*, probably due to the presence of the seed coats in the product, which might hinder more active protein degradation. The other differences in chemical composition between *Dikouanyouri* and *Tayohounta* are also possibly due to the presence of the seed coat in *Dikouanyouri*, which is rich in fibrous compounds and lignin and probably other

compounds. Proteolysis leads to an increase of free amino acids such as lysine (Odunfa, 1985). In *Soumbala* (also known as *Afitin* and very similar to *Tayohounta*) made from African locust beans, the quantity of total free amino acids and essential free amino acids such as lysine increased sharply between 24 and 48h of fermentation; cysteine, methionine, leucine, isoleucine, tyrosine and phenylalanine appeared during fermentation. These changes were mainly induced by strains of *Bacillus subtilis* and *Bacillus pumilus* (Ouoba *et al.*, 2003). We expect that strains of microorganisms that improve the nutritional quality through fermentation by inducing an increase of free amino acids, including essential free amino acids, are also present in *Tayohounta* and *Dikouanyouri*

Ash content increases only slightly in *Tayohounta* after fermentation, whereas a 2.3 fold increase was found for *Dikouanyouri*. We ascribe this to the addition of potash to *Dikouanyouri* during the fermentation process.

Apart from the fat content that increases from 0.7 to 1.6 g/ 100g dm, the characteristics of *Mutchayan* before and after 1 day of fermentation are quite unchanged. The increase in the fat content may be due to possible metabolism of carbohydrates to fat as noticed in the fermentation of cassava and African locust beans (Oboh *et al.*, 2002; Oboh *et al.*, 2008). *Mutchayan* is characterised by an acidic pH (4.2) and relatively low dry matter content (22.5 g/ 100g). The acidity is mainly caused by the addition of baobab pulp, which has a low pH (about 2.9-3.3) and by the effect of lactic acid bacteria. The free amino nitrogen content *Mutchayan* was not detectable, both before and after fermentation. This may in part be explained by the much lower protein content (Table 2), as well as the inability of lactic acid bacteria to degrade protein.

The most frequently consumed product is *Mutchayan*, followed by *Tayohounta*; *Dikouanyouri* is the least frequently consumed. *Mutchayan* is used as a drink (during the day) as well as for lunch or dinner, while *Dikouanyouri* and *Tayohounta* are only used in smaller quantities for flavouring of stews and sauces. Moreover, the preparation of *Mutchayan* is easier than that of the other foods. In general, every *Otamari* is able to prepare *Mutchayan*, while the preparation of *Tayohounta* and *Dikouanyouri* is done by experienced people, mainly women. The use of *Mutchayan* as anti-fatigue is not surprising, considering the ascorbic acid content of baobab pulp, most of which is in the reduced form (Carr, 1955). Most traditional foods are still well appreciated by urban consumers, who, however, usually do not have the required skills to prepare them and therefore rely on the market. Their increasing interest in these products may boost the improvement of traditional techniques to facilitate production at a larger scale and make traditional foods accessible for a larger public who can pay a better price for them.

The preparation of *Dikouanyouri* requires much time due to a long cooking process, which is needed to soften the seed coats, but the preparation of *Tayohounta* may require as much time when the seed decortication process is included in the calculation. This high energy consumption for the preparation of *Dikouanyouri* contributes to the use of fire wood and thus to deforestation. Therefore it is desirable to find another way of softening the seed coats that will require less use of fire wood. At present the use of vinegar or fermentation of the seeds with some pulp are investigated as aids to soften the seed coats (Debróci *et al.*, 2008). This will help to find an easier way to soften the seed coats and thus promote the utilisation of baobab whole seeds e.g. production of *Tayohounta*.

It is suggested that future studies address the identification of the most important microbial strains in baobab fermented foods, which is necessary for specification of authenticity and future development of starter cultures for controlled fermentation. Controlled fermentation is necessary to produce more standardized, hygienic and stable products with an improved nutritional composition. Moreover, starter culture development will facilitate the production of *Mutchayan* during pulp shortage period. The present study provides new knowledge about traditional fermented baobab foods and sets research priorities for further studies that should lead to an improvement of the processing techniques for the nutritional and economic benefits of local populations. Similar work should be done on other baobab foods identified in Benin, also to stimulate research projects in other African countries where the tree is important for food purposes.

Acknowledgement

We thank NUFFIC and Codesria SGTR-04/T07 for their financial support. We are also thankful to local populations in Northern Benin for their cooperation.

