Baobab (*Adansonia digitata* L.) **foods from Benin**: **composition, processing and quality**

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Abstract

In 2007, more than 923 million people did not have enough to eat (FAO, 2008), especially in developing countries. Simultaneously, several forest foods are available but under-utilized. Among them, the baobab tree (*Adansonia digitata* L.) is a key economic tree, a multipurpose, widely-used species, rich in nutrients in its leaves and fruits, used daily by local populations in several African countries for food, medicines and other purposes. The tree contributes significantly to nutrient supply in those areas where it grows. So far, few studies have been undertaken on the valorization of the species and on its contribution to food security in Africa. The present work focused on indigenous knowledge and nutritional aspects concerning baobab foods, necessary for further valorisation and improvement of local food processing practices.

The objectives of the study were to (i) review the nutritional value of baobab parts, (ii) document ethno-food knowledge related to baobab food products in rural areas in Benin, (iii) assess the effect of traditional processing on in vitro digestibility and bioavailability of minerals and carotenoids in sauce made of baobab leaves, (iv) characterise the microbiological flora of baobab fermented food products, and (v) build quantitative information on degradation of baobab pulp quality during storage.

The review on baobab foods showed that pulp from baobab fruits is particularly rich in vitamin C (150 to 360 mg/100 g dw). The leaves are particularly rich in calcium (307 to 2640 mg/100 g dw). The whole seeds and the kernels have a relatively high lipid content, viz. 12 to 33 g/100 g dw and 19 to 35 g/100 g dw, respectively. The pulp and leaves exhibit antioxidant properties with a higher activity in the pulp than in the leaves.

Two hundred and fifty-three processors of baobab food products were surveyed in Benin, to investigate the ethno-food knowledge related to baobab and the variation among socio-cultural groups. Local populations reported up to 35 baobab food products processed from the leaves, the pulp, the seeds and the kernels, most of which have never been characterized. Multivariate analysis showed that the types of foods processed from baobab differed among socio-cultural groups. The survey revealed that seed decortication is considered to be the most laborious processing operation and that the preservation of pulp and kernels is difficult in local conditions.

The effect of processing on the quality of traditionally processed baobab leaves was evaluated. Assessment of in vitro digestibility and bioavailability of Ca, Fe, and Zn in non processed and processed baobab leaves showed that 10-30% of total Ca was available and that lutein and beta-carotene are the most important carotenoids. Moreover, “bitter leaves” are richer than “sweet leaves”, though the latter are the preferred ones. However, the experiments on Fe and Zn in vitro digestibility require further attention because of experimental difficulties caused by the sliminess of the leaves.

The fermented baobab products recorded during the survey have been characterised and results show that two of them, namely *Tayohounta* (fermented kernels) and *Dikouanyouri* (fermented seeds), are flavouring agents and the third one, *Mutchayan* (sorghum paste with baobab pulp), is used as a drink and a main dish. Fermentation of

Dikouanyouri and *Tayohounta* appeared to be induced mainly by *Bacillus* spp. (8.5 and 9.5 Log cfu/g, respectively) and that of *Mutchayan* mainly by lactic acid bacteria (8.1 Log cfu/g) and yeasts (7.2 Log cfu/g). Detailed microbiological studies on *Tayohounta* showed that the microbiota responsible for the fermentation were sporeforming bacteria, mainly *Bacillus subtilis* and other *Bacillus* spp.

One preservation problem reported during the survey, namely quality degradation of baobab pulp during storage has been investigated. The results show that as storage time increases, pulp colour looses its lightness with decreasing L* values and becomes more brownish with increasing a* value (reddish) and b* value (yellowish); vitamin C degraded following roughly a first order kinetic reaction. Reaction rate is faster at higher temperatures and water activity. More data are necessary to build prediction models of quality degradation of baobab pulp during storage.

The work presented here shows the potential of baobab for food and nutrition in rural Benin, and may open up research on other forest foods.

Contents

Chapter 1

General introduction

1.1 Justification of the study

In 2007, more than 923 million people in the world did not have enough food (FAO, 2008), mostly people living in the developing countries. The sub-Saharan population of Africa, notably women and children, suffer from insufficient intake of protein and energy, and a lack of micronutrients, mainly iron, iodine, and vitamin A (FAO/WHO/UNU, 1985). In Benin, a health and demographic survey showed that in 2006, 78% of the 6-59 month-old children were anaemic. Since the year 2000, vitamin A deficiency has been recognized as a public health problem (INSAE-Bénin and Macro International Inc., 2007).

Benin is characterized by: 1) high rates of rural poverty; 2) low basic health services; 3) problematic and unstable food security, and 4) unsustainable use of natural resources (UNDP, 2008). Any attempt to find sustainable solutions to these problems should take into account locally known, used and available natural resources. In fact, it is recognized by the Government of Benin that for poverty reduction, small-scale farmers can profit from the sustainable utilisation and valorisation of agro-forestry systems (IMF, 2007). Several programmes have been implemented to alleviate the nutritional and poverty problems in the country, e.g. Projet d'Appui au Développement de la Sécurité Alimentaire (PADSA), Projet d'Activités Génératrices de Revenus (PAGER), Projet Développement Racines et Tubercules (PDRT), Projet Manioc, Projet d'Intervention Locale pour la Sécurité Alimentaire (PILSA), Projet de Gestion des Ressources Naturelles (PGRN). Forest food resources are scarcely dealt with in these programmes, although they are of great importance and already part of the food habits of local poor populations.

The Ministry of Nature Protection of the Government of Benin and Bioversity International (formerly known as the International Plant Genetic Resources Institute –IPGRI-) expect that wild edible tree products can play a major role in future crop development programmes and in the development of agro-forestry systems in Benin (IPGRI, 2002). Among the 3000 plants species found in the country, 172 were reportedly consumed by the local population (Codjia *et al.*, 2003). Although there is no specific legislation on the forest food resources in Benin, one of the objectives of the national forest policy is to integrate the management and the preservation of forest resources to the benefit of rural development.

1.2 Forest food resources

Local communities of West Africa, as well as Bioversity International, selected ten forest tree species with food value as priority species. These species will therefore be given special attention with respect to their conservation, domestication and valorisation. They have been selected for a number of reasons, namely their social and economic importance, their availability on the market, ease of access, the threats to which they are subjected according to their use and preservation, their extinction risks, and because no research or development programme had been devoted to these species (IPGRI, 2002). They are: the cashew (*Anarcardium occidentale*), the shea tree (*Vitellaria paradoxa*), the locust bean tree (*Parkia biglobosa*), the bush mango (*Irvengia gabonensis*), the black tamarind (*Dialium guineense*), the butter tree (*Pentadesma butyracea*), the breadfruit (*Artocarpus altilis*), the white star apple (*Chrysophyllum albidum*), the baobab (*Adansonia digitata*), and the tamarind (*Tamarindus indica*).

Among these, the baobab (*Adansonia digitata*), is a key economic tree of which several parts are used daily in the diet of rural communities in West Africa (Codjia *et al.*, 2001; Sidibe and Williams, 2002). The Forestry Department of the Food and Agriculture Organisation of the UN (FAO) has issued information on *Adansonia digitata* (e.g. FAO, 1988), and the International Centre for Research in Agroforestry (ICRAF) continues to promote its use as a multipurpose species. A number of bilateral agencies promoted the species in the past e.g. NORAD (Norway) and SIDA (Sweden) but also countries such as Kenya, and Tanzania. Regional consultations organised by the International Centre for Underutilised Crops (ICUC) have accorded high priority to, enhanced research and development of baobab. National research efforts, especially in Benin, Nigeria, Burkina Faso, Mali and Senegal have provided relatively recent data on the agronomy, ethnobotany, ecology and genetic diversity of the baobab (Assogbadjo *et al.*, 2005a, 2005b, 2008; Diop *et al.*, 2005). However, most of these studies did not address the potential of the species with respect to solving food-related problems. This study on the use of the baobab for food products was undertaken to fill that gap.

1.3 Baobab: *Adansonia digitata*

The African baobab tree (Figure 1.1) and its related species belong to the family of Bombacaceae and the genus *Adansonia*. The tribe, which is pantropical, includes *Bombax* and *Ceiba* with species producing fruit fibres used as kapok. The family includes about 30 genera, six tribes and about 250 species (Baum, 1995a). A number of these species are used locally for leaves, wood, fruits, seeds or gum. The African baobab (*Adansonia digitata*) occurs naturally in most countries of the Sahara as a scattered tree in the savannah, and is also associated with habitation (i.e. baobab trees are present where man lived or is living). In the past some ethnic groups in Mali such as the Dogon, Kagolo and Bambara used to take seedlings from the wild to plant them around their villages (Sidibe *et al.*, 1996). The tree has been introduced in many countries for its oddity and its use as an ornamental plant. It is also known as the dead-rat tree (from the appearance of the fruits), monkey-bread tree (the dry fruit is a source of food for monkeys), upside-down tree (as the bare branches resemble roots) and cream of tartar tree (due to the acidic taste of the fruits) (Sidibe and Williams, 2002).

Figure 1.1 African baobab tree (*Adansonia digitata* L.)

The available literature on the African baobab is well presented by Baum (1995a) and Wickens (2008). The binomial *Adansonia digitata* was given by Linnaeus, the generic name honouring Michel Adanson who visited Senegal in the eighteenth century and described *Baobab* (Adanson, 1771). African baobab is a very long-lived tree with multipurpose uses. It is thought that some trees are over 1000 years old. The African baobab tree is characterized by its massive size, reaching to a height of 18-25 m. It has a rounded crown and shows a stiff branching habit. The trunk is swollen and stout, up to 10 m in diameter, usually tapering or cylindrical and abruptly bottle-shaped. Giant individuals can reach a girth of up to 28 m. Branches are large and distributed irregularly. The bark is smooth, reddish brown to grey, soft and fibrous (Baum, 1995a). Biogeography and floral evolution of *Adansonia* species, and their systematic relationship with other taxa of Bombacaceae were studied and described in the literature. Flowers of the African baobab are large, pendulous, solitary or paired in leaf axils, and hermaphrodite (Baum, 1995b). The flowering time varies greatly; in West Africa, the flowering period is from May to June (Wickens, 1982; Baum, 1995a; Baum *et al.*, 1998; Assogbadjo *et al.*, 2005b). The African baobab is known to be bat-pollinated (Pijl, 1936; Jaeger, 1945, 1954). The fruits (Figures 1.2a and 1.2b) develop 5-6 months after flowering and tend to fall from the late rainy season onwards. Seeds (Figure 1.3a) are reniform and embedded in the pulp, dark brown to reddish black with a smooth testa; kernels (Figure 1.3b) are obtained after seed decortication. Leaves (Figure 1.4a) are 2-3-foliate at the start of the season and are early deciduous, more mature ones are 5-7(-9)-foliate. Leaves are alternate at the ends of branches or occur on short spurs on the trunk. Leaves of young trees are often simple. Leaves are processed into leaf powder (Figure 1.4b).

Figure 1.2a Baobab fruits

Figure 1.2b Baobab fruit contents

Figure 1.3a Baobab seeds

Figure 1.3b Baobab kernels

Figure 1.4a Baobab leaves

Figure 1.4b Baobab leaf powder

1.4 Uses of the baobab

The baobab tree is known to be important for many communities. The following tale over the hare and the magic baobab is an illustration of its relevance. The hare was living in an African village where famine has become severe; there was nothing to eat. The hare decided to go to the savannah with the hope to find food and bring this to his village. After walking for hours, he felt tired and decided to rest a bit under a baobab tree because of its nice shade. Suddenly, the baobab started to talk to him and said: "Do you want to taste my leaves? You will see how refreshing they are". The hare enjoyed the baobab leaves and got rid of his thirst and the baobab asked him what he was actually looking for. The hare explained the situation of his village and the baobab asked him to go step by step to his top and to explore him in details. The higher the hare went on the baobab, the more dishes and foods he found. At the top, he even found other non-food articles needed for daily life. The hare filled his bags, thanked the baobab and went back to his village where he was received as a hero (http://blog.crdp-versailles.fr/lespetitsecoliers/index.php/post/27/01/2010/Le-baobab-magique-d-apr%C3%A8s-les-contes-de-Souleman-M-Bog).

Although the baobab is mostly regarded as a fruit-bearing forest tree, it is a widely-used species with numerous food uses of various tree parts, and bark fibres that are used for a variety of applications such as artistic products and rope production (Codjia *et al.*, 2001, 2003; Sidibe and Williams, 2002). According to Wickens (2008), the baobab is an important source of human nutrition today in Africa. Chemical analysis of baobab parts showed the presence of proteins, amino acids, iron, vitamins C, A and E (in abundant amounts compared to daily needs) in leaves, seeds and fruit pulp (Codjia *et al.*, 2001; Sidibe and Williams, 2002). Baobab pulp and leaves have a high antioxidant activity when compared to other fruits (Vertuani *et al.*, 2002; Besco *et al.*, 2007) and can consequently be considered as so-called functional foods, which may have a positive impact on health in addition to their role as a food. In 2008, baobab dried fruit pulp has been acknowledged as a novel food by the European Union (The Commission of the European Communities, 2008) and approved in 2009 as a food ingredient in the US (Addy, 2009), which may boost the trade of this product from African countries, and thus provide a valuable source of foreign exchange.

1.5 Research questions

The studies performed on the species provide, among others, ample information on the nutritional value of raw baobab parts (leaves, pulp, seeds, kernels), but little on the nutritional value of processed ones and none on the solubility and bioavailability of the minerals and vitamins in baobab foods (Sidibe and Williams, 2002; Assogbadjo, 2006). Moreover, within the species *Adansonia digitata*, a number of local types have been characterised that differ in habit, vigour, size, and quality of the fruits (Assogbadjo *et al.*, 2008). It is not known whether the nutritional value varies from one morphotype to another. In addition, many studies reported general information about uses of baobab as food but little on the link between socio-cultural groups and their knowledge about baobab-derived foods, the importance of these foods for the population, their processing and preservation.

1.6 Objectives of the thesis

In view of the above, the present work focused on indigenous knowledge, some food uses and nutrition aspects of baobab parts, necessary for any further valorisation and improvement of local practices related to the species. To achieve this goal, the specific objectives of the thesis are to:

(i) review the nutritional value of baobab parts;
(ii) document the ethnofood knowledge related to baobab in Benin;
(iii) study the effect of home cooking on the in vitro digestibility and bioavailability of minerals and carotenoids in baobab leaves;
(iv) study some baobab fermented products and in particular *Tayohounta*, a food from fermented baobab seed kernels;
(v) study the quality degradation of baobab pulp during storage.

1.7 Outline of the thesis

The present chapter describes the justification and relevance of the PhD research, its objective and outline and provides general information about the baobab. In chapter 2, the food uses and nutritional value of baobab parts are critically reviewed and discussed. Studies that need to be done based on this critical analysis are outlined. In chapter 3, the indigenous knowledge on baobab foods, their importance and the problems related to their processing are documented, investigated and linked with socio-cultural groups. Based on chapter 2 and 3, it became clear that further research was needed on bioavailability of minerals and carotenoids and on fermented baobab food products. Consequently, chapter 4 investigated the in vitro digestibility of iron, zinc and calcium and carotenoids in baobab leaf sauce considering the different morphotypes of leaves mentioned by local populations, and also the effect of traditional cooking on these minerals and carotenoids. Subsequently, chapter 5 characterises the three baobab fermented foods identified during our survey, while in chapter 6 a further microbiological characterization is given of a particular fermented product, namely *Tayohounta* (i.e., fermented kernels). Chapter 7 investigated the quality degradation of baobab pulp during storage in terms of colour change and loss of vitamin C. Finally, in chapter 8, a general discussion is given on the obtained results and a view on the degree in which the objectives have been achieved. Moreover, recommendations for further research are given.

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Chapter 2

Baobab food products: A review on their composition and nutritional value

This chapter has been published as:
Baobab food products: a review on their composition and nutritional value.
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Abstract

Several authors have published about baobab food products. Data on macronutrients, micronutrients, amino acids and fatty acids were collected from literature for pulp, leaves, seeds and kernels of the baobab tree. The results show that baobab pulp is particularly rich in vitamin C; consumption of 40 g covers 84 to more than 100% of the Recommended Daily Intake (RDI) of pregnant women (19 - 30 years). The leaves are particularly rich in calcium (307 to 2640 mg/100 g dw), and they are known to contain good quality proteins with a chemical score of 0.81. The whole seeds and the kernels have a relatively high lipid content, 11.6 to 33.3 g/100 g dw and 18.9 to 34.7 g/100 g dw, respectively. The pulp and leaves exhibit antioxidant properties with a higher activity in the pulp than in the leaves. Reported nutrient contents of different baobab parts show a large variation, which may have arisen from various factors. Three recommendations are given for future research: 1. more attention should be given to precision of analytical methods, 2. research about digestibility and bioavailability of baobab products is needed, 3. the effect of storage and processing on the nutritional value of baobab products needs to be assessed.

Keywords: Nutrients, antioxidant capacity, baobab pulp, baobab leaves, baobab seeds, baobab kernels.

2.1 Introduction

The African baobab tree (*Adansonia digitata*) and its related species belong to the family of Malvacea (Alverson, 1999). The tree occurs naturally in dry areas of Africa, mainly in the Sahelian, Soudano-Sahelian and Soudanian zones; the distribution extends through the woodlands, savannas and grasslands of sub-Saharan Africa to about 25°S. It is characterized by its massive size, reaching to a height of 18-25 m. Crown shape ranges from depressed ovoid through globose to obovoid. The bark is smooth, reddish-brown, greyish-brown or purplish-grey, soft and fibrous. Leaves are 2-3 foliate at the start of the season and they are early deciduous; more mature ones are 5-7 (-9) foliate. Leaves of young trees are often simple. They are glabrous to tomentose; the former are preferred for food. The fruits are very variable, usually globose to ovoid but sometimes oblong-cylindrical, often irregular in shape, apex pointed or obtuse, covered by velvety yellowish or greenish hairs (Sidibe and Williams, 2002). The fruits are indehiscent; they are broken open by chimpanzees, baboons, etc. or will crack open if they fall on a stony surface. They contain reniform seeds and powdery pulp (Baum, 1995).

The baobab is a multi-purpose tree with products having numerous food uses and medicinal properties, and a fibrous bark that is used for various applications (Wickens, 1982; Codjia *et al.*, 2001; Sidibe and Williams, 2002). The pulp of the fruit, the seeds and the leaves are all utilized and are essentially wild-gathered foods. They are consumed daily by rural populations in Africa and are also commercialized. The tuberous taproot of seedlings and young saplings are also eaten, especially in times of famine. Baobab products (leaves, fruits, craft products and bark) are sold on local, informal markets. Middlemen also operate and trade in the larger urban markets (Sidibe and Williams, 2002). In Benin, for instance, 6923 kg of pulp was sold for about 1370 euros in a rural market of Boukoumbe district in northern Benin by 48 vendors from January to March 2001 (Codjia *et al.*, 2003). In developed countries, e.g. Italy, baobab fruits are used to produce dietary supplements and cosmetics.

Previously published biochemical analyses revealed that baobab's edible parts (pulp, leaves, seeds) are rich in nutrients (Yazzie *et al.*, 1994; Nordeide *et al.*, 1996; Sidibe *et al.*, 1996; Barminas *et al.*, 1998; Sena *et al.*, 1998; Codjia *et al.*, 2001; Sidibe and Williams, 2002). Literature reviews on baobab by Sidibe and Williams (2002) and Diop *et al*. (2005) provide substantial information on the species taxonomy, distribution, properties, utilization, agronomy and agro-ecology. However, these reviews did not deal in detail with the nutritional value of baobab food products. Information on the nutrient composition of food is essential to estimate adequate nutrient intake both at individual and group levels (Joyanes and Lema, 2006). The present review investigates the nutritional value of baobab food products based on data from various authors and critically evaluates the similarities and divergences of the values in relation to the research methods used. Research needs are identified on the basis of our review. For each component, the reported values are, as much as possible, converted into the same unit, and their average, minimum and maximum values are calculated and reported in table 2.1. Non converted data were shown as reported originally.

2.2 Leaves

2.2.1 Macronutrients

Nordeide *et al.* (1996) and Lockett *et al.* (2000) found that the water content (Table 2.1) of dried baobab leaves was 6.4 and 8.2%, respectively. The average water content is 7.3%. Oomen and Grubben (1978) investigated the moisture content of fresh leaves and found a value of 77%. The reported energy value varies from 1180 kJ/100 g dw (Becker, 1983) to 1580.6 kJ/100 g dw (Nordeide *et al.*, 1996) with an average of 1380.3 kJ/100 g. The carbohydrate content varies from 40 g/100 g dw (Lockett *et al.*, 2000) to 69 g/100 g dw (Nordeide *et al.*, 1996); the average is 56.4 kJ/100 g. Crude protein contents vary from 10 g/100 g dw (Yazzie *et al.*, 1994; Lockett *et al.*, 2000) to 14.9 g/100 g dw (Nordeide *et al.*, 1996). The reported values for fat content are generally low and vary from 4 g/100 g dw (Becker, 1983) to 6.3 g/100 g dw (Lockett *et al.*, 2000). The ash content ranges from 11.5 g/100 g dw (Nordeide *et al.*, 1996) to 15.9 g/100 g dw (Lockett *et al.*, 2000).

The variability in the reported values for baobab leaves is lower than for the macronutrient composition of the pulp (described farther), despite the use of different measurement methods by the authors. The time-temperature combination used to determine water and ash contents were different. Energy was calculated using Atwaters' coefficients while carbohydrates were determined by difference. The fat content was determined either by the Soxtec system or gravimetrically, while the protein content was determined by Kjeldahl analysis. However, the number of authors who investigated the macronutrient composition of the leaves, is rather low.

2.2.2 Minerals

Baobab leaves are very rich in Calcium according to literature (Table 2.1). With an average of 1582.3 mg/100 g dw, the reported values range between 1470 mg/100 g dw (Sena *et al.*, 1998) and 2640 mg/100 g dw (Yazzie *et al.*, 1994). However, lower values of 307 and 315 mg/100 g dw were mentioned by Yazzie *et al.* (1994), who studied different specimens of baobab leaves. Boukari *et al.* (2001) also measured the Ca content of selected African foods and mentioned that baobab dried leaves contain 2240 mg/100 g dw; among the 24 foods analysed, baobab was ranked fifth after sorrel leaves (*Hibiscus sabdariffa*) (3630 mg/100 g dw), amaranth (*Amaranthus spp.*) leaves (3590 mg/100 g dw), okra (*Hibiscus esculentus*) leaves (2850 mg/100 g dw) and onion (*Allium cepa*) leaves (2540 mg/100 g dw). The reported magnesium content ranges from 93.6 mg/100 g dw (Smith *et al.*, 1996) to 549 mg/100 g dw (Glew *et al.*, 1997). Smith *et al.* (1996) investigated the mineral content of dark, fine light, and rough light leaves and found a large variability in Mg content (93.6 mg/100 g dw for the dark leaves, 121.7 mg/100 g dw for the fine light leaves and 274.2 mg/100 g dw for the rough light leaves). It is presumed that the connotations “fine” and “rough” –with respect to the leaves- refer to their pubescence. The Potassium contents vary greatly from 140 mg/100 g dw to 1080 mg/100 g dw (Yazzie *et al.*, 1994) with an average of 531 mg/100 g. The reported Sodium contents range from 3.8 mg/100 g dw (Sena *et al.*, 1998) to 163 mg/100 g dw (Glew *et al.*, 1997); the average of the reported values is 83.4 mg/100 g dw. Smith *et al.* (1996) reported a Copper content of 0.29 mg/100 g dw and most of the other values are between the latter and 1.6 mg/100 g dw, which is the highest value reported by Glew *et al.* (1997). The Manganese content varies from 1.9 to 9.8 mg/100 g dw (Yazzie *et al.*, 1994) , and Phosphorus contents range from 115 mg/100 g dw (Lockett *et al.*, 2000) to 875.6 mg/100 g dw (Barminas *et al.*, 1998). Zinc levels generally vary between 0.7 mg/100 g dw (Smith *et al.*, 1996) and 4.0 mg/100 g dw (Yazzie *et al.*, 1994). However, a higher value of 22.4 mg/100 g dw was reported by Barminas (1998). The Iron content varies greatly from 1.2 mg/100 g dw for rough

leaves (Smith *et al.*, 1996) to 100 mg/100 g dw (Yazzie *et al.*, 1994). The Molybdenum content is generally lower than 2 mg/100 g dw.

Lockett *et al.* (2000) used atomic absorption spectroscopy to determine most elements except P, whereas Sena *et al.* (1998) and Glew *et al.* (1997) used inductively coupled argon plasma atomic emission spectroscopy. Barminas *et al*. (1998) and Nordeide *et al*. (1996) used atomic absorption spectrophotometry, Smith *et al.* (1996) atomic absorption and Yazzie *et al.* (1994) atomic emission spectrophotometry.
The structure and the colour of the leaves seem to be related to the mineral content (Smith *et al.*, 1996). This apparent relation requires further investigation. Moreover, in future research better descriptions of analysed leaf material are required to allow comparison.
To our knowledge, the bioavailability of these minerals has not been investigated. However, this is necessary to determine to which extent baobab leaves can be used to combat certain micronutrient deficiency problems.

2.2.3 Vitamins

Only few authors have investigated the vitamin A content of baobab leaves. Scheuring *et al*. (1999) found that the simple practice of drying baobab leaves in the shade protects against deterioration of provitamin A. The selection of small leaves (which is tree specific) further increased provitamin A by 20%. The combination of small leaves and shade drying enabled the retention of the provitamin A content up to 27 µg retinol equivalent per gram of dried leaf powder (Table 2.2). Other authors mention the carotenoid content of baobab leaves (Table 2.3). Vertuani *et al*. (2002) found that baobab leaves have an Integral Antioxidant Capacity (IAC) of 8.7 mmol/g. The IAC represents the sum of the antioxidant capacity of hydrophilic and lipophilic antioxidants, calculated as mmol equivalents in activity of Trolox, determined in the best experimental conditions for the sample. The antioxidant activity may be due to the presence of carotenoids or other phenolic compounds, but this needs to be checked.

The type of leaves analyzed, young or old, sun or shade dried, is not indicated by Sena *et al.* (1998) and Nordeide *et al.* (1996) and their reported values are far below the general range of the ones reported by Scheuring *et al.* (1999). Becker (1983) reported thiamine, riboflavin and niacin content of 0.13, 0.82 and 8.06 mg/100 g dw, respectively.

2.2.4 Amino acids

Concerning the amino acid content, the highest averages from the reported values were found for aspartic acid (10.6 g/100 g protein), glutamic acid (10.5 g/100 g protein), leucine (8.3 g/100 g protein) and arginine (7.7 g/100 g protein), whereas the lowest values were found for methionine (1.7 g/100 g protein) and tryptophan (1.9 g/100 g protein) (Table 2.1). Nordeide *et al.* (1996) mentioned that the limiting amino acid for baobab leaf products is lysine. The authors also computed the chemical score (FAO/WHO/UNU, 1985) using the reference amino acid pattern for preschool children (2-5 years) and found that it was 81% for baobab leaves. The amino acid chemical score should be in the order of 60 and above (Nordeide *et al.*, 1996). This implies that the leaves of *A. digitata* are potentially valuable protein sources to be used to complement the amino acid profile of local dishes, namely in order to improve the protein quality of agricultural products that are not deficient in lysine, e.g. cereals. Similarly, Yazzie *et al.* (1994) calculated the chemical score using tryptophan, the most limiting essential amino acid and found that baobab leaf contains significant amounts of all the essential amino acids.

Table 2.1 Composition of baobab pulp, leaves, seeds and kernels

	Pulp				Leaves				Whole seeds				Kernels			
	Average	**min**	**max**	**References**	**Average**	**min**	**max**	**References**	**Ave-rage**	**min**	**max**	**References**	**Average**	**min**	**max**	**References**
Macronutrients																
Water (%)	11.6	2.0	27.5	(Busson, 1965; Wehmeyer, 1966; Becker, 1983; Obizoba and Amaechi, 1993; Saka and Msonthi, 1994; Lockett *et al.*, 2000; Murray *et al.*, 2001; Osman, 2004; Soloviev *et al.*, 2004)	7.3	6.4	8.2	(Nordeide *et al.*, 1996; Lockett *et al.*, 2000)	7.2	6.1	8.2	(Proll *et al.*, 1998; Lockett *et al.*, 2000)	7.24	6.38	8.1	(Obizoba and Amaechi, 1993; Igboeli *et al.*, 1997)
Energy (kJ/100 g dw)	1274	849	1495	(Saka and Msonthi, 1994; Murray *et al.*, 2001; Osman, 2004)	1380	1180	1581	(Becker, 1983; Nordeide *et al.*, 1996)	1762	1590	1935	(Proll *et al.*, 1998; Osman, 2004)	1965	1965	1965	(Igboeli *et al.*, 1997)
Carbohydrates (g/100 g dw)	74.9	46.6	87.7	(Wehmeyer, 1966; Becker, 1983; Obizoba and Amaechi, 1993; Saka and Msonthi, 1994; Lockett *et al.*, 2000; Murray *et al.*, 2001; Osman, 2004)	56.4	40.2	69.0	(Becker, 1983; Nordeide *et al.*, 1996; Lockett *et al.*, 2000)	31.7	5.2	56.8	(Arnold *et al.*, 1985; Proll *et al.*, 1998; Lockett *et al.*, 2000; Osman, 2004)	34.52	22.1	48.1	(Obizoba and Amaechi, 1993; Igboeli *et al.*, 1997; Ajayi *et al.*, 2003; Nnam and Obiakor, 2003)
Crude protein (g/100 g dw)	5.3	2.5	17	(Wehmeyer, 1966; Nour *et al.*, 1980; Becker, 1983; Obizoba and Amaechi, 1993; Saka and Msonthi, 1994; Glew *et al.*, 1997; Sena *et al.*, 1998; Lockett *et al.*, 2000; Murray *et al.*, 2001; Osman, 2004)	12.8	10.1	15.0	(Becker, 1983; Nordeide *et al.*, 1996; Lockett *et al.*, 2000)	21.4	14.4	36.7	(Arnold *et al.*, 1985; Glew *et al.*, 1997; Proll *et al.*, 1998; Lockett *et al.*, 2000; Osman, 2004)	24.7	14	32.7	(Obizoba and Amaechi, 1993; Igboeli *et al.*, 1997; Ajayi *et al.*, 2003; Nnam and Obiakor, 2003)
Crude lipids (g/100 g dw)	3.6	0.2	15.5	(Wehmeyer, 1966; Nour *et al.*, 1980; Becker, 1983; Obizoba and Amaechi, 1993; Saka and Msonthi, 1994; Glew *et al.*, 1997; Sena *et al.*, 1998; Lockett *et al.*, 2000; Murray *et al.*, 2001; Osman, 2004)	4.9	4.0	6.3	(Becker, 1983; Nordeide *et al.*, 1996; Lockett *et al.*, 2000)	18.4	11.6	33.3	(Arnold *et al.*, 1985; Glew *et al.*, 1997; Proll *et al.*, 1998; Lockett *et al.*, 2000; Osman, 2004)	27.8	18.9	34.7	(Obizoba and Amaechi, 1993; Igboeli *et al.*, 1997; Ajayi *et al.*, 2003; Nnam and Obiakor, 2003)
Fibre (g/100 g dw)	13.7	6.0	45.1	(Busson, 1965; Wehmeyer, 1966; Nour *et al.*, 1980; Becker, 1983; Saka and Msonthi, 1994; Lockett *et al.*, 2000; Murray *et al.*, 2001; Osman, 2004)	19.2	11.0	27.5	(Becker, 1983; Lockett *et al.*, 2000)	28.3	16.9	49.7	(Arnold *et al.*, 1985; Lockett *et al.*, 2000; Osman, 2004)	21.2	21.2	21.2	(Ajayi *et al.*, 2003)
Ash (g/100 g dw)	4.9	1.9	6.4	(Busson, 1965; Wehmeyer, 1966; Nour *et al.*, 1980; Obizoba and Amaechi, 1993; Saka and Msonthi, 1994; Lockett *et al.*, 2000; Murray *et al.*, 2001; Osman, 2004)	13.7	11.5	15.9	(Nordeide *et al.*, 1996; Lockett *et al.*, 2000)	5.3	4.0	6.4	(Arnold *et al.*, 1985; Proll *et al.*, 1998; Lockett *et al.*, 2000; Osman, 2004)	6.50	5	7.9	(Obizoba and Amaechi, 1993; Igboeli *et al.*, 1997; Ajayi *et al.*, 2003; Nnam and Obiakor,

	Pulp				Leaves				Whole seeds				Kernels			
	Average	min	max	References	Average	min	max	References	Ave-rage	min	max	References	Average	min	max	References
																2003)
Minerals (mg/100 g dw)																
Ca	302	3.0	701	(Nour *et al.*, 1980; Arnold *et al.*, 1985; Obizoba and Amaechi, 1993; Prentice *et al.*, 1993; Saka and Msonthi, 1994; Glew *et al.*, 1997; Sena *et al.*, 1998; Lockett *et al.*, 2000; Osman, 2004)	1582	307	2640	(Oomen and Grubben, 1978; Prentice *et al.*, 1993; Yazzie *et al.*, 1994; Nordeide *et al.*, 1996; Glew *et al.*, 1997; Barminas *et al.*, 1998; Sena *et al.*, 1998; Lockett *et al.*, 2000; Boukari *et al.*, 2001)	252	29.6	395	(Arnold *et al.*, 1985; Glew *et al.*, 1997; Lockett *et al.*, 2000; Osman, 2004)	1.36	0.43	3.76	(Obizoba and Amaechi, 1993; Odetokun, 1996; Ajayi *et al.*, 2003; Nnam and Obiakor, 2003)
Cu	0.9	0.2	1.8	(Obizoba and Amaechi, 1993; Sena *et al.*, 1998; Lockett *et al.*, 2000; Osman, 2004)	0.8	0.3	1.6	(Smith *et al.*, 1996; Glew *et al.*, 1997; Barminas *et al.*, 1998; Lockett *et al.*, 2000)	2.3	1.3	3.0	(Arnold *et al.*, 1985; Lockett *et al.*, 2000; Osman, 2004)	1.54	0.02	5.36	(Obizoba and Amaechi, 1993; Odetokun, 1996; Ajayi *et al.*, 2003; Nnam and Obiakor, 2003)
Fe	4.3	1.1	10.4	(Arnold *et al.*, 1985; Obizoba and Amaechi, 1993; Saka and Msonthi, 1994; Glew *et al.*, 1997; Sena *et al.*, 1998; Lockett *et al.*, 2000; Osman, 2004)	65.3	1.2	254	(Yazzie *et al.*, 1994; Nordeide *et al.*, 1996; Smith *et al.*, 1996; Glew *et al.*, 1997; Barminas *et al.*, 1998; Sena *et al.*, 1998; Lockett *et al.*, 2000)	5.1	1.8	7.1	(Arnold *et al.*, 1985; Glew *et al.*, 1997; Lockett *et al.*, 2000; Osman, 2004)	1.39	0.63	2.39	(Obizoba and Amaechi, 1993; Odetokun, 1996; Ajayi *et al.*, 2003; Nnam and Obiakor, 2003)
K	1794	726	3272	(Saka and Msonthi, 1994; Sena *et al.*, 1998; Osman, 2004)	531	140	1080	(Yazzie *et al.*, 1994; Sena *et al.*, 1998)	908	428	1387	(Arnold *et al.*, 1985; Osman, 2004)	6.6	0.6	17.4	(Odetokun, 1996; Ajayi *et al.*, 2003; Nnam and Obiakor, 2003)
Mg	195	100	300	(Arnold *et al.*, 1985; Saka and Msonthi, 1994; Glew *et al.*, 1997; Sena *et al.*, 1998; Lockett *et al.*, 2000; Osman, 2004)	339	93.6	549	(Yazzie *et al.*, 1994; Smith *et al.*, 1996; Glew *et al.*, 1997; Barminas *et al.*, 1998; Sena *et al.*, 1998; Lockett *et al.*, 2000)	402	282	696	(Arnold *et al.*, 1985; Glew *et al.*, 1997; Lockett *et al.*, 2000; Osman, 2004)	0.65	0.2	1.1	(Odetokun, 1996; Ajayi *et al.*, 2003)

	Pulp				Leaves				Whole seeds				Kernels			
	Average	**min**	**max**	**References**	**Average**	**min**	**max**	**References**	**Ave-rage**	**min**	**max**	**References**	**Average**	**min**	**max**	**References**
Mn	0.7	0.4	1.0	(Sena *et al.*, 1998; Lockett *et al.*, 2000)	6.0	1.9	9.8	(Yazzie *et al.*, 1994; Smith *et al.*, 1996; Glew *et al.*, 1997; Sena *et al.*, 1998; Lockett *et al.*, 2000)	1.1	1.1	1.1	(Glew *et al.*, 1997; Lockett *et al.*, 2000)	1.49	0.15	2.84	(Odetokun, 1996; Ajayi *et al.*, 2003)
Na	14.8	0.8	31.1	(Saka and Msonthi, 1994; Glew *et al.*, 1997; Sena *et al.*, 1998; Osman, 2004)	83.4	3.8	163	(Glew *et al.*, 1997; Sena *et al.*, 1998)	2.3	1.9	2.7	(Arnold *et al.*, 1985; Glew *et al.*, 1997)	0.91	0.17	1.64	(Ajayi *et al.*, 2003; Nnam and Obiakor, 2003)
P	106	0.0	425	(Nour *et al.*, 1980; Arnold *et al.*, 1985; Obizoba and Amaechi, 1993; Saka and Msonthi, 1994; Glew *et al.*, 1997; Sena *et al.*, 1998)	274	115	875	(Prentice *et al.*, 1993; Yazzie *et al.*, 1994; Glew *et al.*, 1997; Barminas *et al.*, 1998; Sena *et al.*, 1998; Lockett *et al.*, 2000)	453	5.6	738	(Arnold *et al.*, 1985; Glew *et al.*, 1997; Lockett *et al.*, 2000)	109	0.38	326	(Obizoba and Amaechi, 1993; Odetokun, 1996; Nnam and Obiakor, 2003)
Zn	1.7	0.5	3.2	(Arnold *et al.*, 1985; Obizoba and Amaechi, 1993; Glew *et al.*, 1997; Sena *et al.*, 1998; Lockett *et al.*, 2000; Osman, 2004)	4.1	0.7	22.4	(Yazzie *et al.*, 1994; Nordeide *et al.*, 1996; Smith *et al.*, 1996; Glew *et al.*, 1997; Barminas *et al.*, 1998; Sena *et al.*, 1998; Lockett *et al.*, 2000)	5.0	2.6	7.3	(Arnold *et al.*, 1985; Glew *et al.*, 1997; Lockett *et al.*, 2000; Osman, 2004)	1.42	0.10	3.57	(Obizoba and Amaechi, 1993; Odetokun, 1996; Ajayi *et al.*, 2003; Nnam and Obiakor, 2003)
Vitamins (mg/100 g)																
B1 Thiamine	0.3	0.0	0.6	(Santos Oliveira, 1975; Becker, 1983; Arnold *et al.*, 1985)					-	-	-	-				
B2 Riboflavin	0.1	0.1	0.1	(Santos Oliveira, 1975; Becker, 1983; Arnold *et al.*, 1985)					-	-	-	-				
B3 Niacin	2.2	1.8	2.7	(Santos Oliveira, 1975; Becker, 1983; Arnold *et al.*, 1985)					-	-	-	-				
Vitamin A	-	-	-	-					-	-	-	-				
Vitamin C	150	209	500	(Nour *et al.*, 1980; Becker, 1983; Arnold *et al.*, 1985)					-	-	-	-				

	Pulp				Leaves				Whole seeds				Kernels			
	Average	min	max	References	Average	min	max	References	Ave-rage	min	max	References	Average	min	max	References
Amino Acids (g/100 g proteins)																
Alanine	5.6	3.3	8.2	(Glew *et al.*, 1997; Sena *et al.*, 1998; Osman, 2004)	6.4	5.8	7.5	(Yazzie *et al.*, 1994; Glew *et al.*, 1997; Sena *et al.*, 1998)	8.0	5.4	11.5	(Glew *et al.*, 1997; Proll *et al.*, 1998; Osman, 2004)				
Arginine	6.8	4.4	8.4	(Glew *et al.*, 1997; Sena *et al.*, 1998; Osman, 2004)	7.7	6.4	11.1	(Yazzie *et al.*, 1994; Glew *et al.*, 1997; Sena *et al.*, 1998)	11.5	1.1	25.5	(Glew *et al.*, 1997; Proll *et al.*, 1998; Osman, 2004)				
Aspartic acid	7.5	5.2	11.0	(Glew *et al.*, 1997; Sena *et al.*, 1998; Osman, 2004)	10.6	8.1	12.5	(Yazzie *et al.*, 1994; Glew *et al.*, 1997; Sena *et al.*, 1998)	16.9	10.3	23.4	(Proll *et al.*, 1998; Osman, 2004)				
Cysteic acid	1.3	1.0	1.7	(Sena *et al.*, 1998; Osman, 2004)	2.3	1.5	3.9	(Yazzie *et al.*, 1994; Glew *et al.*, 1997; Sena *et al.*, 1998)	2.8	1.5	5.2	(Glew *et al.*, 1997; Proll *et al.*, 1998; Osman, 2004)				
Glutamic acid	8.4	4.1	14.6	(Glew *et al.*, 1997; Sena *et al.*, 1998; Osman, 2004)	10.5	7.4	12.9	(Yazzie *et al.*, 1994; Glew *et al.*, 1997; Sena *et al.*, 1998)	35.9	23.7	59.2	(Glew *et al.*, 1997; Proll *et al.*, 1998; Osman, 2004)				
Glycine	6.2	2.9	11.4	(Glew *et al.*, 1997; Sena *et al.*, 1998; Osman, 2004)	5.5	4.8	6.7	(Yazzie *et al.*, 1994; Glew *et al.*, 1997; Sena *et al.*, 1998)	8.8	5.3	12.5	(Glew *et al.*, 1997; Proll *et al.*, 1998; Osman, 2004)				
Histidine	2.0	1.2	3.4	(Glew *et al.*, 1997; Sena *et al.*, 1998; Osman, 2004)	2.1	1.7	2.6	(Yazzie *et al.*, 1994; Nordeide *et al.*, 1996; Glew *et al.*, 1997; Sena *et al.*, 1998)	3.4	2.2	5.4	(Glew *et al.*, 1997; Proll *et al.*, 1998; Osman, 2004)				
Isoleucine	3.6	2.2	5.1	(Glew *et al.*, 1997; Osman, 2004)	5.7	4.7	7.5	(Yazzie *et al.*, 1994; Nordeide *et al.*, 1996; Glew *et al.*, 1997; Sena *et al.*, 1998)	5.8	3.6	9.6	(Glew *et al.*, 1997; Proll *et al.*, 1998; Osman, 2004)				
Leucine	5.4	4.1	7.6	(Glew *et al.*, 1997; Sena *et al.*, 1998; Osman, 2004)	8.3	7.2	9.7	(Yazzie *et al.*, 1994; Nordeide *et al.*, 1996; Glew *et al.*, 1997; Sena *et al.*, 1998)	10.6	7.0	17.8	(Glew *et al.*, 1997; Proll *et al.*, 1998; Osman, 2004)				
Lysine	4.0	1.7	6.0	(Glew *et al.*, 1997; Sena *et al.*, 1998; Osman, 2004)	5.6	4.7	6.7	(Yazzie *et al.*, 1994; Nordeide *et al.*, 1996; Glew *et al.*, 1997; Sena *et al.*, 1998)	6.9	5.0	10.1	(Glew *et al.*, 1997; Proll *et al.*, 1998; Osman, 2004)				
Methionine	1.9	0.2	4.9	(Glew *et al.*, 1997; Sena *et al.*, 1998;	1.7	0.9	2.6	(Yazzie *et al.*, 1994;	1.9	1.0	3.4	(Glew *et al.*, 1997;				

	Pulp				Leaves				Whole seeds				Kernels			
	Average	min	max	References	Average	min	max	References	Ave-rage	min	max	References	Average	min	max	References
				Osman, 2004)				Nordeide *et al.*, 1996; Glew *et al.*, 1997; Sena *et al.*, 1998)				Proll *et al.*, 1998; Osman, 2004)				
Phenylalanine	3.5	2.1	4.4	(Glew *et al.*, 1997; Sena *et al.*, 1998; Osman, 2004)	5.5	4.8	6.5	(Yazzie *et al.*, 1994; Nordeide *et al.*, 1996; Glew *et al.*, 1997; Sena *et al.*, 1998)	7.2	4.0	12.3	(Glew *et al.*, 1997; Proll *et al.*, 1998; Osman, 2004)				
Prolamine	3.7	2.2	5.1	(Sena *et al.*, 1998; Osman, 2004)	-	-	-	-	6.9	6.9	6.9	(Osman, 2004)				
Proline	7.0	5.4	8.7	(Glew *et al.*, 1997; Sena *et al.*, 1998)	5.6	4.9	6.6	(Yazzie *et al.*, 1994; Glew *et al.*, 1997; Sena *et al.*, 1998)	9.1	4.9	13.3	(Glew *et al.*, 1997; Proll *et al.*, 1998)				
Serine	3.3	2.2	4.4	(Glew *et al.*, 1997; Sena *et al.*, 1998; Osman, 2004)	4.3	3.6	5.6	(Yazzie *et al.*, 1994; Glew *et al.*, 1997; Sena *et al.*, 1998)	8.3	5.8	12.9	(Glew *et al.*, 1997; Proll *et al.*, 1998; Osman, 2004)				
Threonine	2.7	2.4	2.8	(Glew *et al.*, 1997; Sena *et al.*, 1998; Osman, 2004)	3.9	3.4	4.8	(Yazzie *et al.*, 1994; Nordeide *et al.*, 1996; Glew *et al.*, 1997; Sena *et al.*, 1998)	5.8	3.8	7.9	(Glew *et al.*, 1997; Proll *et al.*, 1998; Osman, 2004)				
Tryptophan	3.5	0.7	6.4	(Glew *et al.*, 1997; Sena *et al.*, 1998)	1.9	1.0	3.0	(Yazzie *et al.*, 1994; Nordeide *et al.*, 1996; Glew *et al.*, 1997; Sena *et al.*, 1998)	2.6	1.4	3.7	(Glew *et al.*, 1997; Proll *et al.*, 1998)				
Tyrosine	8.5	0.9	20.6	(Glew *et al.*, 1997; Sena *et al.*, 1998; Osman, 2004)	4.0	3.4	5.1	(Yazzie *et al.*, 1994; Nordeide *et al.*, 1996; Glew *et al.*, 1997; Sena *et al.*, 1998)	3.9	1.5	7.4	(Glew *et al.*, 1997; Proll *et al.*, 1998; Osman, 2004)				
Valine	4.9	3.8	6.0	(Glew *et al.*, 1997; Sena *et al.*, 1998; Osman, 2004)	6.0	5.2	7.0	(Yazzie *et al.*, 1994; Nordeide *et al.*, 1996; Glew *et al.*, 1997; Sena *et al.*, 1998)	8.5	5.9	13.5	(Glew *et al.*, 1997; Proll *et al.*, 1998; Osman, 2004)				
Fatty acids (mg/g dw)																
C:8 (Caprylic)	-	-	-	-	0.01	0.01	0.01	(Sena *et al.*, 1998)								
C:12 (Lauric)	-	-	-	-	0.09	0.09	0.09	(Sena *et al.*, 1998)								
C14:0 (Mystiric)	0.2	0.2	0.2	(Sena *et al.*, 1998)	0.37	0.37	0.37	(Sena *et al.*, 1998)								
C16:0 (Palmitic)	13.6	0.2	27.0	(Glew *et al.*, 1997; Sena *et al.*, 1998)	1.72	0.24	3.2	(Glew *et al.*, 1997; Sena *et al.*, 1998)								
C16:1	-	-	-	-	0.11	0.01	0.21	(Glew *et al.*, 1997;								

	Pulp				Leaves				Whole seeds				Kernels			
	Average	**min**	**max**	**References**	**Average**	**min**	**max**	**References**	**Ave-rage**	**min**	**max**	**References**	**Average**	**min**	**max**	**References**
(Palmitoleic)								Sena *et al*., 1998)								
C18:0 (Stearic)	3.3	3.3	3.3	(Sena *et al.*, 1998)	0.19	0.04	0.35									
C18:1 (Oleic)	25	25	25	(Sena *et al.*, 1998)	0.22	0.06	0.39	(Glew *et al.*, 1997; Sena *et al*., 1998)								
C18:2 (Linoleic)	13.5	0.0	27.0	(Glew *et al.*, 1997; Sena *et al.*, 1998)	0.55	0.1	1	(Glew *et al.*, 1997; Sena *et al*., 1998)								
C18:3 (Linolenic)	0.5	0.2	0.9	(Glew *et al.*, 1997; Sena *et al.*, 1998)	2.09	0.08	4.1	(Glew *et al.*, 1997; Sena *et al*., 1998)								
C20:0 (Arachidic)	0.7	0.7	0.7	(Sena *et al.*, 1998)	0.15	0.15	0.15	(Sena *et al.*, 1998)								
C20:1 (Gadoleic)	0.04	0.04	0.04	(Sena *et al.*, 1998)												

The variation in the reported amino acid contents is relatively low between authors, and also between different specimens analyzed by the same author. A comparison with other forest leaves showed that the chemical scores of *Tamarindus indica* dried leaves, *Parkia biglobosa*, *Amaranthus viridis* dried leaves, and *Allium cepa* dried leaves are 79%, 61%, 51% and 47%, respectively (Nordeide *et al.*, 1996).