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Table 1: Microorganisms (Log cfu/g) in *Mutchayan*, *Dikouanyouri* and *Tayohounta*

<i>Microorganisms group</i>	<i>Mutchayan</i>	<i>Dikouanyouri</i>	<i>Tayohounta</i>
Total mesophilic aerobic bacteria (TMC)	7.5 ± 0.8 b	9.5 ± 0.6 a	9.5 ± 0.6 a
Total sporeformers (TS)	ND	8.5 ± 1.0 a	9.1 ± 1.1 a
Lactic Acid Bacteria (LAB)	7.6 ± 0.4 c	8.9 ± 0.4 a	8.4 ± 0.8 b
Yeasts (Y)	7.2 ± 0.6 a	5.5 ± 0.5 b	5.3 ± 1.3 b

Values represent the mean scores (n=6 samples and 2 replications of each ± standard deviation)

ND: not detected

For each parameter (each row), means with the same letter are not significantly different (p<0.05).

Table 2: Effect of fermentation on the physico-chemical characteristics of *Mutchayan*, *Dikouanyouri* and *Tayohounta*

<i>Parameters</i>	<i>Dikouanyouri</i>		<i>Tayohounta</i>		<i>Mutchayan</i>	
	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented
Dry matter (g/100g)	36.6 ± 0.2 c	37.5 ± 0.1 c	49.7 ± 0.5 a	46.1 ± 1.9 b	22.2 ± 0.3 d	22.5 ± 1.5 d
Crude lipids (g/100g dm)	13.0 ± 0.5 d	17.2 ± 1.06 c	36.9 ± 0.9 a	42.5 ± 0.6 b	0.7 ± 0.06 e	1.6 ± 0.4 e
Ash (g/100 g dm)	4.7 ± 0.2 b	10.9 ± 3.2 a	8.6 ± 0.2 a	9.6 ± 0.4 a	2.2 ± 0.0 b	2.2 ± 0.4 b
pH	6.7 ± 0.0 a	6.5 ± 0.7 a	6.4 ± 0.0 a	7.0 ± 0.5 a	4.2 ± 0.0 b	4.2 ± 0.3 b
Titrateable acidity (mmol NaOH/100 g dm)	6.9 ± 0.2 d	14.3 ± 1.7 b	11.6 ± 01.7 c	17.5 ± 2.8 b	23.2 ± 0.4 a	17.5 ± 3.7 b
Total nitrogen (mmol N/ 100 g dm)	342.5 ± 17.2 b	290.5 ± 42.4 c	476.9 ± 11.6 a	496.5 ± 16.6 a	106.6 ± 1.1 d	120.2 ± 6.9 d
Free amino nitrogen (mmol AN/ 100 g dm)	7.9 ± 0.5 c	39.7 ± 5.0 b	11.0 ± 0.3 c	105.7 ± 10.8 a	0.0 ± 0.0 d	0.00±0.0 d
Index of protein cleavage = free amino nitrogen / total nitrogen (%)	2.3	13.7	2.3	21.3	0	0

Values represent the mean scores (n=6 samples and 2 replications of each ± standard deviation)

For each parameter (each row), means with the same letter are not significantly different (p<0.05).

Table 3: Consumption frequency (recorded in July-August 2008) of *Mutchayan*, *Dikouanyouri* and *Tayohounta* in periods of abundance (A) (January – May/June) and shortage (S) in 3 municipalities in Benin

<i>Consumption frequency</i>	<i>6-7 times/week</i>		<i>4-5 times/week</i>		<i>2-3 times/week</i>		<i>1 time/ week</i>		<i>Rarely</i>	
	<i>A</i>	<i>S</i>	<i>A</i>	<i>S</i>	<i>A</i>	<i>S</i>	<i>A</i>	<i>S</i>	<i>A</i>	<i>S</i>
Naitingou (N=50)										
<i>Mutchayan</i>										
Male	20	12	2	5	0	3	1	1	0	2
Female	25	15	5	6	0	7	0	0	0	1
Total	45	27	7	11	0	10	1	1	0	3
<i>Dikouanyouri</i>										
Male	5	3	3	0	9	0	1	5	2	12
Female	8	0	10	0	9	2	5	4	2	16
Total	13	3	13	0	18	2	6	9	4	28
<i>Tayohounta</i>										
Male	4	3	0	0	1	0	8	2	3	9
Female	4	0	3	0	9	0	3	4	8	17
Total	8	3	3	0	10	0	11	6	11	26
Boukoumbe (N=50)										
<i>Mutchayan</i>										
Male	10	3	7	3	6	5	1	8	0	3
Female	10	8	9	1	8	9	0	7	0	4
Total	20	11	16	4	14	14	1	15	0	7
<i>Dikouanyouri</i>										
Male	4	0	8	3	3	4	3	8	1	4
Female	5	0	3	2	6	4	6	5	7	15
Total	9	0	11	5	9	8	9	13	8	19
<i>Tayohounta</i>										
Male	4	2	3	2	6	2	2	2	1	8
Female	5	4	4	0	7	3	9	9	1	8
Total	9	6	7	2	13	5	11	11	2	16
Korontière (N=50)										
<i>Mutchayan</i>										
Male	4	3	2	1	12	5	0	3	7	13
Female	5	5	3	1	6	2	0	5	3	6
Total	9	8	5	2	18	7	0	8	9	19
<i>Dikouanyouri</i>										
Male	2	0	1	0	6	3	8	5	8	17
Female	0	0	1	0	5	2	8	5	6	13
Total	2	0	2	0	11	5	16	10	14	30
<i>Tayohounta</i>										
Male	17	9	5	1	2	8	1	5	2	2
Female	14	8	2	2	2	8	1	1	1	3
Total	31	17	7	3	4	16	2	6	3	5