Table 2.2 Vitamin A contents of baobab leaves

Leaves	Sun dried			Shade dried		
	α carotene (µg/g)	ß carotene (µg/g)	Retinol Equivalent (µg/g)	α carotene (µg/g)	ß carotene (µg/g)	Retinol Equivalent (µg/g)
Young trees, small leaves	5.7	74.5	12.9	12.9	156	27.2
Young trees, large leaves	6.7	54.0	9.3	5.1	130	22.0
Old trees, small leaves	9.9	87.0	15.3	19.4	147	26.2
Old trees, large leaves	4.1	69.0	11.5	7.1	107	18.5

Source: Scheuring *et al.* (1999)

Table 2.3 Carotenoid contents of baobab leaves

Authors	Lutein	α-Carotene	ß-carotene	Vitamin A total Retinol Equivalent
Sena *et al*. (1998) (µg/g dw) (Niger)	50.9	0.92	17.2	
Nordeide *et al*.(1996) (µg/100 g) (Mali)		Trace	670	112
Nordeide *et al.* (1996) (µg/g)*			6.7	1.12

*Converted values

2.2.5 Fatty acids

Few authors investigated the fatty acid content of baobab leaves, and the reported data by Sena *et al.* (1998) and Glew *et al.* (1997) show many differences. The leaves mainly seem to contain oleic and palmitic acid.

2.2.6 Antinutrients

Baobab leaves contain some toxicants (Andy and Eka, 1985). Phytic acid content varies from 0.04 mg/100 g in market samples to 0.05 mg/100 g in field samples. Total oxalic acid ranges from 4.37 mg/100 g in field samples (with 40% soluble oxalate) to 5.26 mg/100 g in market samples (with 37% soluble oxalate). Baobab leaves contain also 37.2 g/100 g (market samples) to 40.4 mg/100 g (field samples) hydrocyanic acid. Tannins levels are 17.8 mg/100 g and 19.8 mg/100 g in market samples and field samples, respectively. These values are below the known toxic levels but may interfere with the nutrients and possibly decrease their digestibility and availability (Andy and Eka, 1985).

2.3 Pulp

2.3.1 Macronutrients

Water. The reported water content (Table 2.1) varies considerably between authors, and ranges from 2% to 27.5%. Values lower than 10% (namely 2% to 8.7%) are mentioned (Busson, 1965; Wehmeyer, 1966; Nour *et al.*, 1980; Becker, 1983; Murray *et al.*, 2001), while higher values varying from 10 up to 27.5% are also reported (Obizoba and Amaechi, 1993; Saka and Msonthi, 1994; Lockett *et al.*, 2000; Osman, 2004; Soloviev *et al.*, 2004). The average of all reported values is 11.6%. This average water content is a bit high for a powdery type of product such as baobab pulp and may negatively influence its shelf life.

Carbohydrates. Reported carbohydrate contents (Table 2.1) range from 46.6 g/100 g dw (Murray *et al.*, 2001) to 88 g/100 g dw (Wehmeyer, 1966). The average of the reported values is 79.9 g/100 g dw. Most authors determined carbohydrates by difference, so these values are not expected to be very accurate. However, Murray *et al.* (2001) determined different types of carbohydrates and only the fraction containing monosaccharides and disaccharides was calculated by difference. The presence of sugar was also mentioned by Soloviev *et al.* (2004), who found a total soluble sugar content of 7.2-11.2 g/100 g dw in baobab pulp, while Nour *et al.* (1980) reported 23.2% of total sugars and 19.9% of reducing sugars. According to Murray *et al.* (2001), simple sugars in baobab pulp account for about 35.6% of the total carbohydrate content. This explains the noticeable sweet taste of the pulp. However, the sweetness may vary for different types of pulp.

Crude proteins. Generally, the reported crude protein content (Table 2.1) varies from 2.5 g/100 g dw (Lockett *et al.*, 2000) to 3.6 g/100 g dw (Osman, 2004). However, Obizoba and Amaechi (1993) reported higher values of 15.3 g/100 g dw for protein in the pulp, using analytical methods similar to those applied by the other researchers. Most authors used Kjeldahl analysis with the conversion factor of 6.25 to determine the protein content of baobab pulp. Moreover, after amino acid analysis, Sena *et al.* (1998) mentioned a total protein content of 17 g/100 g dw. The value mentioned by Sena *et al.* (1998) is comparable to the one of Obizoba and Amaechi (1993), despite the hydrolysis procedure before amino acid analysis.

Crude lipid. The reported crude lipid contents (Table 2.1) of baobab pulp vary from 0.21 g/100 g dw (Nour *et al.*, 1980) to 15.5 g/ 100 g dw (Glew *et al.*, 1997) with an average of 3.6 g/100 g dw. The value reported by Glew *et al*. (1997) was obtained after fatty acids analysis (hydrolysis of sample before determination) and was quite similar to the one reported by Sena *et al*. (1998), who mentioned a total lipid content of 12.7 g/100 g dw. The highest values without fatty acid analyses, 4.3 and 4.1 g/100 g dw respectively, were reported by Saka and Msonthi (1994) and Obizoba and Amaechi (1993), who however used (a) dilute acid hydrolysis and hexane extraction and (b) the method using the Soxtec system (extractable materials readily passed from the sample and dissolved in the organic solvent -similar to a tea bag in hot water for a duration of 1 hour), respectively. The latter method was also used by Lockett *et al.* (2000), who found a very low fat content of 0.41 g/100 g dw. The Soxhlet and the gravimetric method were also mentioned, but all results were different; the great variation observed may have an origin other than the method used.

Energy. The energy value varies from 848.9 kJ/100 g dw (Murray *et al.*, 2001) to 1494.9 kJ/100 g dw (Osman, 2004) (Table 2.1). The values reported averaged 1275 kJ/100 g. Note

that the method for carbohydrate determination of Murray (2001) was different from the generally used method of difference, and that this may have affected his result. Furthermore, the coefficients used by authors to compute the energy value are sometimes slightly different.

Fibers. Fiber contents are lower than 12.5% in most cases (Table 2.1), ranging generally from 6.0 g/100 g dw (Osman, 2004) to 12.5 g/100 g dw (Lockett *et al.*, 2000). However, Murray *et al.* (2001) mentioned a high value of 45.1 g/100 g dw, which is the maximum of the reported values. The average of the reported values is 13.7 g/100 g dw. Murray *et al.* (2001) measured the fibers (after extraction of fat) by a gravimetric method, while the others used AOAC methods (Nour *et al.*, 1980; Osman, 2004) or an acid and alkaline hydrolysis method (Saka and Msonthi, 1994).

Ash. The reported ash contents (Table 2.1) are from 4.1 (Busson, 1965) to 6.4 g/100 g dw (Lockett et al., 2000) with the exception of the very low value of 1.9 g/100 g dw reported by Obizoba and Amaechi (1993). The methods used by the authors vary considerably with respect to the time temperature combinations.

Other measurements. The pH of the pulp is about 3.3 (Nour et al., 1980). Soloviev *et al.* (2004) investigated total free acidity and their results show that baobab pulp contains 6.5-11.2 g equivalent malic acid per 100 g dw (Table 2.1). These results indicate the acidic character of the pulp.

2.3.2 Minerals

The reported mineral contents of baobab pulp show a great variability between authors (Table 2.1). The values for Magnesium vary from 100.5 mg/100 g dw (Osman, 2004) to 300 mg/100 g (Sena *et al.*, 1998) with an average value of 195.1 mg/100 g. For Potassium, the reported average value is 1793.8 mg/100 g dw and it varies from 726 mg/100 g dw to 3272 mg /100 g dw (Saka and Msonthi, 1994). Reported values for the Calcium content are generally between 390 mg/100 g dw (Prentice *et al.*, 1993) and 700.9 mg/100 g dw (Nour *et al.*, 1980). However, a very low value of 3.0 mg/100 g dw was reported by Obizoba and Amaechi (1993). The average of the reported values is 301.8 mg/100 g dw. The reported Sodium content varies from 0.8 (Sena *et al.*, 1998) to 31.2 mg/100 g dw (Osman, 2004) with an average of 14.8 mg/100 g dw. For Copper, it goes from below the detection level (Glew *et al.*, 1997) to 1.8 mg/100 g dw (Osman, 2004); the average is 0.9 mg/100 g dw. The Manganese content varies from below the detection level (Glew *et al.*, 1997) to 1.0 mg/100 g dw (Sena *et al.*, 1998). The average value is 0.7 mg/100 g dw. For Phosphorus, reported contents vary greatly from 0.04 mg/100 g dw (Obizoba and Amaechi, 1993) to 425 mg/100 g dw (Sena *et al.*, 1998). The reported Zinc content is generally low, ranging from 0.5 (Lockett *et al.*, 2000) to 3.2 mg/100 g dw (Sena *et al.*, 1998) with an average of 1.7 mg/100 g dw. The Iron content varies strongly from 1.1 mg/100 g (Arnold *et al.*, 1985) to 10.4 mg/100 g dw (Osman, 2004).

The methods used by the different researchers to determine minerals were generally atomic absorption methods. For instance, apart from P for which Lockett *et al*. (2000) used the Technicon Auto-analyser methodology, they used a flame atomic absorption spectroscopy method for the other minerals. Sena *et al*. (1998) used the same method as Glew *et al*. (1997), namely the inductively coupled argon plasma atomic emission spectroscopy. Saka and Msonthi (1994) estimated P colorimetrically by the ammonium molybdate method, determined Ca, Mg and Fe by the atomic absorption technique using a Perkin-Elmer 500 spectrophotometer, and analyzed K and Na using a corning 400 flame photometer. Obizoba

and Amaechi (1993) used the polarized Zeeman atomic absorption spectrophotometry. In contrast to the others, Nour *et al.* (1980), who also used a molybdenum colorimetric method to determine P, determined the other minerals using a thiocyanate method for Fe, oxalate precipitation for Ca, and estimated Mg. The large variation in the reported data may be due to the methods used, but may also have other origins, that will be discussed further.

2.3.3 Vitamins

Authors have investigated mainly vitamin C. There is a great variability and the average of the reported values is 283 mg/100 g. Scheuring *et al*. (1999) found that there is a remarkable tree-to-tree variability in the vitamin C content of the fruit pulp, ranging from 150 to 500 mg/100 g, which constitutes actually the minimum and the maximum of the reported values. The figures were found to be quite stable from one year to the next (Scheuring *et al.*, 1999). Possible explanations given for this variability are soil type, genetic make-up and morphotypes. Sena *et al.* (1998) investigated the carotenoid content of the pulp and found that it contains 1.53 µg/g dw of lutein, 0.17 µg/g dw of α-carotene and 0.17 µg/g dw of β-carotene.
The investigation of group B vitamins in the pulp also showed large variations. Data ranged from 1.8 to 2.7 mg/100 g for vitamin B3, niacin, as reported by Santos (1975) and Arnold *et al.* (1985), respectively. The riboflavin content ranges from 0.07 mg/100 g (Becker, 1983) to 0.14 mg/100 g (Arnold *et al.*, 1985).
The methods used to determine each vitamin seldom are described, which makes it hard to evaluate these figures critically.

2.3.4 Amino acids

There is a large variability in the reported amino acid contents of baobab fruit pulp (Table 2.1), despite the fact that the authors (Glew *et al.*, 1997; Sena *et al.*, 1998; Osman, 2004) used similar methods for determination. They all hydrolyzed the samples in the described procedures. The results show that most essential amino acids are present. The highest average contents were found for tyrosine (8.5 g/100 g protein), glutamic acid (8.4 g/100 g protein), aspartic acid (7.5 g/100 g protein), arginine (6.8 g/100 g protein) and glycine (6.2 g/100 g protein). The lowest values were found for the sulphur containing amino acids, namely cystein (1.3 g/100 g protein) and methionine (1.9 g/100 g protein).

2.3.5 Fatty acids

Most fatty acids in the pulp do not reach detectable levels. Similarly to the amino acids, the variability in the reported values is high (Table 2.1), despite the use of identical methods by the researchers. The content of oleic acid is the highest reported value among all fatty acids (25 mg/g dw).

2.3.6 Antinutrients

The baobab fruit pulp, as other plant fruits, contains naturally occurring antinutritional substances. Tannin content of the pulp varies between 0.0051% and 0.0062% (Ghani and Agbejule, 1986). This level is too low to cause any harmful effects on consumers (Ghani and Agbejule, 1986). Other harmful components estimated by Ghani and Agbejule (1986) include: hydrocyanic acid (HCN) (0.0049% dw) and total oxalate (0.0044% dw). However, the detected levels were not alarming (Ghani and Agbejule, 1986).

2.4 Whole seeds

2.4.1 Macronutrients

Two authors reported the water content of the whole seeds to be 6.1% (Proll *et al.*, 1998) and 8.2% (Lockett *et al.*, 2000). They also reported an energy value of 1935 kJ/100 g dw and 1589.8 kJ/100 g dw, respectively. The average carbohydrate content is 31.7g/100 g dw, with values ranging from 5.2 g/100 g dw (Arnold *et al.*, 1985) to 56.8 g/100 g dw (Proll *et al.*, 1998). The crude lipid content ranges from 9 g/100 g dw (Glew *et al.*, 1997) to 33.3 g/100 g dw (Arnold *et al.*, 1985). Ezeagu *et al.* (1998) mentioned a fat content of 14.8% on fresh weight basis, which is lower than that of some soybean varieties (*Glycine max* TGX 1660-15F, TGX 1740-6F, TGX 1740-2F, TGX 1649-11F, TGX 1681-3F), of which the fat content varied from 18.6 to 22.2% on fresh weight basis. The fiber content is reported to vary from 16.9 g/100 g dw (Osman, 2004) to 49.7 g/100 g dw (Lockett *et al.*, 2000), while the ash content has an average value of 5.3 g/100 g dw. The crude protein content varies from 14.4 g/100 g dw (Proll *et al.*, 1998) to 36.7 g/100 g dw (Arnold *et al.*, 1985) with an average value of 21.4 g/100 g dw (Table 2.1). The time-temperature combination for the moisture and ash measurements differs from one author to another. The crude protein contents are mostly measured according to Kjeldahl methods using 6.25 as conversion factor, except for a determination after measurement of amino acids by Glew *et al.* (1997).

2.4.2 Minerals

The mineral levels in whole seeds are presented in table 2.1. The Magnesium content of the whole seeds is reported to vary from 282.2 mg/100 g dw (Osman, 2004) to 696.3 mg/100 g dw (Arnold *et al.*, 1985) with an average value of 402 mg/100 g dw. This is much higher than reported values for the kernels. The same was observed for Potassium and Calcium, that range from 428.5 mg/100 g dw (Osman, 2004) to 1387.2 mg/100 g dw (Arnold *et al.*, 1985) with an average of 908 mg/100 g dw; and from 29.6 mg/100 g dw (Osman, 2004) to 395 mg/100 g dw (Glew *et al.*, 1997) with an average of 252 mg/100 g dw, respectively. The Sodium content varies from 1.9 mg/100 g dw (Glew *et al.*, 1997) to 2.7 mg/100 g dw (Arnold *et al.*, 1985) and the average is 2.3 mg/100 g dw. The average Copper content is 2.3 mg/100 g dw and values range from 1.3 mg/100 g dw (Lockett *et al.*, 2000) to 3 mg/100 g dw (Arnold *et al.*, 1985). The average Manganese content 1.1 mg/100 g dw. The average Zinc content is 5.0 mg/100 g dw and values range from 2.6 mg/100 g dw (Glew *et al.*, 1997) to 7.3 mg/100 g dw (Arnold *et al.*, 1985). The Iron content varies from 1.8 mg/100 g dw (Glew *et al.*, 1997) to 7.1 mg/100 g dw (Arnold *et al.*, 1985) with an average value of 5.1 mg/100 g dw. The Phosphorus contents vary greatly from 5.6 mg/100 g dw (Arnold *et al.*, 1985) to 738.3 mg/100 g dw (Lockett *et al.*, 2000) (Table 2.1) with an average value of 453 mg/100 g dw. A comparison of these data with those of the kernels (described below) shows how rich the coat is in several minerals, namely Ca, P and Mg.

2.4.3 Vitamins

Not much has been reported on the vitamin content of the whole seeds. However, Arnold *et al.* (1985) found that the seeds contain 0.25 mg/100 g dw, 0.14 mg/100 g dw and 1.0 mg/100 g dw of thiamine, riboflavin and niacin, respectively. The method used for the determinations was not specified.

2.4.4 Amino acids

The levels of amino acids in whole seeds are presented in table 2.1. Considering the average value, whole seeds are very rich in glutamic acid (35.9 g/100 g protein), aspartic acid (16.9 g/100 g protein), arginine (11.6 g/100 g protein), leucine (10.6 g/100 g protein), proline (9.1 g/100 g protein), glycine (8.8 g/100 g protein), serine (8.3 g/100 g protein), phenylalanine (7.2 g/100 g protein) and lysine (6.9 g/100 g protein). The lowest average value was found for methionine (1.9 g/100 g protein).

2.4.5 Fatty acids

The fatty acid content of the whole seeds is presented in table 2.4. The presence of a relatively high quantity of oleic acid can be observed (Glew *et al.*, 1997).

2.4.6 Antinutritional factors

Baobab seeds contain some antinutritional factors that can be removed or inactivated by different processing methods. Osman (2004) investigated the antinutritional factors in baobab seeds and found that they contain a Trypsin Inhibitor Activity of 5.7 TIU/mg sample, 73 mg/100 g of phytic acid and 23% catechin equivalent of tannin. Igboeli *et al.* (1997) investigated the effects of some processing techniques on the antinutrient composition of baobab seeds and found that cold water, hot water, hot alkali and acid treatments reduced the tannic acid concentration in baobab seeds significantly. However, dehulling did not lead to significant decreases (from 400 µg tannic acid /g in the whole seeds to 390 µg tannic acid/g in the dehulled seeds). The activity of amylase inhibitors in the seeds was reduced significantly by dehulling (from 35 to 10 µg/ 100 g), cold water and hot alkali treatments. Moreover, fermentation reduced the antinutrient contents (phytate and tannins) of baobab seeds (Nnam and Obiakor, 2003).

2.5 Kernels

The cream-coloured kernels are obtained by removing the shell from the whole seeds. Traditionally, the whole seeds are soaked and boiled for 4-6 hours. Afterwards, the seeds are individually and manually dehulled. Next, the kernels are dried. This operation is one of the most difficult ones in the traditional processing of baobab parts.

2.5.1 Macronutrients

As presented in table 2.1, the average reported water content is 7.2%. It ranges from 6.4% (Igboeli *et al.*, 1997) to 8.1% (Obizoba and Amaechi, 1993). The energy value mentioned by Igboeli *et al.* (1997) is 1965.5 kJ/100 g dw. The carbohydrate content ranges from 22.1 g/100 g dw (Ajayi *et al.*, 2003) to 48.1 g/100 g dw (Nnam and Obiakor, 2003). The average of the reported crude protein contents is 24.7 g/100 g dw, ranging from 14 g/100 g dw (Nnam and Obiakor, 2003) to 32.7 g/100 g dw (Obizoba and Amaechi, 1993). The crude lipid content has an average of 27.8 g/100 g dw. A fiber content of 21.2 g/100 g dw is mentioned (Ajayi *et al.*, 2003) and the average ash content is 6.5 g/100 g dw. The kernels appear to be a good source of protein and energy and may be an interesting ingredient for designing formulated infant foods.

Table 2.4 Reported fatty acid content of baobab whole seeds

Authors	**C14:0 mystiric**	**C16:0 palmitique**	**C16:1 palmitoleic**	**C18:0 stearic**	**C18:1 oleic**	**C18:2 linoleic**	**C18:3 linolenic**	**C20:0 arachidic**
Glew *et al.* (1997) (mg fatty acid/g dw material)	Trace	1.43	0.02	0.16	2.14	1.38	0.016	Trace
Glew *et al.*, (1997) (g fatty acid/100 g dw material)*		0.14	0.002	0.016	0.21	0.13	0.002	

*Converted values

2.5.2 Minerals

The kernels were investigated for their mineral content by several authors (Table 2.1). They were reported to have an average Potassium content of 6.6 mg/100 g dw, with a minimum of 0.6 mg/100 g dw (Nnam and Obiakor, 2003) and a maximum of 17.3 mg/100 g dw (Ajayi *et al.*, 2003). The Magnesium content ranges from 0.2 mg/100 g dw (Odetokun, 1996) to 1.1 mg/100 g dw (Ajayi *et al.*, 2003) with an average of 0.7 mg/100 g dw. The Calcium content ranges from 0.4 mg/100 g dw (Ajayi *et al.*, 2003) to 3.8 mg/100 g dw (Obizoba and Amaechi, 1993); the Sodium content from 0.2 mg/100 g dw (Ajayi *et al.*, 2003) to 1.6 mg/100 g dw (Odetokun, 1996); Copper from 0.02 mg/100 g dw (Nnam and Obiakor, 2003) to 5.4 mg/100 g dw (Odetokun, 1996); Manganese from 0.2 mg/100 g dw (Ajayi *et al.*, 2003) to 2.8 mg/100 g dw (Odetokun, 1996); Zinc from 0.1 mg/100 g dw (Ajayi *et al.*, 2003) to 3.6 mg/100 g dw (Obizoba and Amaechi, 1993); and Iron from 0.6 mg/100 g dw (Nnam and Obiakor, 2003) to 2.4 mg/100 g dw (Obizoba and Amaechi, 1993). The Phosphorus content is reported to vary greatly from 0.4 mg/100 g dw (Obizoba and Amaechi, 1993) to 326.3 mg/100 g dw (Nnam and Obiakor, 2003).

For the determination of minerals, Ajayi *et al.* (2003) used the method described by Idouraine *et al.* (1996), while Nnam and Obiakor (2003) used the atomic absorption spectrophotometer. Odetokun (1996) determined Na and K by a flame photometer and the other minerals by atomic absorption spectrophotometry. Obizoba and Amaechi (1993) determined Ca, Zn, Cu, P and Fe by polarized Zeeman atomic absorption spectrophotometry.
The huge differences found may be due to the use of different methods, but may also have other causes.

2.5.3 Vitamins

No published reports on the vitamin content of the kernels were encountered.

2.5.4 Amino acids

Amino acid contents have been investigated in the whole seeds, but not specifically for the kernels.

2.5.5 Fatty acids

The data reported by Ajayi *et al.* (2003) and Odetokun (1996) show that the kernels are rich in oleic acid (26.1 to 58.2%) and linoleic acid (23.3% to 39.4%) as indicated in table 2.5. Moreover, Ajayi *et al.* (2003) mentioned 2.1% cerotic acid and 3.2% of other (unspecified) acids; in total, the seed oil contains 26.9% of saturated fatty acids and 73.1% of unsaturated fatty acids. It is recognized that an increased dietary intake of saturated fat and (to a lesser extent cholesterol), raises plasma/serum total and low-density lipoprotein (5LDL)-cholesterol, and of polyunsaturated fatty acids (PUFA) decreases these levels (Li and Sinclair, 2002). Because of its high proportion of unsaturated fatty acid, baobab kernels may have a positive effect on human health.

2.6 Some characteristics of seed oil

2.6.1 Sterols

The Baobab Fruit Company (www.baobabfruitco.com) collected data from literature and presented some results on sterols. Some data are also provided by Gaydou (1979). Table 2.6 shows that the seed oil contains 75-81% of β-sitosterol followed by campesterol (6-6.3%). β-sitosterol, campesterol and stigmasterol are the main sterols in plants and constitute bioactive compounds that can decrease the plasma/serum levels of lipids and lipoprotein lipids (Li and Sinclair, 2002).

2.6.2 Fatty acids

According to Osman (2004), baobab seed oil is an excellent source of monounsaturated and polyunsaturated fatty acids (Table 2.7a). The oil is composed of approximately 31.7% saturated fatty acids, 37% monounsaturated fatty acids, and 31.7% polyunsaturated fatty acids. The major fatty acid is oleic acid, which comprises 35.8%, followed by linoleic (30.7%) and palmitic (24.2%) acids. Reported by Gaydou (1979), saturated fatty acids accounted for 34.6% of the fatty acids in baobab seed oil. However, the genus *Adansonia* has been reported to contain a particularly large proportion of cyclopropenoic fatty acids in their seed oil. Baobab oil from *Adansonia* sp. was reported to contain 6.3% malvalic acid, 6.5% sterculic acid (12.8% total cyclopropenes). More specifically, *Adansonia digitata* seed oil contains 3.1-6.2% malvalic acid; 1.0-1.9% sterculic acid and then 4.1-8.1% total cyclopropenes (Table 2.7a) (Aitzetmüller, 1996). Cyclopropenoic fatty acids are toxic with a higher toxicity with sterculic acid compared to malvalic acid (Andrianaivo-Rafehivola *et al.*, 1994b). Biological effects are growth restriction and dysfunction of genital systems in chicken, rats and mice as well as an induction of liver cancer in hardhead (*Oncorhynchus mykiss*), when cyclopropenes are combined with aflatoxins (Andrianaivo-Rafehivola *et al.*, 1994b). Cyclopropenoic fatty acids, when present in an edible oil, have to be removed during refining to render the oil edible; Andrianaivo-Rafehivola *et al.*, (1994a) showed that heating at 180°C or 220°C reduces the cyclopropenoic fatty acid content of oil by 60% or 96%, respectively. This implies that locally produced baobab seed oil should be refined before consumption.

2.6.3 Indices

The specific gravity, refractive index at different temperatures as well as the iodine value, saponification value and other indexes are presented in table 2.7b. The reported values for the specific gravity do not vary much and have a value of approximately 0.9 at 25°C. The refractive index is in the range of 1.5. The iodine value varies from 49.5 (Ajayi *et al.*, 2003) to 88 (Osman, 2004).

2.7 Discussion and conclusion

2.7.1 Variation in reported data

This review shows that the reported values of nutrient contents of baobab parts vary greatly. The causes of these variations are not well known but several assumptions can be made. This variation may be due to the quality of the sample (mixture of samples, or samples obtained from markets or samples from individual trees), the provenance of the samples, the age of the sample, the treatment before analysis, the analytical methods used, the storage conditions, a genetic variation, and the soil structure and its chemical composition.

Table 2.5 Fatty acids content of the baobab kernels.

Authors	C12:0 lauric	C14:0 mystiric	C16:0 palmitique	C16:1 palmitoleic	C18:0 stearic	C18:1 oleic	C18:2 linoleic	C18:3 linolenic	C20:0 arachidic	C20:1 gadoleic	C22:0 behenic	C24:0 lignoceric
Ajayi *et al.* (2003)			4.43		3.98	26.07	39.4		2.26	4.01	3.46	10.7
(% of total fatty acid)												
Odetokun (1996) (% of total fatty acid)	0.34	1.46	2.22	1.65		58.71	23.3	8.2		3.64		

Table 2.6 Sterols in baobab seeds oil

Sterol Compostion	(Sidibe and Williams, 2002) (% of total sterol)	(Gaydou et *al.*, 1979) (% of total sterol)
Cholesterol	2	1.9
Campesterol	6	6.3
Stigmasterol	1-2	
β-Sitosterol	75	81
Δ5-Avenasterol	0.5	3.4
Δ7-Stigmasterol	0.6	4.8
Δ7-Avenasterol	12	0.6
Stigmasterol		2.0

Tables 2.7 Characteristics of baobab seed oil

(a) Fatty acids content of baobab seed oil

Authors	C14:0 mystiric	C16:0 palmitic	C16:1 palmitoleic	C16:2 Hexade cadienic	C18:0 stearic	C18:1 oleic	C18:2 linoleic	C18:3 linolenic	C20:0 arachidic	C20:1 gadoleic	C22:0 behenic	C24:0 lignoceric	malvalic	sterculic
Ezeagu *et al*. (1998) (g fatty acid/100 g oil)	0.19	15.5	0.20	0.70	3.12	25.4		1.97	0.74	0.19	0.36	0.31		
Osman (2004) (% of total fatty acid)	0.2	24.2			0.3	4.6	35.8	30.7	1.0	1.3	0.9	0.7		
Eteshola and Oraedu (1996) (% of total fatty acid)	38.4	19.7			3.2	22.4	16.2							
Gaydou *et al* (1979) (% of total fatty acids)		26.7				41.9	20.6							
Aizetmüler (1996) (%)													3.1-2.6	1.0-1.9

(b) Indices of baobab seed oil

Authors	Specific gravity	Refractive Index		Iodine value	Saponification value	Acid value	Free fatty acid as oleic acid	Peroxide value	Ester value	Density	Viscosity	Colour
	25/25°C	25°C	27°C									
Osman (2004)	0.9		1.5	88	210							
Ajayi *et al.* (2003)	0.85	1.46*		49.5	230	5.2	2.6	5.2	224.8	0.42	11.5	
Odetokun (1996)	0.94	1.46		82.4	133	7.8	6.5	5.1				Light yellow

*not sure about temp

The composition of a food can be influenced significantly by the environment such as soil type, fertilizer, water or sunlight intensity. For instance, Maranz *et al.* (2004) investigated the chemical composition of 42 populations of the Shea butter or Karité tree (*Vitellaria paradoxa)* in 11 countries and found very high variability in all measured parameters, both within and between populations. The mineral content of the soils needs to be considered when dealing with the mineral content of the plant. The variation found in the reported data on the composition of baobab parts may be partially explained by the fact that baobab trees grow on a wide range of soils, ranging from deep, consolidate sands to well-drained clayey soils and coal limestone.

If the biochemical composition of a food depends on its genetic make-up, variability can also be attributed to genetic factors. In Benin, for instance, a genetic variability has been identified for baobab populations by Assogbadjo *et al.* (2006) but the relation with the composition of the food products from these baobab populations is not yet known. However, in Mali, measurements of bulked fruit samples from many baobab trees consistently resulted in vitamin C values of around 220 mg/100 g. It was only when researchers measured bulked fruit from individual trees that a threefold range of values from 150 to 500 mg/100 g vitamin C was discovered (Anonymous, 1998). According to Diop *et al.* (2005), variations are obviously also due to the variability of the raw material (habitat, maturity, storage conditions of samples). Indeed, the investigated literature shows that the analyzed samples were selected and handled differently. For instance, some researchers purchased their baobab material from local markets, e.g. Nnam and Obiakor (2003), Lockett *et al.*(2000), Obizoba and Amaechi (1993) and Yazzie *et al.*(1994). In Mali for instance, it has been noticed that β-carotene and vitamin C levels were much lower in market samples than in any sample that was gathered directly from trees; market samples are frequently adulterated with worthless material such as cereal stalk pulp (Scheuring *et al.*, 1999, Anonymous, 1998). However, some authors like Soloviev *et al.* (2004) and Scheuring *et al.* (1999), collected their material in the field. In this case, the degree of maturity of the biological material matters, and is linked to the method of harvesting; fruit harvested by knocking on branches or cutting from the tree will invariably include unripe fruit. Such unripe fruits do not show any ascorbic acid content until the moisture of baobab pulp decreases below 75% (Carr, 1958). Moreover, the storage conditions before analysis differ; while Osman (2004) stored his samples at –20°C in tight plastic jars before analysis, Nnam and Obiakor (2003) boiled and dried the seeds before keeping them in Kilner jars for analysis. The form in which the product is analysed also matters (Joyanes and Lema, 2006). In the present literature review, it was observed that leaves, for example, were analysed after drying but that the method of drying (sun or shade for instance) was seldom specified. It was found in Mali that baobab leaves dried in the shade may contain twice as much Retinol Equivalent (pro-vitamin A) than sun-dried leaves, even though sun drying is the common local practice (Anonymous, 1998). The age of the samples may also induce some variability in their composition. In the case of vitamin C, for instance, pulp samples stored in clear glass bottles and partly exposed to sunlight showed 6.4 to 14.1% loss of ascorbic acid. Samples exposed to a longer storage (14 months), direct sunlight and exposure to air showed losses of up to 45.5% of ascorbic acid (Carr, 1958). In addition, an entire leaf consists of a petiole and a number of leaflets and it is often not specified whether the analyses concern leaflets or entire leaves.

Apart from the variability in the material, the analytical methods (sampling methods, analytical methods, analytical quality control) and inherent variability may also be a cause of variability. Considering seed oil, for instance, the method of extraction of the oil may affect its composition.

Moreover, some of the micronutrients, such as vitamins and minerals, are biologically active. They can interact with other nutrients and change in their bioavailability; because they are

biological material, there is a natural variation in their composition (Joyanes and Lema, 2006).
Our review shows that more attention must be paid to all stages involved in performing analyses before we can make reliable statements on the variation in composition of baobab food products.

2.7.2 Contribution to Recommended Daily Intake

Note: in the following calculation, digestibility and bioavailability could not be taken into account, because of lack of data. Therefore, the values given should be seen as maximum values; in reality they are probably lower.

Leaves

Without considering the conversion factor or the effect of processing, such as cooking, the consumption of 20 g of dry leaf material would cover 10 to 16% of the protein RDI for children (4-8 years). Considering the highest reported values, 20 g would be enough to cover 89% of the zinc RDI and 66% of the calcium RDI for children (Table 2.8). Similarly, consumption of 20 g will cover 53% of the calcium RDI and 41% of the zinc RDI for pregnant women when considering the highest reported values (Table 2.9). The large gap between the lowest and highest reported values of iron makes it difficult to make a reliable prediction for this mineral. A bioavailability study is necessary to obtain a reliable assessment. Finally, the introduction of baobab nurseries for leaf production is becoming increasingly popular. The nutritional composition and digestibility of these leaves also require investigation.

Pulp

Irrespective of the variation in the reported values, the data reveal the high vitamin C content of the pulp. A comparison with the Recommended Daily Intake (RDI) for children (4-8 years) and for pregnant women (19-30 years) is presented in tables 2.8 and 2.9, respectively.
A consumption of 20 g of pulp by a child (4-8 years) will cover 143% of the RDI, considering the lowest reported vitamin C content by the authors. If the highest reported vitamin C content is considered, the coverage will even be 240% for the same quantity. In other words, 13.9 g of pulp with the lowest reported vitamin C content and 8.3 g of pulp with the highest reported vitamin C content is enough to cover the RDI of such a child. Moreover, according to Carr (1955), the bulk of the vitamin C in baobab pulp is present in the reduced form (307 mg/100 g of reduced form out of 328 mg/100 g vitamin C). As the major metabolites of ascorbic acid in the human body are dehydroascorbic acid, 2,3-diketogulonic acid and oxalic acid (the reduced form of vitamin C), it can be concluded that about 93% of the vitamin C present in baobab pulp may be well absorbed.
The reported lowest and highest carbohydrate content of the pulp allows coverage of 21.5% and 40.6% of the RDI when 60 g is consumed by a child. The great variation in the reported iron, zinc and calcium contents renders it difficult to estimate the contribution of baobab pulp to the RDI of these minerals.

Table 2.8 Baobab pulp, leaf and kernel composition with the Recommended Daily Intakes (RDI) for individuals: children, 4-8 years

Nutrients	**Energy**		**Carbohydrates**		**Proteins**		**Ca**		**Zn**		**Fe**		**Vit C**	
RDI for children (4-8 years) (g/d)	6691 kJ/d	6691 kJ/d	130	130	19	19	0.8	0.8	0.005	0.005	0.01	0.01	0.025	0.025
Pulp composition (g/100 g)	highest*	lowest*	highest	lowest	highest	lowest	highest	lowest	highest	lowest	highest	lowest	highest	lowest
	1495 (kJ/100 g)	849 (kJ/100 g)	88	46.6	15.3	2.5	0.7	0.0029	0.0032	0.00053	0.01	0.001	0.3	0.2
% RDI covered by consumption of 20 g/d	4.5	2.5	13.5	7.2	16.1	2.6	17.5	0.1	12.7	2.1	20.8	2.0	240.0	143.2
% RDI covered by consumption of 40 g/d	8.9	5.1	27.1	14.3	32.2	5.2	35.0	0.1	25.4	4.2	41.5	4.0	480.0	286.4
% RDI covered by consumption of 60 g/d	13.4	7.6	40.6	21.5	48.3	7.7	52.6	0.2	38.2	6.4	62.3	6.0	720.0	429.6
% RDI covered by consumption of 100 g/d	22.3	12.7	67.7	35.8	80.5	12.9	87.6	0.4	63.6	10.6	103.8	10.0	1200.0	716.0
Leaf composition (g/100 g)	highest*	lowest*	highest	lowest	highest	lowest	highest	lowest	highest	lowest	highest	lowest	highest	lowest
	1581 (kJ/100 g)	1179 (kJ/100 g)	69	40.2	14.9	10	2.6	0.3	0.022	0.00074	0.3	0.0012	0.05	0.05
% RDI covered by consumption of 20 g/d	4.7	3.5	10.6	6.2	15.7	10.5	66.0	7.7	89.6	3.0	500.0	2.3	40.0	40.0
% RDI covered by consumption of 40 g/d	9.5	7.1	21.2	12.4	31.4	21.1	132.0	15.4	179.2	5.9	1000.0	4.7	80.0	80.0
% RDI covered by consumption of 60 g/d	14.2	10.6	31.8	18.6	47.1	31.6	198.0	23.0	268.8	8.9	1500.0	7.0	120.0	120.0
% RDI covered by consumption of 100 g/d	23.6	17.6	53.1	30.9	78.4	52.6	330.0	38.4	448.0	14.8	2500.0	11.7	200.0	200.0
Kernels composition (g/100 g)	one value		highest	lowest	highest	lowest	highest	lowest	highest	lowest	highest	lowest	-	-
	1966 (kJ/100 g)		48.1	22.1	32.7	14.0	0.0037	0.0004	0.0036	0.0001	0.0024	0.00063	-	-
% RDI covered by consumption of 20 g/d	5.9		7.4	3.4	34.4	14.7	0.1	0.0	14.3	0.4	4.8	1.3	-	-
% RDI covered by consumption of 40 g/d	11.8		14.8	6.8	68.8	29.5	0.2	0.0	28.6	0.8	9.6	2.5	-	-
% RDI covered by consumption of 60 g/d	17.6		22.2	10.2	103.3	44.2	0.3	0.0	42.8	1.2	14.3	3.8	-	-
% RDI covered by consumption of 100 g/d	29.4		37.0	17.0	172.1	73.7	0.5	0.1	71.4	2.1	23.9	6.3	-	-

* Highest and lowest values reported by different authors for nutrient composition of baobab pulp

Source: Recommended Daily Intakes for individuals for Energy: http://www.fnri.dost.gov.ph/reni/renitable1.htm, 06-03-2006
Source: Other Recommended Daily Intakes for individuals: http://www.iom.edu/CMS/3788.aspx, 07-03-2006

Table 2.9 Baobab pulp, leaf and kernels composition with the Recommended Daily Intakes (DRI) for pregnant women, 19-30 years

Nutrients	**Energy**		**Carbohydrates**		**Proteins**		***Ca***		**Zn**		**Fe**		**Vit C**	
RDI for pregnant women, 19-30 years (g/d)	9033 kJ/d	9033 kJ/d	175	175	71	71	1	1	0.011	0.011	0.027	0.027	0.085	0.085
Pulp composition (g/100 g)	highest	lowest	highest	lowest	highest	lowest	highest	lowest	highest	lowest	highest	lowest	highest	lowest
	1495 (kJ/100 g)	849 (kJ/100 g)	88	46.6	15.3	2.5	0.7	0.0029	0.0032	0.00053	0.01	0.001	0.3	0.2
% RDI covered by consumption of 20 g/d	3.3	1.9	10.1	5.3	4.3	0.7	14.0	0.1	5.8	1.0	7.7	0.7	70.6	42.1
% RDI covered by consumption of 40 g/d	6.6	3.8	20.1	10.7	8.6	1.4	28.0	0.1	11.6	1.9	15.4	1.5	141.2	84.2
% RDI covered by consumption of 60 g/d	9.9	5.6	30.2	16.0	12.9	2.1	42.1	0.2	17.3	2.9	23.1	2.2	211.8	126.4
% RDI covered by consumption of 100 g/d	16.5	9.4	50.3	26.6	21.5	3.5	70.1	0.3	28.9	4.8	38.4	3.7	352.9	210.6
Leaf composition (g/100 g)	highest	lowest	highest	lowest	highest	lowest	highest	lowest	highest	lowest	highest	lowest	highest	lowest
	1581 (kJ/100 g)	1179 (kJ/100 g)	69	40.2	14.9	10	2.6	0.3	0.022	0.00074	0.3	0.0012	0.05	0.05
% RDI covered by consumption of 20 g/d	3.5	2.6	7.9	4.6	4.2	2.8	52.8	6.1	40.7	1.3	188.1	0.9	11.8	11.8
% RDI covered by consumption of 40 g/d	7.0	5.2	15.8	9.2	8.4	5.6	105.6	12.3	81.5	2.7	376.3	1.7	23.5	23.5
% RDI covered by consumption of 60 g/d	10.5	7.8	23.7	13.8	12.6	8.5	158.4	18.4	122.2	4.0	564.4	2.6	35.3	35.3
% RDI covered by consumption of 100 g/d	17.5	13.1	39.4	23.0	21.0	14.1	264.0	30.7	203.6	6.7	940.7	4.3	58.8	58.8
Kernels composition (g/100 g)	one value	highest	lowest	highest	lowest	highest	lowest	highest	lowest	highest	lowest	-	-	
	1966 (kJ/100 g)	48.1	22.1	32.7	14.0	0.0037	0.0004	0.0036	0.0001	0.0024	0.00063	-	-	
% DRI covered by consumption of 20 g/d	4.4	10.1	5.3	9.2	3.9	0.1	0.01	6.5	0.2	1.8	0.5	-	-	
% DRI covered by consumption of 40 g/d	8.7	20.1	10.7	18.4	7.9	0.2	0.02	13	0.4	3.5	0.9	-	-	
% DRI covered by consumption of 60 g/d	13.1	30.2	16.0	27.6	11.8	0.2	0.03	19.5	0.6	5.3	1.4	-	-	
% DRI covered by consumption of 100 g/d	21.8	50.3	26.6	46.1	19.7	0.4	0.04	32.5	0.9	8.9	2.3	-	-	

* Highest and lowest values reported by different authors for nutrient composition of baobab pulp

Source: Recommended Daily Intakes for individuals for Energy: http://www.fnri.dost.gov.ph/reni/renitable1.htm, 06-03-2006
Source: Recommended Daily Intakes for individuals: http://www.iom.edu/CMS/3788.aspx, 07-03-2006

However, considering the highest reported values, the consumption of 40 g of baobab pulp is enough to cover 41.5% of the RDI for iron; 25.4% of the RDI for zinc, and 35% of the RDI for calcium. The energy content of the pulp is rather low when compared with the RDI for children.
A similar trend is observed for pregnant women (19-30 years). The consumption of 40 g of pulp by a pregnant woman will cover 84 to 141% of her RDI of vitamin C, considering the lowest and the highest vitamin C content of the pulp reported by authors. Moreover, a consumption of 100 g pulp will cover 26 to 50% of the carbohydrate RDI for pregnant women. As mentioned for the children, the coverage of iron, zinc, and calcium RDI is possible only when the highest reported values are considered for the pulp. Consumption of 60 g and 100 g would cover 23.1% and 38.4% of the RDI for iron; 17.3% and 28.7% of the RDI for zinc; 42.1% and 70.1% of the RDI for calcium; 30.2% and 50.3% of the RDI for carbohydrates, respectively. The energy content is also low for the RDI for pregnant women.
There is no doubt that baobab pulp is a valuable source of vitamin C. If an added value would be given to the pulp by improving its handling, its quality and storage stability using adequate processing methods, this might help to enhance interest about the pulp and lead to a better organization of this food chain in developing countries where the tree occurs and its food is well appreciated. At present, the preservation of the pulp, despite all its importance, is not properly controlled by the population, leading to undesirable losses. Subsequently, research is important to overcome problems in prolonging the shelf-life of the pulp in order to retain its nutritive value and sensorial properties. Bioavailability studies are necessary for a better appreciation of the contribution to human health since the dietary intake can never be fully utilized by the human body.