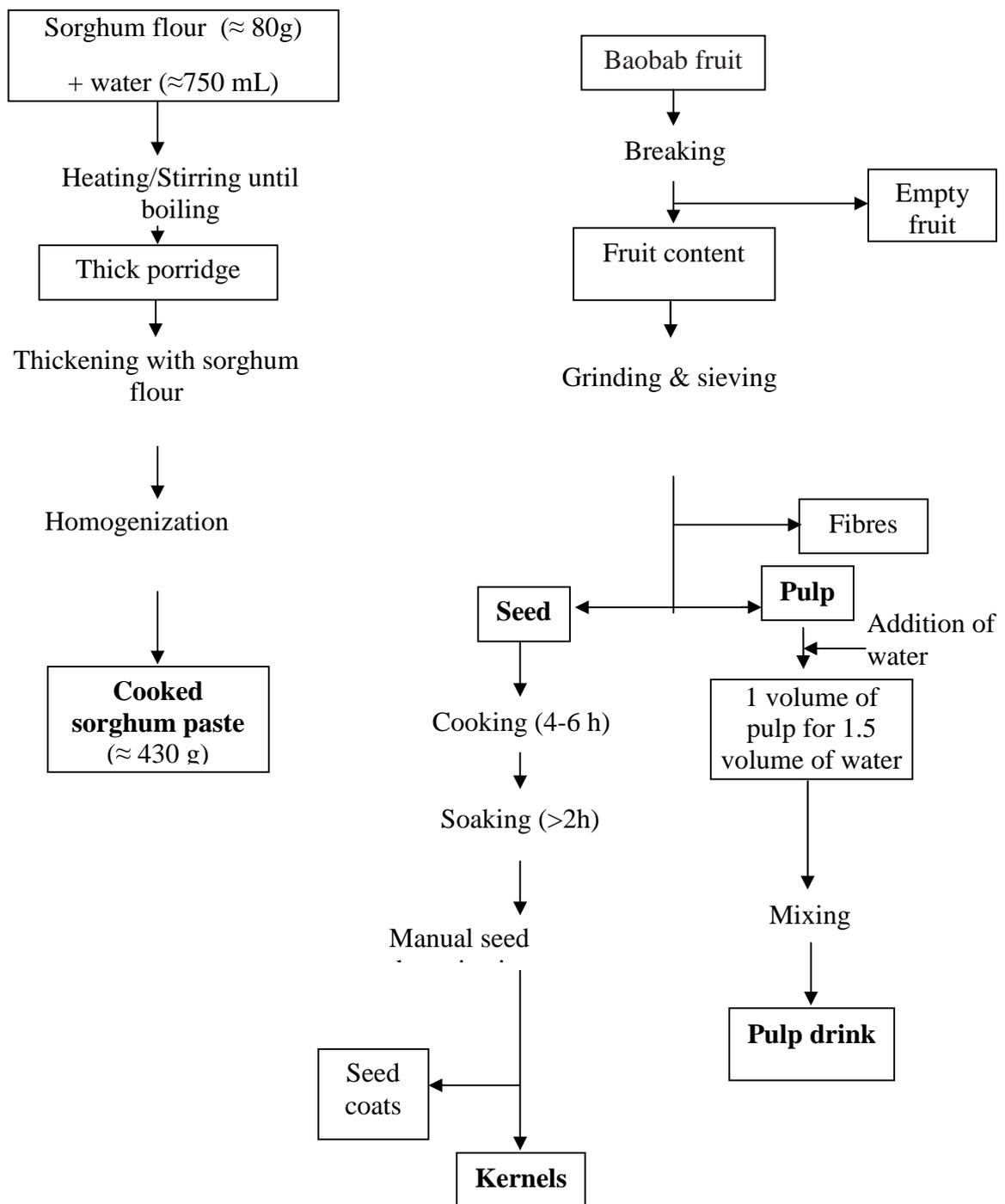


Figure 1: Sorghum paste preparation and seeds, kernels and pulp extraction from Baobab fruit and

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