Kernels

The kernels are known for their high protein content. Consumption of 20 g can cover 15 to 34% of the protein RDI for children (Table 2.8), while for pregnant women 60 g can cover 27% of the RDI based on the highest reported content (Table 2.9). Moreover, consumption of 100 g can cover 22% of the energy RDI for pregnant women and 29.4% of energy RDI for children. Oil is extracted from the kernel and used for food and medicinal purposes. It is important to keep in mind that these data are related to the raw product and that further studies are required to evaluate the effect of cooking or other processing operations on the nutritive value of these products.

2.7.3 Antioxidant capacity of pulp and leaves

Consuming antioxidant-rich foods can contribute to the prevention of oxidation in the human cell, hence of some diseases. In addition to the general chemical composition of baobab pulp and leaves discussed previously, Vertuani *et al.* (2002) investigated their antioxidant capacity and compared this with that of other common fruits (Table 2.10), using the photochemiluminescence method. They indicated that the antioxidant property of the pulp (measured as the Integral Antioxidant Capacity -IAC- value) was 100 times higher than that of orange pulp. This antioxidant capacity may vary depending on the measuring method used, but the comparison with other fruits could still give similar trends. Cook *et al.* (1998) also investigated the antioxidant content of the aqueous extract of wild plants and found that baobab leaves have an antioxidant content of 7.7 µmol/g dw expressed as Trolox equivalents. This result is almost 1000 times lower than

the one reported by Vertuani *et al.* (2002), who found that the water-soluble antioxidant capacity of dry baobab leaves was 6.4 mmol Trolox equivalent/g.

These antioxidant activities were measured in fresh raw material and the effect of cooking and storage is not well known. Only Tarwadi and Agte (2005) investigated the antioxidative activity of some fruits and root vegetables before and after cooking. The antioxidant activity was measured as the inhibition of thiobarbituric acid reactive substances (TBARS), superoxide radical scavenging activity (SOSA), and ferrous iron chelating ability (FICA). They found that there were significant cooking losses for each of the assessed antioxidant parameters.

More research is needed on the antioxidative activity of baobab pulp and leaves during various processing operations, as well as the concentration of the related water soluble and fat-soluble compounds.

Table 2.10 Antioxidant capacity of baobab pulp compared to other fruits

Integral antioxidant capacity (IAC) corresponding to the sum of the corresponding water and lipid soluble antioxidants capacity	
Products	IAC (mmol Trolox equivalent/g fresh weight, uncooked portion)
Baobab Fruit pulp	11.1
Baobab Dry leaves	8.7
Baobab Fruit glycolic extract	1.02
Baobab Leaves glycolic extract	4.41
Kiwi fruit pulp	0.34
Orange fresh pulp	0.10
Strawberry fresh pulp	0.91
Apple fresh pulp	0.16

Source: Vertuani *et al*. (2002)

2.8 Concluding remarks

From this literature review on baobab pulp, leaves, whole seeds and kernels, it can be concluded that reported data show considerable variation although a fair number of investigations have been published about baobab products. We suggested some reasons to explain those variations. There is a lack of information on the vitamin content of baobab parts except for vitamin C. Whatever the variability, baobab pulp is apparently rich in vitamin C, the leaves are rich in good quality proteins and minerals and the kernels in fat. Most essential amino acids are present in the leaves. The pulp and the leaves also exhibit antioxidant activity. During future research, care should be taken to limit variability in collecting material, and in chemical analysis, and the data related to the environment of the samples should be described in detail. Further research is necessary to improve the quality and the shelf-life of the pulp, to investigate the antioxidant activity of the pulp and the leaves, to provide information about the bioavailability of macronutrients and micronutrients and the effects of cooking and other processing techniques on the overall nutritional value of the products in order to

improve present day practices as a measure to support the nutritional status of rural populations that incorporate baobab food products in their diet.

With respect to future research we recommend that:
1. More attention should be given to sampling, sample pretreatment, precision in analyses in order to get more reliable information about biological variation.
2. Nutritional research should focus on digestibility and bioavailability for a better nutritional evaluation of baobab products.
3. Detailed studies should be carried out on the effects of processing and storage on nutrient composition.

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Chapter 3

Indigenous Knowledge and Processing of *Adansonia digitata* L. Food Products in Benin

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Abstract

Indigenous knowledge related to baobab food products was investigated in Benin among 253 food processors from 15 ethnic groups. Descriptive statistics and correspondence analysis (CA) were used for data analysis. The following food categories were identified: dough, gruel, drinks (from pulp); sauces (from leaves, seeds and kernels); and flavouring agents (from kernels). CA showed that the food use of baobab parts varies among ethnic groups. Most ethnic groups have similar opinions about the difficulty of certain processing operations, in particular seed decortication, grinding and sieving operations for product recovery. Storage and preservation problems were mentioned for kernels and pulp.

Keywords: baobab, food uses, processing, storage, ethnic groups, Benin

3.1. Introduction

Among the numerous forest food resources available in the wild, the baobab tree (*Adansonia digitata* L.) is of key economic importance, being used daily by local populations in Africa (Wickens, 1982, Baum, 1995, Sidibe and Williams, 2002, Diop et al., 2005, Assogbadjo et al., 2006). It is characterized by its massive size, reaching to a height of 18-25 m, with a rounded crown and showing a stiff branching habit. Fruits are dehiscent, containing kidney-shaped seeds and powdery fruit pulp (Baum, 1995). A number of bilateral agencies promoted the species in the past, e.g. NORAD (Norway) in Kenya and SIDA (Sweden) in Tanzania. The World Agroforestry Centre (ICRAF) and the International Centre for Underutilized Crops (ICUC, UK) accorded high priority to research and development of baobab and continue to promote its use as a multipurpose species. Bioversity International classifies the species amongst the most important edible forest trees to be conserved, domesticated and valorized in Benin (IPGRI, 2002). National research efforts, especially in Benin, Nigeria, Burkina Faso, Mali and Senegal, have provided relatively recent data on agronomy, ethno-botanical knowledge, ecology and genetic diversity of baobab (Codjia et al., 2001, Assogbadjo et al., 2005a, 2005b, 2006, Diop et al., 2005). In Benin, studies on baobab are quite recent and relate to its general importance (Codjia et al., 2001, 2003) and ecological and genetic diversity (Assogbadjo et al., 2005a, 2005b, 2006). However, the ethno-food knowledge and economic potential of baobab for local populations are poorly documented whereas local populations have outstanding knowledge on the processing of several forest trees with edible parts such as shea tree (*Vitellaria paradoxa*) and the African locust tree (*Parkia biglobosa*) (Teklehaimanot, 2004).
There is a recent awakening of interest and concern about the lack of documentation about traditional and indigenous food cultures which are important not only for their own sake, but for the legacy of food knowledge which they can confer on future generations, provided that they are not lost; hence, the value of special focus on African food cultures (Wahlqvist, 2007). Effective valorization through improvement of traditional techniques and products, and production of added value products (*i.e.* with functional properties) for a larger market will increase income of rural, poor populations. Unfortunately, there is no sustained research capitalizing endogenous processing and food knowledge. Such research, however, is a prerequisite for any valorization and promotion of the products (Sidibe and Williams, 2002), and to better orient and prioritize further research. The present study aims at filling this gap.

3.2. Materials and Methods

3.2.1 Sampling of informants

First, a random check was performed on 198 processors offering their foods for sale on local markets to determine the proportion of processors of baobab food products. This proportion was used to compute the sample size N_i of baobab processors to be interviewed, using the following formula: $N_i = \frac{4pi(1-p_i)}{d^2}$, (Dagnelie, 1998) where N_i is the total number of processors to be surveyed for the study; p_i is the proportion of baobab processors among the 198 randomly checked persons; d is the expected error margin in the conclusion, which is fixed at 0.05 (Dagnelie, 1998). Next, the number of processors to be interviewed in each municipality was calculated on the basis of its

population size. If T is the proportion of the population of a community among the total number of people living in the study area, N, according to $T_j = \frac{n_j}{N}$ where n_j is the number of people in community j, then N_{ij} is the number of processors to be surveyed in community j, according to $N_{ij} = N_i \times T_j$.

3.2.2 Field data collection

Field data were collected from December 2006 to January 2007 to establish ethno-food knowledge related to baobab among different communities in Benin where baobab foods are commonly used. Questionnaires were used which were tested with local inhabitants prior to the formal survey, and adjusted if needed. Discussions were conducted in the villages of the selected localities, based on the adjusted questionnaires. Interviews were conducted in the language/dialect that was best understood by the informants with translation when necessary.

Table 3.1 Study localities and ethnic groups

Localities	Municipality	Ethnic groups
Badjoude	Waké	Lokpa
Dassa-zoume	Dassa-zoume	Datcha, Mahi, Fon
Dassari	Materi	Berba, Yom
Birni-Lafia	Karimama	Dendi
Kandi	Kandi	Bariba, Dendi, Peulh*
Karimama	Karimama	Dendi
Korontiere	Boukoumbe	Otamari, Comcombè*, Tchokossi*, Peulh*
Kouaba	Natitingou	Otamari
Koussoucoingou	Boukoumbe	Otamari
Natitingou	Natitingou	Otamari
Tanguieta	Tanguieta	Brouba, Wama
Tantega	Materi	Berba
Tayakou	Cobli	Gourmantche, Nateri

* Not native of that area

In total, 253 processors of baobab food products were interviewed. Informants (223 women and 30 men), from different ethnic groups and localities (Table 3.1, Figure 3.1) and of various ages (young, adults, elderly) were randomly selected and interrogated on baobab food uses, traditional processing technologies, specific problems related to baobab traditional processing, and preservation and storage problems of products.

3.2.3 Data processing and analyses

The collected data were recorded in a database. Statistical analyses were performed using SAS.v8 software (SAS Institute Inc.). The importance of processed food products from baobab parts was evaluated by asking the population to rank the derived products from the most (ranked 1) to the least important (ranked 5) considering each baobab part. Descriptive statistics and correspondence analysis (CA) were used as mathematical tools.

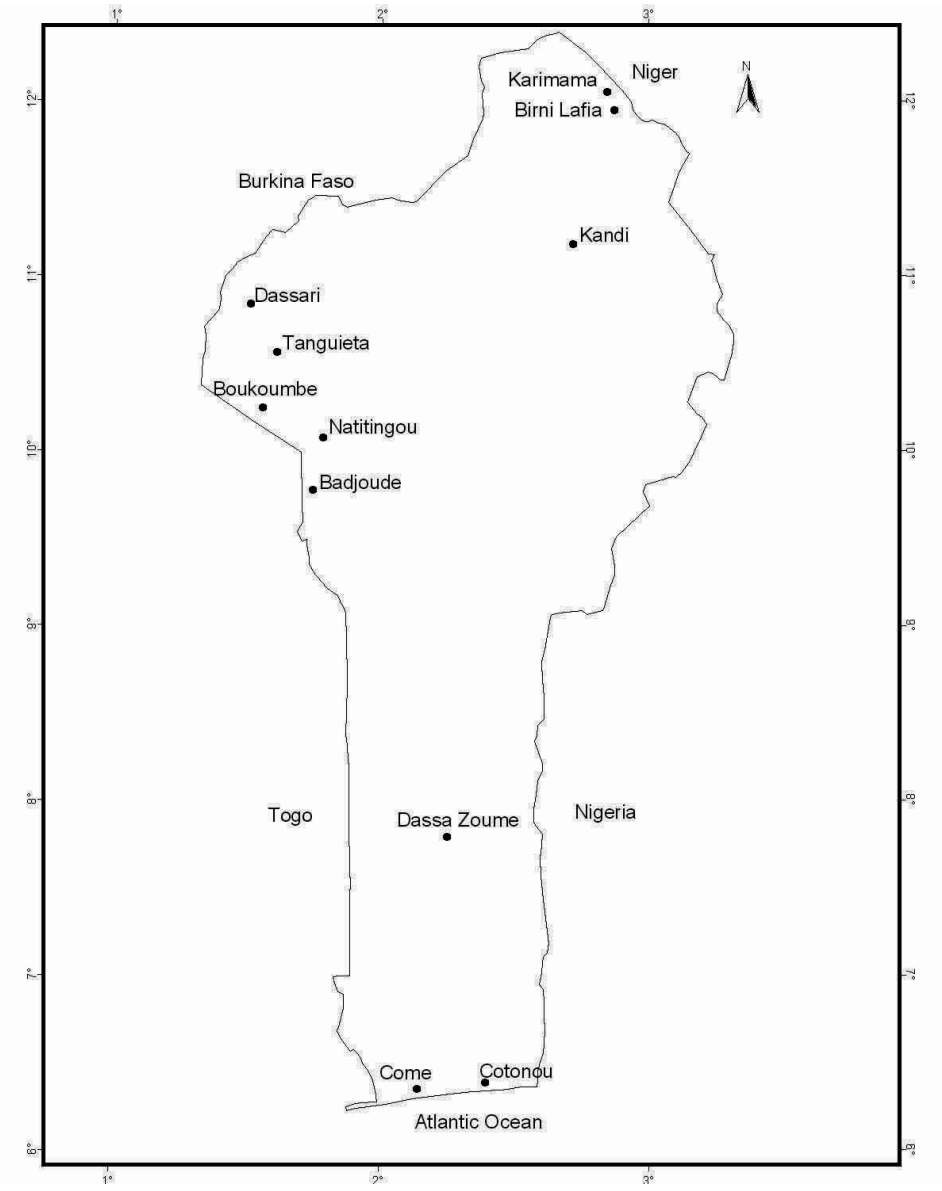

Figure 3.1 Map of Benin showing the municipalities included in the study

CA is similar to principal component analysis (PCA) but has more flexibility, since it inherently includes weights on both rows and columns of tables (Greenacre, 1993). This method was used to link socio-cultural groups according to local perception of various parameters. For CA, correlations and partial contributions of the modality under consideration allowed to obtain the best represented modalities on each axis. Projections of modalities on axes allowed to describe linkages between choices and perceptions of indigenous knowledge related to baobab food products by different ethnic groups.
Table 3.1 presents the ethnic groups encountered in each study locality, as shown in figure 3.1. The interpretation of CA graphs and tables was according to this table. In total, 15 ethnic groups were interviewed during the survey on baobab food processing.

3.3 Results

3.3.1 Food uses of baobab: importance and processing techniques

Table 3.2 presents an overview of the utilization, perceived importance and unit operations involved in the processing of a wide variety of baobab-derived products. According to local respondents, foods from baobab are mainly derived from the following baobab parts: leaves (27.5% of informants), pulp (27.2%), fruits (23.4%), seeds (14%), kernels (7.6%) and roots (0.3%). Each baobab part is used to prepare several types of foods with different importance for local people.

Food derived from baobab leaves

Leaves are used mainly for fresh leaf sauce, classified as the most important product from leaves (37% of informants), for dry leaf powder, classified as the second most important product from leaves (29%) and for dry leaves (14% of informants) (Table 3.2). Dry leaf powder and dry leaves are also used to prepare sauce. Ranking scores confirmed that sauce from fresh leaves is understood to be the most important leaf product and is ranked 1 while "*Kouimkoundi*" is the least important one, ranked 4. *Kouimkoundi* sauce is dry leaves sauce + "*Afitin",* a traditional fermented condiment from African locust bean (*Parkia biglobosa* (Jacq.) G.Don).
There are two main types of fresh leaves sauce (with or without added potash). In general, the fresh leaves are washed, ground (using mortar and pestle or millstone). The ground product is added to boiling water and cooked for approximately 5 min; during cooking, spices and animal protein (meat, fish) are added. In some cases, potash may be added as an ingredient.
To obtain dry leaf powder, fresh leaves are dried under shade or not, ground and sieved. The powder thus obtained is stored and used to make dry leaf powder sauce. However, fresh leaves are also dried without any grinding to obtain dry leaves that can be ground later when needed or used to make dry leaf sauce.
Yatirankounti sauce and *Touwoundou* sauce are quite similar. The difference is that *Yatirankounti* is made with whole baobab fresh leaves while *Kouimkoundi* is made with ground fresh or dry baobab leaves. In both cases, beans (*Vigna unguiculata*) are wet cleaned, dehulled and cooked. Whole fresh leaves (for *Yatirankounti*) or ground fresh or dry leaf (in the case of *Touwoundou*) are added and the mixture is cooked further. Flavouring agents such as spices and oil are then added to obtain the final product.

Table 3.2 Proportion and importance of baobab food products for baobab parts

Baobab parts	Processing products	Frequency of informants ranking the product according to their relative importance (%)	Importance of product (median rank)[1]	Other ingredients added	Main unit process operations
Leaves	Fresh leaves sauce	36.9	1	Flavouring agents	Grinding/milling, cooking
	Dry leaf powder	29.3	2	-	Drying, grinding, sieving
	Dry leaf sauce	3.4	2	Flavouring agents	Cooking
	Dry leaf	14.4	3	-	Drying
	Dry leaf powder sauce	3.9	3	Flavouring agents	Drying, cooking
	Yatirankounti sauce (whole fresh leaves+ dehulled beans)	1.5	3	Whole beans (*Vigna unguiculata*)	Beans dehulling, cooking
	Touwoundou sauce (ground dry or fresh leaves + dehulled beans)	8.7	3	Dehulled beans (*Vigna unguiculata*)	Beans dehulling, leaves grinding, cooking
	Kouimkoundi sauce (leaves sauce or touwoundou + afitin)	2.0	4	"Afitin"	Cooking
Pulp	Gruel	29.9	1	Maize or millet flour	Cooking
	Sour dough or *Mutchayan*[2]	9.1	2	Maize or millet flour	Dough cooking, fermentation
	Nanganfirou	8.8	2	Millet flour	Millet milling
	Yewowi beverage	10.6	2	Broken millet	Millet breaking/grinding
	Solani	0.2	2	Sugar	Cooking (facultative), freezing
	Pulp beverage	15.0	2	-	Pulp extraction
	Tcho beverage	10.0	2	Maize or millet dough	Dough cooking
	Iced pulp beverage	13.7	3	Sugar	Freezing
	Norendoorou	0.7	4	Locust bean pulp	Pulp extraction
	Baobab pulp syrup	0.7	4	Sugar	Cooking
	Moukou-moukou	1.4	5	Sugar	Pulp extraction

Baobab parts	Processing products	Frequency of informants ranking the product according to their relative importance (%)	Importance of product (median rank)[1]	Other ingredients added	Main unit process operations
Fruit	Pulp	40.7	1	-	Grinding, sieving
	Seeds	41.1	2	-	Grinding
	Potash	18.3	3	-	Incineration, filtration
Seeds	Kernels	42.1	1	-	Decortication
	Moutokpei sauce	25.3	2	Common sauce ingredients[3]	Roasting, grinding, cooking
	Matofaman sauce[2]	18.1	2	Common sauce ingredients[3]	Breaking, fermentation, sieving, cooking
	Mougoundoro	0.3	2	Salt, sugar	Roasting
	Batokoue sauce	10.9	2.5	Common sauce ingredients[3]	Grinding, sieving, cooking
	Powder from roasted seed	1.3	3	-	Roasting, grinding, sieving
	Dikouanyouri sauce[2]	0.7	3	Common sauce ingredients[3]	Cooking, soaking, grinding, fermentation, drying, milling
	Mougou-Mougou	1.3	4	Sugar	Roasting, grinding
Kernels	Kernels sauce	71.1	1	Common sauce ingredients[3]	Roasting, grinding, cooking
	Sarai sauce (kernels + dehulled beans)	16.3	2	Dehulled beans, cowpea leaves	Bean dehulling, kernels grinding, cooking
	Tayohounta type 2[2]	3.6	2	Locust beans	Locust beans decortication, cooking, fermentation
	Tayohounta type 1[2]	6.6	2	-	Cooking, fermentation
	Roasted Kernels	2.4	2.5	-	Roasting
Root	Potash	100.0	2	-	Incineration, filtration

[1] 1=most; 5= least important; [2] Fermented food; [3] Tomato, spices, animal proteins.

Foods derived from the fruit

The pulp is the most important product from the fruit and is ranked 1, followed by the seed ranked 2 and potash ranked 3 (Table 3.2). The pulp is a powdery product. Its extraction is achieved by dry or wet manual operations. After breaking the fruit, the whole content of the fruit (consisting of pulp, fibers and seeds) is crushed using mortar and pestle; the crushed product is sieved to separate the pulp from seeds and fiber. Wet pulp extraction is easier for the local population. Water is added to the whole content of the fruit, thereby dissolving the pulp in the water. A sieving process allows separation of the seeds and fiber. The liquid extract is used immediately.
The fabrication of potash requires incineration of baobab parts. Water is added to the obtained ash and the mixture is filtered through several layers of filtering material (*e.g.* wood, charcoal). The potash solution can be used as such for food purposes, or is concentrated and dehydrated to obtain solid potash.

Foods derived from baobab pulp

According to the interviewees, the most important food from baobab pulp is gruel, which is ranked 1, followed by sour dough (*Mutchayan*), and most beverages (*Nanganfirou, Yewowi*, pulp beverage, and *Tcho*) and *Solani*, which are ranked 2. The least important product is *Moukou-Moukou* (mixture of pulp and sugar), ranked 5 (Table 3.2).
Gruel is made from cereal (*i.e.* maize or millet) flour and baobab pulp. There are two techniques. In the first case, water is boiled and cereal flour is mixed with cold water. This mixture is subsequently added to the boiling water to make cereal gruel. This gruel is removed from the fire and diluted baobab pulp is added, and mixed to obtain baobab gruel. For the second method, baobab pulp is diluted in water and boiled, and diluted cereal flour is added to the boiling baobab juice to obtain the pulp gruel. The first method seems to be more convenient and may retain more nutrients in the gruel, since many nutrients are heat sensitive and may be destroyed while boiling the baobab juice.
To make sour dough, a fermented paste from baobab pulp, diluted baobab pulp is required. This can be obtained by soaking the content of baobab fruits in water or by diluting baobab pulp in water. The second important ingredient for sour dough is cereal (*i.e*. maize, millet or sorghum) dough (prepared with cereal flour and water). The cereal dough is mixed with diluted baobab pulp, or alternatively the diluted pulp is boiled and used to make dough with cereal flour. The mixture is put in a jar, covered and fermented for at least 24 h. This dough keeps on fermenting up to 7 days without any deterioration. After 5-7 days, part of this dough can be used as starter in the preparation of another sour dough. In this case, the starter will be mixed with the freshly prepared cereal dough and will be left again for 1 to 7 days to ferment like the previous one. This “back-slopping” technique is used mainly during periods of pulp shortage.
To prepare *Solani,* pulp is mixed with water, and heated with crystalline sugar until it turns brown. Sugar can also be added without heating and then there will be no brown colour. Sometimes, aroma (e.g. vanilla aroma bought from town) is added and the mixture is put in a top-freezer refrigerator (the generator of which is heated by kerosene) before consumption. One *tohoungolo* (*i.e.* 450 g) of pulp, 1 kg of sugar, 6 L of water and aroma are required for making this product.
Pulp beverage is obtained either by soaking the whole contents of the fruit in water to extract the drink from it, or by diluting baobab pulp in water. The consistency is adjusted according to consumer preference.

Various beverages can be prepared with a cereal to which baobab is added. *Nanganfirou* is a beverage made from millet flour and baobab pulp. Millet flour is added to diluted baobab extract and mixed. *Yewowi* beverage is made from broken/ground (using mortar and pestle) millet seeds and baobab pulp. The two ingredients are mixed and water is added. *Tcho* beverage is obtained by mixing cereal dough with water to which baobab pulp is added.
Iced pulp beverage is prepared by diluting baobab pulp in water, adding sugar and freezing the mixture. One volume of pulp for 5 volumes of water, and 1 kg of sugar are required.
Norendoorou is a beverage made from a mixture of baobab pulp, locust bean pulp and water.
Baobab pulp syrup is prepared by adding sugar and water to the pulp and boiling the mixture for 1 to 2 h to concentrate the syrup.
Moukou-Moukou is a dry mixture of baobab pulp and sugar.

Foods from the whole seeds

The most important products from the seeds are kernels ranked 1, followed by *Moutokpei* and *Matofaman* sauces ranked 2. *Batokoue* is ranked 2.5, *Dikouanyouri* sauce is ranked 3, while the least important product is *Mougou-Mougou,* ranked 4 (Table 3.2).

Kernels are obtained by decortication of the seed. Seed decortication is a tedious process comprising the following steps: cooking (for 4-6 h), soaking (in cooking water) and finally manual coat removal. An overcooking or undercooking may render the decortication nearly impossible. Seed coats need to be removed from individual seeds to obtain the kernels. This fastidious operation requires time and experience. If seeds are difficult to decorticate, a mild roasting and boiling process before seed coat removal may follow the soaking process.
For *Moutokpei* sauce, whole seeds are roasted, ground and added to boiling common sauce (*i.e.* tomato or spiced sauce). The mixture is further boiled for a few minutes to obtain *Moutokpei* sauce.
Matofaman sauce is a sauce from fermented baobab seeds. The seeds are broken using mortar and pestle and water is added to the broken seeds. The mixture is exposed to the sun for 24 to 72 h and then sieved. The liquid thus obtained is used as the basis to prepare *Matofaman* sauce. Usually some of the fermented liquid is kept and used later as starter for the fermentation of newly broken seeds. This will shorten the fermentation time to a maximum of 12 h.
To make *Batokoue* sauce, seeds are roasted, ground, sieved and the powder obtained is added to common sauce (tomato or spiced sauce), further cooked for 5 min to obtain *Batokoue* sauce. *Dikouanyouri* sauce is usually made from seeds that cannot be decorticated. When after cooking and soaking the seeds, decortication turns out to be impossible, the cooked seeds are fermented for 72 h, ground (using mortar and pestle), put in a pot, mixed with potash and further fermented for 24 h. The fermented paste is then dried and preserved as such. To make sauce, the powder is both used directly in common sauce (tomato or spiced sauce), or ground further (on a millstone), mixed with water and sieved. The liquid obtained is used to make *Dikouanyouri* sauce.
To obtain *Mougoundoro*, baobab seeds are roasted, ground, sieved and salt and sugar are added to the obtained flour. This mixture is used as a snack. *Mougou-Mougou* is made of a mixture of powder from roasted seeds and sugar. It is quite similar to *Mougoudoro* (*Mougoudoro* = *Mougou-mougou* + salt).

Foods from the kernels

Baobab kernels are used for kernel sauce, the most important product from kernels (71% of informants), with a median rank of 1. Other foods from kernels are: *Sarai* sauce, *Tayohounta* type 2 (from locust bean + baobab kernels) and *Tayohounta* type 1 (from baobab kernels only) ranked 2, and finally the least important kernel product is roasted kernels with a median rank of 2.5.
Kernel sauce is prepared by optional roasting of the kernels, followed by grinding; the resulting product is used as protein concentrate in tomato or spiced sauce (Table 3.2). *Sarai* sauce is made from dehulled locust beans, cowpea (*Vigna unguiculata* (L.) Walp.) leaves and roasted baobab kernels. The locust beans are wet cleaned, dehulled and cooked; baobab kernels are roasted and ground; cowpea leaves are added to the ground baobab kernels and cooked dehulled locust beans. The mixture is cooked further with addition of spices and potash to obtain the *Sarai* sauce.
Tayohounta type 1 is a fermented product made from baobab kernels only. The kernels are roasted and further cooked for approximately 30 min, drained and packed in a container covered with plant leaves. They are left for 48 h of fermentation and further sun dried. The obtained product is shaped, usually sun dried and used as flavouring agent in sauce. *Tayohounta* type 2 is made from a mixture of baobab kernels and locust bean kernels. Locust beans are cooked, decorticated and mixed with baobab kernels. Both are boiled for 45-60 min, drained off, spread out over a clean surface and covered. Next, the mixture is left to ferment for 48 h and then sun dried, ground and shaped. The shaped *Tayohounta* type 2 may also be sun dried to increase its shelf life. *Tayohounta* is a pungent, nutritious spice or condiment used in sauces and stews.

Foods from baobab roots

Potash is the only food ingredient made from baobab roots and is thus the most important one, ranked 1. Potash solution is made with the ash from incineration of the roots, as was described in the section “Foods derived from the fruit”.

3.3.2 Relationship between final food uses and socio-cultural groups

Some ethnic groups have similar uses of parts of the baobab tree, while others differ. The result of the correspondence analysis (CA) (Table 3.3, Figures 3.2a and 3.2b) performed on the final food uses and socio-cultural groups showed that the first three axes explained 55.2% of the observed variation. Partial correlations and contribution of each of the considered modalities allow identification of the socio-cultural groups and the end uses that are the best represented on each axis. The projection of the different modalities in the axes system shows that the food uses of baobab parts are specific for the ethnic groups.

Considering axis 1 (Table 3.3, Figure 3.2a), Berba, Wama, and Yom ethnic groups use baobab pulp to make *Yewowi* (P36 = product n°36), *Tcho* (P35) and *Nanganfirou* (P10) beverages. These ethnic groups, especially the women, process leaves in dry leaf (P5), dry leaf sauce (P22), and *Touwoundou* sauce (P28). Kernels are mainly used for *Sarai* sauce (P27). By contrast, Otamari and Tchocossi ethnic groups, especially adult and elderly people, use baobab pulp for making sour dough/*Mutchayan* (P12), pulp drink (P3) and baobab syrup (P30). In these ethnic groups, seeds are used for making *Matofaman* (P17), *Batokoue* sauce (P19), and *Dikouanyouri* (P20) especially from the seeds that cannot be decorticated.

Specifically, the Otamari people use baobab kernels mixed with locust beans for making *Tayohounta* type 2 (P34) and *Mougoun-Mougoun* (P7).
With respect to axis 2 (Table 3.3, Figure 3.2a), the following ethnic groups: Datcha, Mahi (locality of Dassa), Otamari, Tchocossi, Comcombè, Dendi, and Lokpa have quite similar uses of some baobab parts; they use baobab fruits for making potash (P13). Apart from Datcha and Mahi, who use the whole fruit to make potash, all other ethnic groups use the empty fruit shell as raw material to make potash. In addition, the other ethnic groups (except Datcha and Mahi) produce baobab pulp (P16) from the fruits; they use pulp for making iced pulp beverage (P32) and they use leaves to produce and prepare dry leaf powder (P14), fresh leaves sauce (P21), and dry leaf powder sauce (P26). Specifically, Bariba use the pulp for *Solani* (P31) production; young people from the Dendi ethnic group produce *Moukou-Moukou* (P9) from pulp; the Dendi from Karimama locality produce *Mougoundoro* (P8) from whole seeds; Otamari, Tchocossi and Comcombè ethnic groups use seeds for making kernels (P1), kernels for making *Tayohounta* type 1 (P33) and roasted kernels (P2), and leaves to make *Yatirankounti* sauce (P29).
Regarding axis 3 (Table 3.3, Figure 3.2b), it can be observed that the Gourmantche ethnic group produces roasted seed powder (P15); and the Nateri use leaves for *Kouimkoundi* sauce (P23); the Wama ethnic group uses pulp to make *Norendoorou* beverage (P11). Most ethnic groups (Berba, Brouba, Gourmantche, Dendi, Peulh, Otamari and Wama), produce seeds (P6) from the fruits, *Moutopkei* sauce (P24) from the seeds, gruel from the pulp and, with the exception of the Dendi, produce kernel sauce (P18) from the kernels.

Table 3.3 Correspondence Analysis to reveal linkages between end uses and socio-cultural groups

Axis 1		**Axis 2**		**Axis 3**	
Products	Groups	Products	Groups	Products	Groups
P3	Berba	P1	Bariba	P4	Berba
P5	Otamari	P2	Berba	P6	Brouba
P7	Tchocossi	P9	Comcombè	P10	Dendi
P8	Wama	P12	Datcha	P11	Gourmantché
P10	Old	P13	Dendi	P15	Nateri
P12	Yom	P14	Lokpa	P18	Peulh
P17		P16	Mahi	P21	Wama
P19		P17	Otamari	P23	
P20		P19	Tchocossi	P24	
P22		P21	Wama	P27	
P27		P26	Women		
P28		P29	Men		
P30		P31	Young		
P34		P32	Adult		
P35		P33			
P36		P35			
		P36			

Legend: P=Product; P1=Kernels; P2 = Roasted kernels; P3 = Pulp drink; P4 = Gruel; P5 = Dry leaf; P6 = Seeds; P7 = Mougou-Mougou; P8 = Mougoundoro; P9 = Moukou-Moukou; P10 = Nanganfirou; P11 = Norendoorou; P12 = Sour dough; P13 = Potash; P14 = Dry leaf powder; P15 = Powder from roasted seeds; P16 = Pulp; P17 = *Matofaman* sauce; P18 = Kernels sauce; P19 = *Batokoue* sauce; P20 = *Dikouanyouri* sauce; P21 = Fresh leaves sauce; P22 = Dry leaf sauce; P23 = *Kouimkoundi* sauce; P24 = *Moutokpei* sauce; P26 = Leaf powder sauce; P27 = *Sarai* sauce; P28 = *Touwoundou* sauce; P29 = *Yatirankounti* sauce; P30 = Baobab syrup; P31 = Solani; P32 = Iced pulp beverage; P33 = *Tayohounta* type 1; P34 = *Tayohounta* type 2; P35 = Tcho; P36 =Yewowi

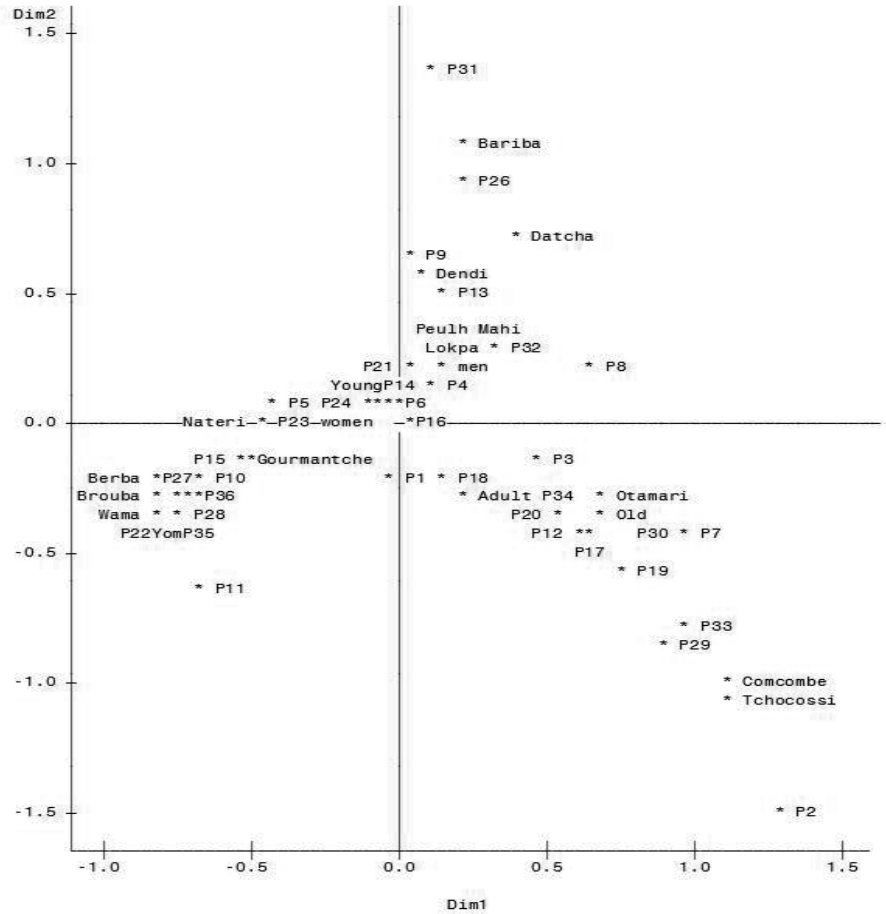

Figure 3.2a Correspondence Analysis to reveal linkages between Baobab uses and socio-cultural groups on axes 1 and 2
The figure shows the linkages between the baobab food uses by different ethnic groups best represented on axes 1 and 2 of Correspondence Analysis. Ethnic groups with similar uses are grouped together and with the uses with respect to the two axes. Groups with different uses are opposed.

Legend: P = Product; P1=Kernels; P2 = Roasted kernels; P3 = Pulp drink; P4 = Gruel; P5 = Dry leaf; P6 = Seeds; P7 = *Mougou-Mougou*; P8 = *Mougoundoro*; P9 = *Moukou-Moukou*; P10 = *Nanganfirou*; P11 = *Norendoorou*; P12 = Sour dough; P13 = Potash; P14 = Dry leaf powder; P15 = Powder from roasted seeds; P16 = Pulp; P17 = *Matofaman* sauce; P18 = Kernels sauce; P19 = *Batokoue* sauce; P20 = *Dikouanyouri* sauce; P21 = Fresh leaves sauce; P22 = Dry leaf sauce; P23 = *Kouimkoundi* sauce; P24 = *Moutokpei* sauce; P26 = Leaf powder sauce; P27 = *Sarai* sauce; P28 = *Touwoundou* sauce; P29 = *Yatirankounti* sauce; P30 = Baobab syrup; P31 = Solani; P32 = Iced pulp beverage; P33 = *Tayohounta* type 1; P34 = *Tayohounta* type 2; P35 = Tcho; P36 =Yewowi

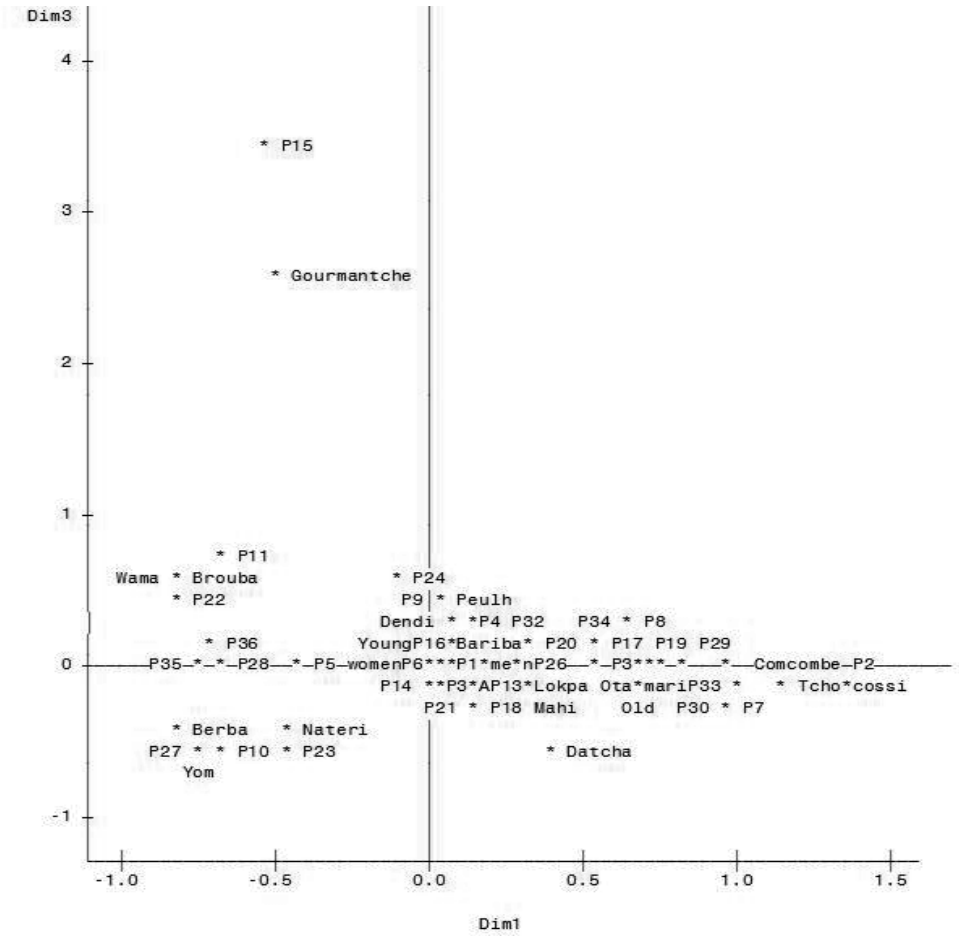

Figure 3.2b Correspondence Analysis to reveal linkages between baobab uses and socio-cultural groups on axis 3

The figure shows the relationships between the baobab food uses by different ethnic groups best represented on axis 3 of the CA. The information on axis 3 is used to complete the ones on axes 1 and 2 in figure 3.2a. Ethnic groups with similar uses are grouped together and with the uses with respect to axis 3. Groups with different uses are opposed.

Legend: P = Product; P1 = Kernels; P2 = Roasted kernels; P3 = Pulp drink; P4 = Gruel; P5 = Dry leaf; P6 = Seeds; P7 = Mougou-Mougou; P8 = Mougoundoro; P9 = Moukou-Moukou; P10 = Nanganfirou; P11 = Norendoorou; P12 = Sour dough; P13 = Potash; P14 = Dry leaf powder; P15 = Powder from roasted seeds; P16 = Pulp; P17 = *Matofaman* sauce; P18 = Kernels sauce; P19 = *Batokoue* Sauce; P20 = *Dikouanyouri* Sauce; P21 = Fresh leaves sauce; P22 = Dry leaf sauce; P23 = *Kouimkoundi* Sauce; P24 = *Moutokpei* Sauce; P26 = Leaf powder sauce; P27 = *Sarai* sauce; P28 = *Touwoundou* sauce; P29 = *Yatirankounti* sauce; P30 = Baobab syrup; P31 = Solani; P32 = Iced pulp beverage; P33 = *Tayohounta* type 1; P34 = *Tayohounta* type 2; P35 = Tcho; P36 =Yewowi

Explanation

If an ethnic group is present on more than one axis, it means that this ethnic group is compatible with the criteria that were explained by those axes. In our case, Berba, Otamari,

Tchocossi and Wama are characterized by the products listed in axis 1 (Table 3.3) for their uses of baobab food products. However, we can see in the graph which axis that ethnic group is more compatible with. Some ethnic groups may be grouped together on the graph if they have similar uses and opposed otherwise. In this case, looking at the graph (Figure 3.2a), Berba, Wama and Yom are opposed to Otamari and Tchocossi for axis 1. This does not exclude the fact that the same ethnic groups have other uses that could be in axis 2 or 3.
Each figure has two axes. Parallel lines represent the same axis. On figure 3.2a axis 1 (dim1) is horizontal and axis 2 (dim2) is vertical. On figure 3.2b, axis 1 is the same, i.e. horizontal, whereas axis 3 is vertical.

3.3.3 Constraints in processing operations according to socio-cultural groups

CA of cumbersome processing operations and socio-cultural groups (Table 3.4, Figure 3.3), shows that with the first two axes, 71.1% of the collected information was explained. Correlations and partial contributions of each modality allowed identifying the socio-cultural groups that are best represented on each axis. The projection of the different modalities in the axes system and their interpretation showed that most ethnic groups find similar operations difficult.

Table 3.4 Correspondence Analysis to reveal linkages between cumbersome processing operations and socio-cultural groups

Axis1		**Axis 2**	
Operation	Groups	Operation	Groups
Op1	young	Op1	Bariba
Op2	Adult	Op2	Dendi
Op4	Old	Op3	Gourmantche
Op5	Women	Op6	Otamari
Op7	Men	Op8	Peulh
	Bariba	Op9	Wama
	Berba		
	Brouba		
	Dendi		
	Lokpa		
	Natéri		
	Tchokossi		
	Yom		

Legend: Op = Operation; Op1 = Fruit breaking; Op2 = Leaves harvesting; Op3 = Fruits harvesting; Op4 = Seeds decortication; Op5 = Leaves grinding; Op6 = Seeds grinding; Op7 = Grinding of the fruit content; Op8 = Pulp sieving; Op9 = Sieving of dry leaf powder

Considering axis 1 (Figure 3.3), all classes of populations from Brouba, Berba, Lokpa, Yom, Tchokossi and Nateri ethnic groups perceive seed decortication (Op4 = Operation n° 4) as a very cumbersome processing operation; these ethnic groups produce kernels from the seeds. In fact, individual seeds are decorticated manually after a long cooking process, followed by soaking. In contrast, the Gourmantche perceive the grinding of the fruit content (Op7) – to separate pulp, seed and fiber before sieving - as a difficult operation, while the Bariba mentioned leaf harvesting (Op2) as a hard operation. Indeed, the baobab tree is very big and high, and climbing sometimes causes accidents. In addition, the Dendi and Peulh consider dry leaf grinding (Op5) a difficult operation.

Considering axis 2 (Figure 3.3), Otamari and Wama also consider fruit breaking (Op1), seed grinding (Op6) and pulp sieving (Op8) - for removal of fibers and seeds - as difficult processing operations. In fact, the shell of the fruit is very hard and requires some strength to break, but the most difficult is that the fruit is covered with short hairs and, during breaking, these get into contact with the human skin and cause itching.

During grinding and sieving of seeds and pulp, a substantial amount of product is lost due to air currents. Dendi and Peulh ethnic groups also mentioned fruit harvesting (Op3), and sieving of dry leaf powder (Op9) as problematic operations.

3.3.4 Storage problems according to socio-cultural groups

CA of storage and preservation problems and socio-cultural groups (Table 3.5, Figure 3.4) showed that 79.2% of the collected information was explained with the first two axes. Correlations and partial contributions of each modality allowed identifying the socio-cultural groups and the problems that are best represented on each axis. The projection of the different modalities in the axes system and its interpretation showed that the recorded storage time varies from one ethnic group to another depending on the storage circumstances (*e.g.* packaging, humidity, drying frequency).
Considering axis 1 (Figure 3.4), Lokpa, Gourmantche, and Peulh ethnicities, especially young persons, found that after 6 months, insect larvae invade the pulp if it is exposed to humidity (Prob 10 = Problem n°10). Similarly, the Comcombè mention the development of insect larvae in the kernels after 6 months, if they are exposed to humidity and not regularly dried (Prob 9). In contrast, the Berba mentioned that particularly roasted kernels are usually attacked by mice (Prob 1) and that insects invading the pulp render it sticky (Prob 11) and unacceptable for consumption.

Considering axis 2 (Figure 3.4), Otamari and Tchokossi, especially adult women, mentioned insect larvae invading the pulp after 3 months if exposed to humidity (Prob 7), discolouration of pulp after 6 months if exposed to humidity, or after 1 year if kept dry. They also mentioned that insect larvae invade kernels after 1 month if not regularly dried or if exposed to humidity (Prob 6) or after 1 year if protected against humidity (Prob 5). In addition, mice attack of kernels (Prob1) was mentioned by the Wama.

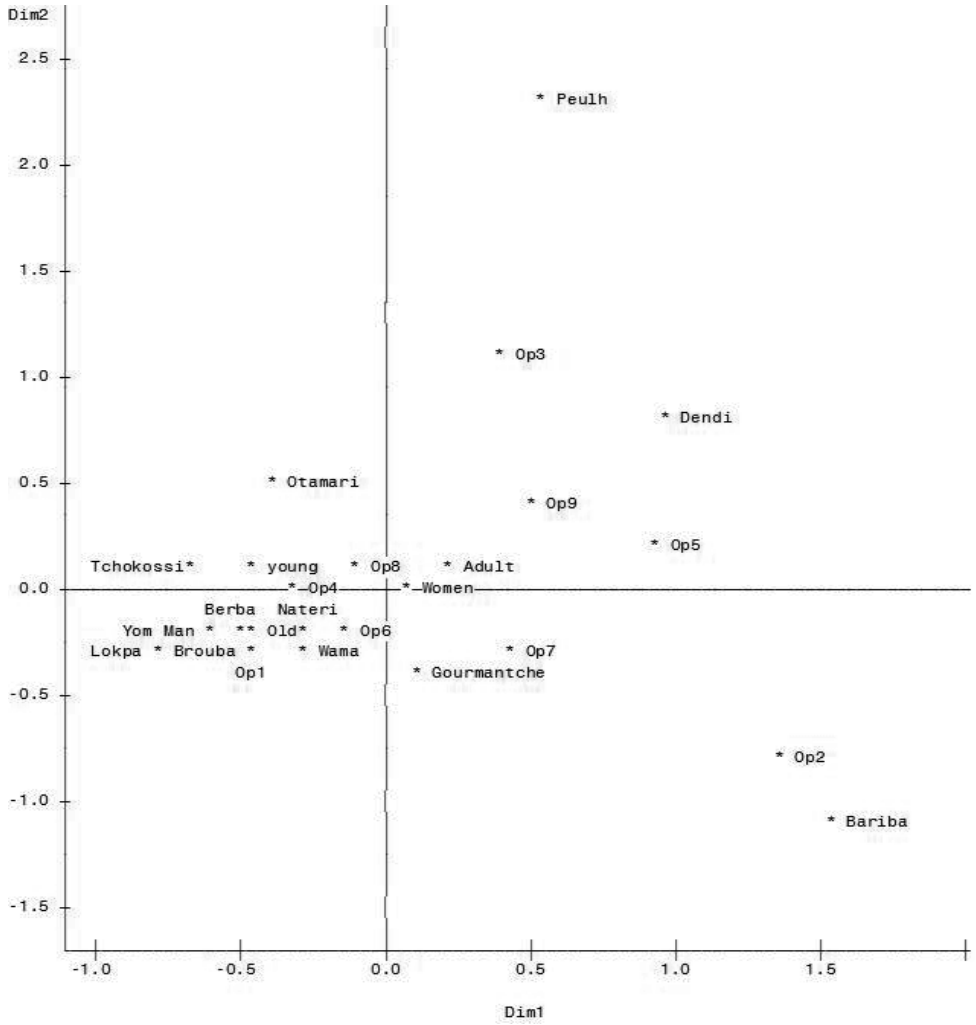

Figure 3.3 Correspondence Analysis to reveal linkages between difficulty of processing operations and socio-cultural groups on the axes 1 and 2
The figure shows correlations between ethnic groups and fastidious processing operations. Ethnic groups that found specific processing operations difficult are grouped together and with the operations.

Legend: Op = Operation; Op1 = Fruit breaking; Op2 = Leaves harvesting; Op3 = Fruits harvesting; Op4 = Seeds decortication; Op5 = Leaves grinding; Op6 = Seeds grinding; Op7 = Grinding of the fruit content; Op8 = Pulp sieving; Op9 = Sieving of dry leaf powder

Table 3.5 Correspondence Analysis to reveal linkages between baobab food storage problems and socio-cultural groups.

Axis1		**Axis2**	
Problems	Groups	Problems	Groups
Prob1	Young	Prob1	Adult
Prob9	Women	Prob2	Old
Prob10	Men	Prob3	Berba
Prob11	Berba	Prob5	Otamari
	Comcombè	Prob6	Tchokossi
	Gourmantché	Prob7	Wama
	Lokpa	Prob8	
	Peulh		

Legend: Prob = Problem ; Prob1 = Mice attack kernels; Prob 2 = Pulp changes colour after 1 year protected against humidity; Prob 3 = Pulp changes colour after 6 months if exposed to humidity; Prob 5 = Appearance of insect larvae in kernels after 1 year if protected against humidity; Prob 6 = Appearance of insect larvae in the kernels after 1 month if exposed to humidity; Prob 7 = Appearance of insect larvae in the pulp after 3 months if exposed to humidity; Prob 8 = Appearance of insect larvae in the kernels after 3 months if exposed to humidity; Prob 9 = Appearance of insect larvae in the kernels after 6 months if exposed to humidity; Prob 10 = Appearance of insect larvae if the pulp after 6 months if not regularly dried Prob 11 = Insects attack pulp rendering it sticky.

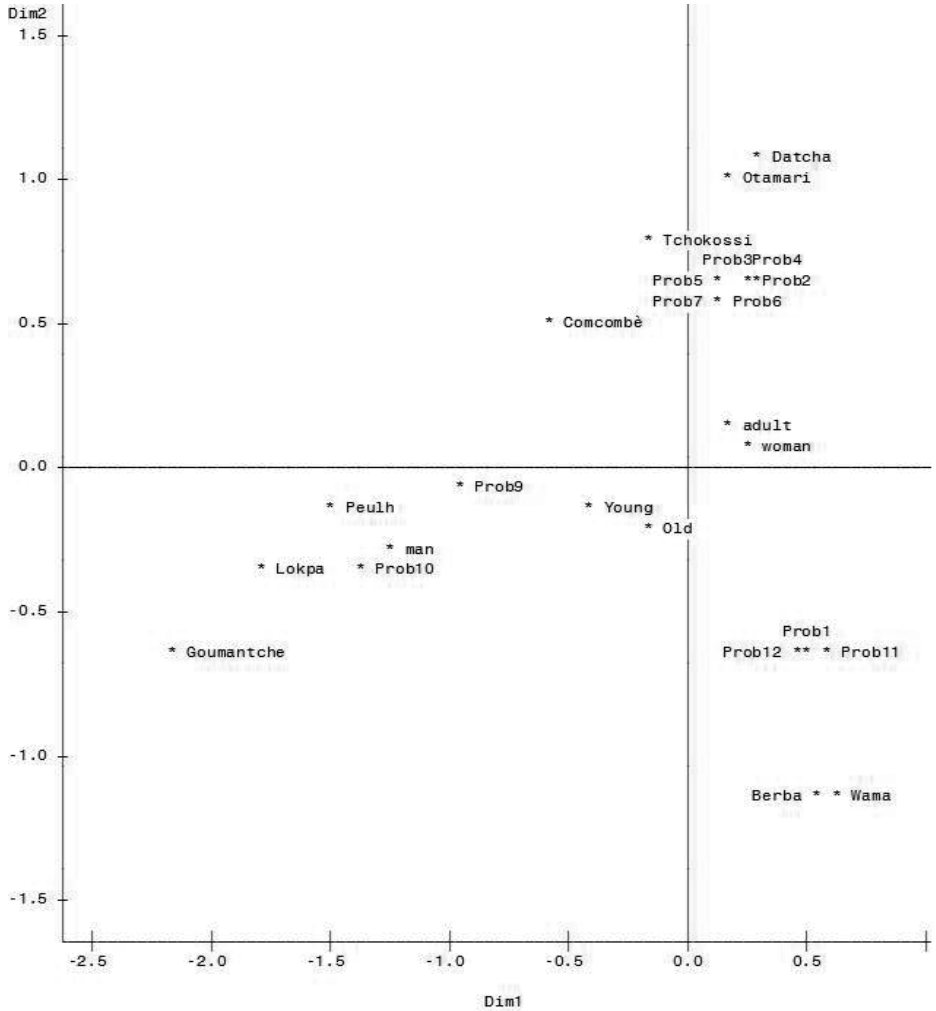

Figure 3.4 Correspondence Analysis to reveal linkages between storage problems and socio-cultural groups represented on each axis
The figure shows that socio-cultural groups that encountered similar storage problems are grouped together and with the problems.

Legend: Prob = Problem ; Prob1 = Mice attack kernels; Prob 2 = Pulp changes colour after 1 year protected against humidity; Prob 3 = Pulp changes colour after 6 months if exposed to humidity; Prob 5 = Appearance of insect larvae in kernels after 1 year if protected against humidity; Prob 6 = Appearance of insect larvae in the kernels after 1 month if exposed to humidity; Prob 7 = Appearance of insect larvae in the pulp after 3 months if exposed to humidity; Prob 8 = Appearance of insect larvae in the kernels after 3 months if exposed to humidity; Prob 9 = Appearance of insect larvae in the kernels after 6 months if exposed to humidity; Prob 10 = Appearance of insect larvae if the pulp after 6 months if not regularly dried Prob 11 = Insects attack pulp rendering it sticky

3.4 Discussion and Conclusion

3.4.1 Preferred end uses of baobab food products

The end uses of baobab parts are quite specific for the ethnic groups interviewed and we observed differences in the processing techniques for similar products among these ethnic groups. Over time, socio-cultural groups have accumulated a rich knowledge on the use of baobab. Over the centuries, various peoples have been using baobab for a variety of purposes and have gained rich experience and knowledge in processing techniques, but mostly with reference to their locality of origin. Moreover, ethnic groups are located in specific localities; the combination of an ethnic group and its locality may be determinant for their food uses since food practices are usually cultural. These findings agree with those by Nguyen (2003) who compared knowledge on 10 traditional Vietnamese fruits and vegetables between urban Vietnamese living in Vietnam and Hawaii; he found that Vietnamese immigrants in Hawaii listed more food uses than those in Vietnam due to adoption of multi ethnic foods found in Honolulu. According to Wahlqvist (2007) food culture is influenced most by the locality of its origin, which will have been one of food acquisition and processing by various means. Rivera et al. (2007), using hierarchical cluster analysis on gathered food plants in the mountains of Castilla-La Mancha in Spain, found that clusters of food plants species form culture-specific logical entities, which allow people to structure and manage their environment.

3.4.2 Processing, preservation and storage problems

Seed decortication appears to be one of the most difficult processing operations. In fact, it is done manually for individual seeds, after a long cooking process followed by soaking. It is a tedious operation and is mainly handled by women in several communities. Because many seeds are very hard to decorticate, a large amount of seeds is thrown away. Some seeds cannot even be decorticated, whatever the cooking and soaking time. It is difficult to visually distinguish seeds that can be decorticated from those which are not decorticable. A mechanical seed decortication method would significantly contribute to the use of seeds as a source of food. The baobab seed coat is waxy, making it slightly elastic (Danthu et al., 1995) and therefore difficult to break compared to other common seeds.
Thus, a sustainable solution is required for seed decortication for improving the livelihood of local populations. Finding a solution for such an operation through research activities resulting in a semi-mechanization of this processing operation, as wished by local populations, will save time for women involved in that activity. The saved time is likely to be spent on other remunerative activities resulting in a higher income that can *e.g.* be spent on education of children supporting their development.
An advantage of the cooking and soaking process (in hot water) before decortication might be the inactivation of antinutritional factors in baobab whole seeds (*e.g.* Trypsin Inhibitor Activity of 5.7 TIU/mg sample, 73 mg/100 g of phytic acid and 23% catechin equivalent of tannin) (Osman, 2004). As indicated by Igboeli et al. (1997) cold water, hot water, hot alkali and acid treatments reduced the tannic acid concentration in baobab whole seeds significantly, and the activity of amylase inhibitors in whole seeds was reduced significantly by dehulling (from 35 to 10 µg/ 100 g) and cold water treatments. The aspect of antinutritional factors merits further investigation.
The preparation of food ingredients such as pulp and leaf powder also faces problems. It requires grinding (with mortar and pestle), generally followed by a sieving process for removal of fiber/seed. These two operations are made difficult by air currents that take away part of the product. For pulp extraction, Baobab Fruit Company

(http://www.baobabfruitco.com) claims to have developed a mechanical process. Extension of this mechanical process may be beneficial, if adequate to local realities; otherwise, it would be interesting to investigate how this operation can best be facilitated for rural local populations.

Apart from separation processes, some storage and preservation problems were recorded, mainly for the kernels and the pulp. Infestation with insect larvae during storage (according to some ethnic groups) is the main problem for kernel preservation, in addition to attack by mice. For the pulp, the two main storage problems are insect larvae infestation and changes in colour. Most of these problems are related to exposure to humidity and consequently depend on the quality of packaging material and the packaging techniques (Ros-Chumillasa et al., 2006). Moreover, apart from humidity, exposure to light and oxygen may affect storage properties. Packaging techniques are indeed very poor and need to be studied and improved to increase the shelf life of the products. Increasing the shelf life of the pulp and the kernels, the two most important commercialized products, will surely add value to these products. Hence it will increase their marketability and improve the income of poor rural populations involved in the production and trade of baobab food products. Moreover, while improving the quality of the products in general and their packaging in particular, attention should be paid to their normalization to increase their chance to be exported and sold on more remunerative markets. Local processing centers need to be established for production of standardized products.

In fact, some baobab products (kernels, leaf powder, pulp) are important on the local market (Benin), as well as at regional and international markets. Many baobab food products can be considered as functional foods because they are claimed to have a therapeutic effect on health in addition to their food properties (Sidibe and Williams, 2002, Diop et al., 2005, Gruenwald and Galizia, 2005, Assogbadjo et al., 2006). In recent years in Europe, a market has developed for food and beverage products that provide a specific positive impact on health. On average, each European spends 15 Euro annually to buy such health-supporting foods (Gruenwald and Galizia, 2005). Baobab foods (based on pulp, leaves) may match this new generation of functional foods. Consequently, any research on valorization and standardization of baobab products will be beneficial for rural people involved in selling these products. Effective valorization through improvement of traditional techniques/products and production of value added products (with functional properties) for a larger market would increase the income of rural, poor populations. For instance, at retail level in Europe, baobab fruit pulp cost 200 Euro per kg, while the supplier in Africa sells it at $ US 3 to 5 (*i.e.* 2.2 to 3.7 Euro) Freight on Board (Gruenwald and Galizia, 2005). It is necessary to perform research on cumbersome processing operations and preservation to solve problems that limit a wider distribution and commercialization of the products for the benefit of local populations.

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Chapter 4

Effect of cooking on in vitro solubility of Ca, Fe, Zn and carotenoids in *Adansonia digitata* (baobab) leaves

This chapter has been submitted as
Effect of cooking on in vitro solubility of Ca, Fe, Zn and carotenoids in *Adansonia digitata* (baobab) leaves
Chadare FJ, Hooiveld GJEJ, Linnemann AR, Nout MJ R and Hounhouigan J (submitted)

Abstract

Effect of home cooking on the content and in vitro solubility of Ca, Fe, Zn and carotenoids from different morphotypes of baobab leaves was assessed. A survey among 302 informants allowed identification of different morphotypes of leaves. In vitro solubility was assessed after simulated gastro-intestinal enzymatic digestion, carotenoid content by HPLC and mineral content by inductively coupled plasma–optical emission spectrometer. Six morphotypes of baobab leaves were distinguished. Effect of morphotype, cooking and digestion on Ca content was significant ($p < 0.05$). The in vitro solubility of Ca ranged from 10-30% of total Ca. Fe (9.2-15.7 mg/100 g dm) and Zn (2.5-4.8 mg/100 g dm) contents were much lower than that of Ca (1371–3655 mg /100 g dm), and their solubility was below detection level. Fe, Zn and carotenoid contents vary significantly between morphotypes while the effect of cooking was significant only for Fe, lutein and betacarotene cis 2.

Key words: *Adansonia digitata* L., leaf sauce, calcium, iron, zinc, lutein, betacarotene.

Practical Applications

The study provides unique data on the nutritional value of baobab foods as prepared and consumed by rural African populations, namely the effect of home cooking on solubility of minerals and carotenoids. Principal Component Analysis showed that leaves of baobab with a high Fe content are also rich in betacarotene cis and lutein. In general, the highest amounts of minerals and carotenoids are found in bitter leaves, young ones as well as old ones. Bitter leaves, though not preferred by the populations, therefore offer the best nutritional opportunities and can be used to improve the health status of rural populations. The findings provide suggestions for improved use of baobab foods and entering new areas of research, especially on the use of acidulants, meat, and fat to increase mineral and carotenoid uptake from consumption of baobab leaf sauce.

4.1 Introduction

Of the world population, more than 923 million people did not have enough food in 2007 (FAO, 2008). In the developing countries more than 40% of women are anaemic and about 25% of children are vitamin A deficient (FAO, 2002). At the same time, several forest foods, rich in nutrients, are available but unfortunately under-utilized. Among the numerous forest food resources, the baobab tree (*Adansonia digitata* L.*;* Bombacaceae) is a key economic tree, used daily by local populations in Africa (Baum, 1995; Wickens, 1982). Studies related to the nutritional importance of the species have shown that the leaves are particularly rich in calcium and iron (Smith*, et al.,* 1996; Yazzie*, et al.,* 1994). However, the content of minerals does not mean much by itself in terms of nutritional value; the uptake of minerals from the food is equally important. Therefore, solubility of minerals from baobab leaves can be a key factor. The effect of traditional cooking techniques on the solubility for uptake of minerals is not known (Sidibe and Williams, 2002). Preliminary investigations among indigenous people of Benin showed that baobab foods in general, and leaves in particular, are consumed frequently (5 to 7 times a week in some areas). However, it is unknown how different types of baobab leaves are distinguished by local dwellers and which type of baobab leaves contains the highest levels of minerals and carotenoids, and to what extent these are available for uptake after home cooking. The objectives of the present work were to (i) determine the criteria that are locally used to identify different morphotypes of baobab leaves; (ii) study the effect of home cooking on levels of minerals and carotenoids; and (iii) study the in vitro solubility of minerals. Knowledge on these aspects is a prerequisite to determine strategies on how to use these products to reduce health disorders due to deficiencies of carotenoids, iron, calcium and zinc among rural populations.

4.2 Materials and methods

4.2.1 Identification of different types of leaves

A survey was conducted in the rural area of Benin where the population has an outstanding knowledge of the baobab tree. In order to determine the number of leaf collectors to be surveyed, a random check was performed with 198 randomly selected potential users of baobab food products to estimate the proportion of collectors of baobab food products. This proportion was then used to compute the sample size N_i of baobab collectors to be interviewed, using the following formula: $N_i = \frac{4p_i(1-p_i)}{d^2}$, where N_i is the total number of collectors to be surveyed for the study; p_i is the proportion of baobab collectors among the 198 randomly checked persons; and d is the expected error margin in the conclusion, which was fixed at 0.05 (Dagnelie, 1998). Three hundreds and two collectors (192 women and 110 men) of baobab foods products were randomly selected among different socio-cultural groups (men, women from different ethnic groups such as Dendi, Lokpa, Namba, Otamari, Wama, Brouba, Bariba, Berba, peulh) and localities. Informants were interrogated on the baobab tree-to-tree variation according to its food products (leaves, fruits, pulp, and seeds) and differentiation criteria. The different types of leaves, distinguished using this method, were analyzed in the present study.

Baobab leaves. From the survey, the criteria taste, age, and sliminess were found to be the most relevant to distinguish baobab leaves by local people as will be shown in the results. Thus, the following morphotypes of leaves were sampled for the present study: the sweet

leaves reported as slimy young leaves (SYL), slimy old leaves (SOL), were sampled from a tree with slimy leaves; and, the sweet leaves reported as non-slimy young leaves (NSYL), non-slimy old leaves (NSOL), were sampled from a tree with non-slimy leaves; the non slimy bitter leaves reported as bitter young leaves (BYL) and bitter old leaves (BOL) were sampled from a tree with bitter leaves.

4.2.2 Processing of baobab leaves

Leaves were stored in plastic bags and preliminary dried over silicagel to avoid decay. They were transported to the laboratory and dried further at 60°C until constant weight. Dry leaves were stored vacuum-packed until they were finely milled (Retsch ZM 200, 12 knives rotor, 10,000 rpm, sieve 4 mm diameter). Dry leaf powder was packed in tightly sealed jars and stored at –20°C until analysis. The traditional home cooking practice (Chadare*, et al.* 2008) was simulated in the laboratory, as follows: for each type of baobab leaves, dry leaf powder was suspended in distilled water in a ratio of 1g dry leaf powder per 15 mL distilled water in a 500 mL Erlenmeyer conical flask. The two components were mixed thoroughly, and the flask containing the suspension was transferred into a water bath that was being warmed up to 100°C. From the time the temperature of the suspension reached 100°C, 5 min were counted, after which the Erlenmeyer was taken from the waterbath and allowed to cool down at room temperature. The cooked material was then freeze-dried before analysis, except for the in vitro digestion process for which cooked material was used directly. Non cooked (i.e., raw) samples were also analysed for comparison.

4.2.3 In vitro digestion of leaves

For the in vitro digestion, food samples were degraded using enzymes similar to those found in the human digestive system. The in vitro digestion (Kiers*, et al.* 2000) was performed with minor modifications. Duplicate samples of dry leaf powder (approximately 2 g) were suspended in 30 mL distilled water and digested under simulated gastro-intestinal conditions with the use of artificial saliva containing human saliva α-amylase (Sigma A-1031; Sigma-Aldrich, Zwijndrecht, The Netherlands), artificial gastric juice containing lipase (Rhizopus F-AP15; Amano Pharmaceuticals, Chipping Norton, UK) and pepsin (Sigma P-6887), and artificial pancreatic solution consisting of pancreatin (Sigma P-1750) and bile (Sigma B-3883), as described before (Kiers*, et al.* 2000). In short, the mixture leaf powder and water (in an Erlenmeyer) was kept in a 37°C water bath while shaking (about 400-500 rpm) for 5-10 min. 2 mL artificial saliva was added; after 30 min of incubation while shaking, the Erlenmeyer was put on ice. pH was measured and adjusted to pH = 4 using 5M HCl. After about 5 min of incubation at 37°C in the water bath, 8 mL artificial gastric juice was added followed by 60 min incubation while shaking; the Erlenmeyer was afterwards put on ice; pH was noted and adjusted to pH 6 using 3M $NaHCO_3$; the samples were further incubated at 37°C for about 5 min and 10 mL pancreatic solution was added and they were incubated for 30 min while shaking. After digestion, the suspension was kept on ice and was centrifuged for 40 min at 20,292 x *g* at 4°C (centrifuge Firlabo SW12R, manuf.: Froilabo SAS, 69330 Meyzieu, France). The supernatant was recovered and the pellet was washed twice with 20 mL distilled water and centrifuged again. Washed pellet was discarded. A blank consisting of 30 mL distilled water was digested and centrifuged as mentioned above. Supernatants and blanks were freeze dried and analyzed for calcium, iron, and zinc. Samples were corrected for added reagents/water by subtracting Ca, Fe and Zn content of blank from that of supernatants from samples. All measurements were performed in duplicate; the results are presented as means. The coefficient of variation (cv) of this technique is 7%.

4.2.4 Determination of micronutrients and carotenoids

Calcium, iron and zinc - Raw dry leaf powder, freeze-dried powder of cooked leaves and freeze-dried powder of digested raw and cooked leaves were digested using hydrofluoric acid (40%) and concentrated nitric acid (65% ww). Concentrations of Ca, Fe and Zn were determined by an inductively coupled plasma–optical emission spectrometer (ICP–OES, Elan 6000, Perkin Elmer, Wellesley, MA, USA) (Temminghof, 1997). All measurements were performed in duplicate (cv < 5%).
Carotenoids - Carotenoids were determined according to Bushway (Bushway and Wilson, 1982). Extractions were carried out under yellow filtered light in a nitrogen atmosphere. Samples (2 g raw leaf powder, or freeze-dried powder of cooked leaves) were weighed in a 100 mL beaker. Anhydrous sodium sulphate (4 g) and magnesium carbonate (0.5 g) were added and mixed well with a glass spatula. The spatula was rinsed with 30 mL tetrahydrofuran HPLC grade (Biosolve, VK 2005) and the suspension was homogenised with an Ultra Turrax homogeniser (IKA-Ultrax Turrax T25 Basic, IKA-Werke GMBH & Co KG, Germany) at 10,000 rpm for 1 min. The Turrax was rinsed with tetrahydrofuran, as often as necessary to be cleaned (at least 150 mL tetrahydrofuran in total). After a few minutes, a clear supernatant was obtained. This supernatant was filtered through a 125 mm diameter filter paper into a 250 mL round bottom flask. Tetrahydrofuran (15 mL) was added to the precipitate and the same procedure was repeated until a colourless filtrate and solid were obtained. The tetrahydrofuran filtrate was concentrated under vacuum using a rotary evaporator. After evaporation, the system was flushed with nitrogen. The concentrate was dissolved in 25 mL methanol/ tetrahydrofuran (3:1). Aliquots of 1 mL were filtered through a 0.45 µm PTFE filter prior to HPLC analysis. For in vitro digested samples, 10 mL of digested supernatant were dissolved in 20 mL tetrahydrofuran, incubated for 30 min and further diluted 4 times in the elution buffer, and filtered through a 0.45 µm PTFE filter prior to HPLC analysis: Vydac column (length 250 mm, diameter 4.0 mm); flow rate of 1 mL/min for 25 min per injection.

4.2.5. Statistical analyses

Data were analysed using SAS v9 software (SAS Institute Inc., 1999). For the survey data, correspondence analysis was used as mathematical tool (Greenace 1993) to link socio-cultural groups according to different types of leaves, differentiation criteria and their importance. Correlations and partial contributions of the modality under consideration allowed to obtain the best represented modalities on each axis. Fixed model Analysis of variance with 3 factors was applied on calcium data. The three factors are morphotypes of leaves (6 levels namely BOL, BYL, NSOL, NSYL, SOL, SYL), Cooking (2 levels namely cooked or raw) and Digestion (2 levels namely digested or not digested). Least square means of the levels of interactions between the three factors were computed because of high significance of all the interactions. Multi comparisons were made on the least square means using Tukey test, in order to obtain homogeneous group of the levels of the 2 ways-interactions of the factors according to calcium content. For Fe, Zn, and carotenoid data, analysis of variance, fixed model with two factors was considered because these nutrients were only determined for leaves that were not digested (carotenoid) or which show data below the detection level after digestion (iron and zinc). For each nutrient, least square means of the levels of interaction between the two factors (morphotype, cooking) were considered because of the high significance of the interaction of most of the nutrients. Principal components analysis was applied to the least square means in order to well describe the effect of the levels of the interaction on the minerals and carotenoid contents of the leaves of baobab. Finally,

descriptive statistics such as mean and standard deviation were used. Student-Newman-Keuls test allowed to check for significant differences between groups.

4.3 Results and discussion

4.3.1 Grouping of baobab leaves according to socio-cultural groups in Benin

Table 4.1 and figure 4.1 present respectively the axes and the grouping for the correspondence analysis of 6 types of leaves, their age, taste, sliminess, versus the importance attached to these characteristics by gender and by socio-cultural group (*Bariba, Berba, Brouba, Dendi, Gourmantche, Lokpa, Namba, Nateri, Otamari, Peulh, Wama*). Of the information collected, 91.1% was explained by two axes. Correlations and partial contributions of each modality revealed the parameters that are best represented on each axis. The interviewees mentioned 3 major criteria to distinguish different types of baobab leaves, namely taste (bitter, vs. sweet), age (young, vs. old) and sliminess (slimy, vs. non-slimy). Socio-cultural groups did give different weights to these criteria. Although all leaves have a somewhat slimy character, the ones characterized as "slimy" are very slimy; the other leaves are referred to as non-slimy. Moreover, when leaves are not perceived as bitter, they are regarded as sweet.

Table 4.1 Correspondence analysis axes for typing baobab leaves

Axis1 (dimension 1)		**Axis2 (dimension 2)**	
Parameters	Socio-cultural group/ethnic groups	Parameters	Socio-cultural group/gender
F1	Bariba	F5	Woman
F2	Berba	Sliminess	Man
F3	Gourmantche	Non Important	Dendi
F4	Dendi	Important	Lokpa
F6	Natéri		Namba
Age	Peulh		Otamari
Taste			Wama
			Brouba

Symbols: F= Leaf types: F1= bitter; F2= sweet; F3= slimy; F4= non-slimy; F5= young; F6= old.

4.3.2 Effect of cooking on levels and in vitro solubility (IVS) of Ca, Zn and Fe

The Ca, Fe and Zn levels in raw and cooked, whole and in vitro digested baobab leaves are presented in table 4.2.

Calcium. Results of ANOVA applied on Ca content of different morphotypes of leaves showed that all the factors morphotypes, cooking, digestion and their interactions were significant ($p < 0.05$). In general, all types of baobab leaves were very rich in Ca, with higher levels in old than in young leaves of all types. Ca levels in raw leaves ranged from 1371 (slimy, young) to 3310 mg/100 g dm (bitter, old) (Table 4.2). Least square means of the levels of the two ways-interaction of the three factors and the results of Tukey test are graphically presented in figure 4.2. In general, cooked samples exhibit higher Ca content. Indeed, after cooking, the Ca content ranged from 1504 (slimy, young) to 3655 mg/100 g dm (bitter, old). The increase of Ca content due to cooking though significant ($p < 0.05$) is most probably an apparent increase caused by loss of other leaf components such as volatile ones, since cooking water was not discarded.

After in vitro digestion, 10 - 24% of Ca present in different types of baobab leaves was available (i.e., dissolved) from raw leaves and 13 - 30% from cooked leaves which is an

expression of the significant effect of digestion on Ca content. The higher Ca solubility in the cooked leaves may have resulted from the degradation of fibrous components.
Results of Tukey test (Figure 4.2) confirmed that there is a clear separation between digested (d1) and not digested (d0) samples with the later at the tail expressing the significant decrease of Ca content of raw and cooked leaves after the in vitro digestion process. Moreover, in each of these two groups, many other sub-group were identified and in general, raw bitter young leaves (BYL) showed to be the richest in Ca. Raw non slimy old leaves (NSOL) and slimy young leaves (SYL) have the lowest content in each of the two important groups based on the digestibility. It is useful to note that the multiplicity of the sub-groups is an expression of big variation in the Ca content according to samples (morphotypes) and treatments (cooking, digestion). The causes of such variability are not well known.

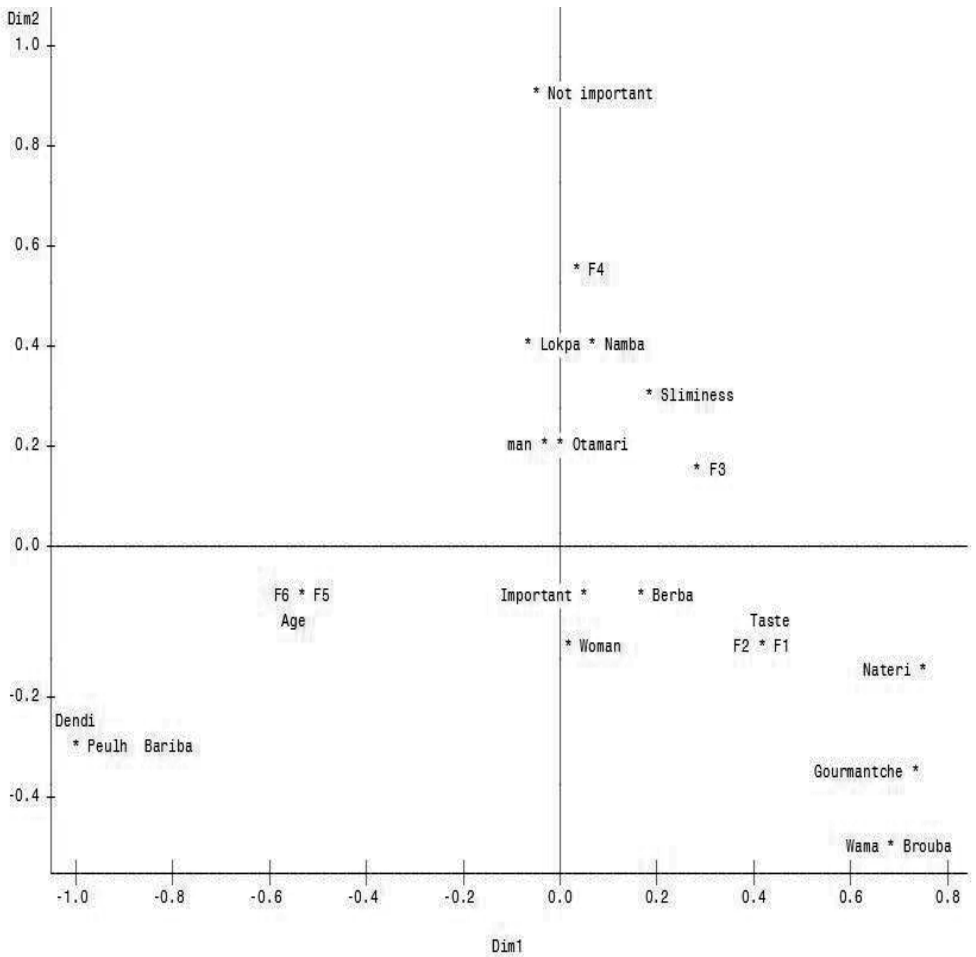

Figure 4.1 Correspondence analysis of major criteria for typing baobab leaves by socio-cultural groups in Benin, showing the linkage between different criteria for typing baobab leaves and the socio-cultural groups in Benin rural area

Symbols: F = Leaf types: F1= Bitter; F2= sweet; F3= Slimy; F4= Non-slimy; F5= Young; F6= Old leaves. F1*F2: both F1 and F2 are associated with e.g., taste.

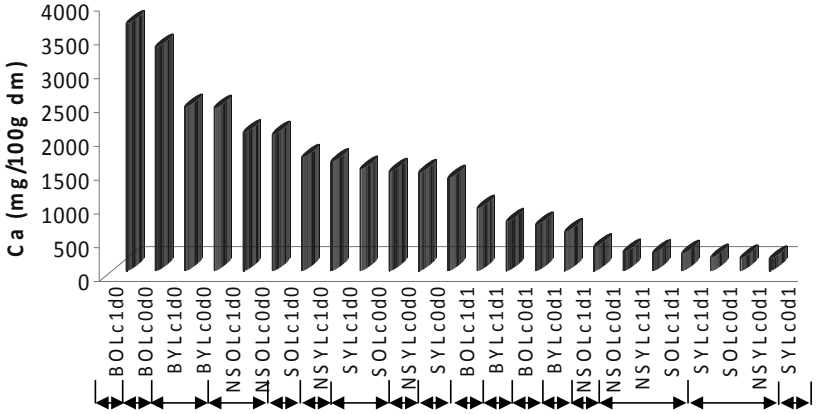

Figure 4.2 Results of Tukey test showing least square means of the levels of two ways interaction of the 3 factors (morphotypes, cooking, digestion)

Symbols: ←→: group identified by the Tukey test.
Cooking: c0= raw; c1= cooked / Digestion: d0 = not digested; d1=digested / Morphotypes: BOL = Bitter Old Leaves; BYL = Bitter Young Leaves; NSOL= Non Slimy Old Leaves; NSYL= Non Slimy Young Leaves; SOL= Slimy Old Leaves; SYL = Slimy Young Leaves. e.g. BOLc1d0 = Cooked and not digested Bitter Old Leaves; BOLc0d0 = Raw and not digested Bitter Old Leaves

Iron - The iron content of different types of raw young and old sweet baobab leaves was quite similar (9 – 10 mg/100 g dm). However, raw, bitter leaves contained a higher quantity of 16 mg/100 g dm. Results of ANOVA showed indeed that there is a significant difference between the different morphotypes for their content in Fe ($p < 0.05$). The reasons for different iron contents of baobab leaves are not known. We observed that dark green leaves contain more iron than light green ones, but it is not clear whether these phenomena (colour vs. iron content) are related. Literature data are also quite diverse, ranging from 1.2 mg/100 g dm (Smith*, et al.* 1996) to 21 and even 100 mg/100 g dm (Barminas*, et al.* 1998, Glew*, et al.* 1997, Lockett*, et al.* 2000, Sena*, et al.* 1998).

The effect of cooking is significant as well as the interaction between the two factors morphotypes and cooking ($p < 0.05$); the effect of cooking on Fe content depends thus on the morphotype. In fact, the Fe levels in most types of leaves varies slightly after cooking and the content is in the range 9 – 11 mg/100 g dm; while in bitter leaves the Fe content after cooking slightly but significantly ($p < 0.05$) increased (from 16 to 21 mg/100 g dm). Iron is not easily lost during cooking; this might be related to stable chemical binding to compounds that probably inhibit iron release such as phytates, calcium, and polyphenols. In addition, it can be estimated that the heat treatment is rather short and as such would not affect to a high extent the mineral content of the leaves. The Fe increase in bitter leaves due to cooking is most probably an apparent increase caused by loss of other leaf components. This is in line with the findings of Kuti and Kuti (1999), who showed that samples of chaya leaves cooked for 15 min in a microwave had slightly higher Fe contents, from 8.1 mg/100 g dm in raw edible leaves to 9 mg/100 g dm in cooked edible leaves. For both raw and cooked leaves the levels of dissolved Fe in in vitro prepared digests were below the detection limit of 3.8 mg/100 g dm. The very low iron content of the supernatant digest is probably caused by the retention of iron

in the undigested pellet, because of chemical complexation and possibly also due to slimy leaf components.

Zinc - The Zn contents of different types of baobab leaves were lower than those of Fe. Zn in bitter leaves was lower than in sweet leaves, which contain 3 – 5 mg/100 g dm. ANOVA showed that there is a significant difference between the different morphotypes for their content Zn ($p < 0.05$). Young slimy leaves had higher Zn levels than old ones. The reasons for these different contents have not been investigated, but our data seem to be compatible with the ones reported for baobab raw leaves (Smith*, et al.* 1996, Yazzie*, et al.* 1994). The effect of cooking as well as the interaction between the 2 factors morphotype and cooking in the present study was not significant ($p > 0.05$). After in vitro digestion, the levels of dissolved Zn of both raw and cooked leaves were below the detection level of 2.1 mg/100 g dm.

Similarly to what happens with iron, this reflects a strong complexation with the pellet of undigested leaf material.

4.3.3 Effect of cooking on carotenoids of baobab leaves

The main carotenoids detected in baobab leaves were lutein, trans -, and cis (1 and 2) - betacarotene. The data are presented in table 4.3. ANOVA showed a significant difference between the different morphotypes for their content in lutein betacarotene, betacarotene cis1, and betacarotene cis2 ($p < 0.05$).

Lutein – the lutein content varies significantly ($p < 0.05$) from one morphotype to another. The levels of lutein in bitter leaves were about twice as high as in sweet leaves. Contents ranged from 55 mg/100 g dm in bitter old leaves, to 13 mg/100 g dm in sweet non-slimy young leaves. There is a tendency for higher contents in old leaves, while cooking significantly ($p < 0.05$) reduced the contents in sweet leaves, ranging from 6 mg/100 g dm (non-slimy young) to 14 mg/100 g dm (non-slimy old). The decrease in the bitter leaves was quite small. Handling methods of the food material such as heat treatment, storage conditions, can change the concentrations of lutein (Calvo, 2005). In the present study, heat treatment reduced its content in baobab leaves.

Table 4.2 In vitro solubility (IVS) of Ca, Fe and Zn in raw and cooked baobab leaves

		Sweet leaves				**Bitter leaves**	
		slimy		non-slimy		non-slimy	
Minerals (mg/100 g dm)	Treatment	young	old	young	old	young	old
Ca	Raw	1371 ± 12 h	1468 ± 31 g	1452 ± 16 g	2018 ± 3 d	2403 ± 6 c	3310 ± 55 b
	IVS	143 ± 0.4 o	189 ± 6 o	177 ± 5 o	279 ± 16 n	580 ± 31 l	680 ± 50 k
	IVS (% of total)	10	13	12	14	24	21
	Cooked	1504 ± 29 g	1676 ± 4 e	1612 ± 4 f	2044 ± 6 d	2421 ± 11 c	3655 ± 0.0 a
	IVS	202 ± 4 o	257 ± 7 n	264 ± 24 n	357 ± 14 m	729 ± 0.4 j	924 ± 17 i
	IVS (% of total)	13	15	16	18	30	25
Fe	Raw	9 ± 0.5 f	10 ± 0.2 ef	10 ± 0.4 ef	10 ± 0.0 ef	16 ± 0.2 c	16 ± 0.2 c
	IVS	< dl	< dl	< dl	< dl	< dl	< dl
	Cooked	9 ± 1.2 f	10 ± 0.3 edf	11 ± 0.2 d	11 ± 0.2 ed	19 ± 0.3 b	21 ± 0.3 a
	IVS (% of total)	< dl	< dl	< dl	< dl	< dl	< dl
Zn	Raw	5 ± 0.4 a	3 ± 0.2 ab	3 ± 0.3 ab	3 ± 0.1 b	< dl	< dl
	IVS (% of total)	< dl	< dl	< dl	< dl	< dl	< dl
	Cooked	5 ± 0.0 a	5 ± 1.5 a	4 ± 0.4 ab	3 ± 0.0 b	< dl	< dl
	IVS (% of total)						

*dl = detection level

For each measured parameter, means (± standard deviation) with the same letter are not significantly different ($p < 0.05$).

Trans-betacarotene or betacarotene - Bitter leaves had significantly ($p < 0.05$) higher levels of trans-betacarotene than sweet leaves, ranging from 32 (bitter, old) to 7 mg/100 g dm (sweet, non-slimy, young). Old leaves had higher levels of trans-betacarotene than young leaves. Differences between bitter and sweet leaves became bigger after cooking, resulting from decreased levels in sweet and increased levels in bitter leaves.
Cis-betacarotene – Cis-betacarotene also reported as betacarotene cis1 reached the highest levels of all carotenoids ranging from 45-74 mg/100 g dm in bitter leaves, to 22-36 mg/100 g dm in sweet non-slimy leaves, and 19-34 mg/100 g dm in sweet slimy leaves. Old leaves clearly had higher levels of cis-betacarotene than young ones. Effect of cooking is significant ($p < 0.05$). In cooked leaves, significant levels were retained, which were of the same order as in raw leaves.
Other carotenoid - Apart from the carotenoids above-mentioned, betacarotene cis2 (Table 4.3) was recorded. For betacarotene cis2, no clear trend is observed. It ranges in raw leaves from 0.6 (non slimy young leaves) to 15.2 mg/100 g dw (slimy young leaves). The effect of heat treatment is significant ($p < 0.05$).

The tendency of old leaves to be richer in carotenoids than the young ones may be due to accumulation of pigments as leaves get more matured. Bitter leaves have a darker green colour hence more accumulated pigments than the other which may explain their higher content in lutein and betacarotene. This is similar as found in *Brassica oleracea* for which mature fully expanded leaves accumulated higher carotenoid concentrations than immature or "baby" leaves (Lefsruda*, et al.,* 2007).

In vitro extractability of carotenoids
After in vitro digestion, extraction of carotenoids with tetrahydrofuran was only partly successful. The sliminess of the leaf components prevented an adequate separation of aqueous and solvent phases. Cooking induced a decrease in lutein content while the trend for betacarotene varied according to the type of leaves. The type of cooking (with or without fat) affects the in vitro accessibility of lutein and betacarotene. Cooking with fat or fat products increases the accessibility hence the contribution of the leaves' all trans betacarotene to the RDA retinol probably due to the fact that they are a fat soluble components (Chandrika*, et al.,* 2006; Hedrén*, et al.,* 2002).

4.3.4 Practical implications of the study

Principal components analysis applied to the least square means of the levels of the interactions revealed that the first two axes concentrated 89.6% of the overall information on the nutrients and are then sufficient to describe the levels of the interaction between the factors according to the nutrients (Figures 4.3a and 4.3b). Projection of the nutrients on these axes shows on axis 1 that leaves of baobab that have a high Fe content are also rich in betacarotene cis and lutein. Axis 2 represented high content in betacarotene cis2 (Figure 4.3a). Projection of morphotypes in axis system is presented in figure 3b. Combination of figures 4.3a and 4.3b helps to notice that; in general, mainly BOL (bitter old leaves) and also BYL (bitter young leaves) are the morphotypes that show the highest content in most of the minerals and carotenoid. SYL (Slimy young leaves), SOL (slimy old leaves) and NSOL (non slimy old leaves) give the lowest values of the nutrients. But non cooked SYL and NSOL showed high values in betacarotene cis2. It can be concluded that bitter leaves, though not preferred by the population are very rich in iron, lutein, betacarotenes and would be interesting for technological applications for improved nutrition among populations.

Table 4.3 Carotenoids in raw and cooked baobab leaves

		Sweet leaves				Bitter leaves	
		slimy		non-slimy		non-slimy	
Carotenoids (mg/100 g dm)	Treatment	young	old	young	old	young	old
Lutein	raw	23 ± 0.0 d	29 ± 1.7 c	13 ± 2.6 e	28 ± 2.1 c	52 ± 0.3 ab	55 ± 0.1 a
	cooked	7 ± 2.7 f	9 ± 1.4 f	6 ± 0.03 f	14 ± 0.4 e	50 ± 2.4 b	48 ± 2.1 b
Trans-β-carotene	raw	10 ± 0.0 fg	12 ± 0.1 e	7 ± 0.1 ih	13 ± 0.4 e	19 ± 0.1 d	32 ± 0.0 b
	cooked	5 ± 1.8 i	8 ±1.6 hg	6 ± 0.2 ih	11 ± 0.4 ef	25 ± 1.8 c	38 ± 1.3 a
Cis-β-carotene	raw	19 ± 0.0 ef	34 ± 0.3	22 ± 0.6 edf	36 ± 2.5 bcde	45 ± 0.4 cb	74 ± 0.1 a
	cooked	13 ± 4.3 f	52 ± 12.4 b	28 ± 0.2 cdef	44 ± 11.6 cb	39 ± 7.2 cbd	80 ± 7.8 a
Betacarotene cis 2	raw	15.2 ± 0.0 a	1.4 ± 0.2 cb	0.6 ± 0.5 c	17.7 ± 1.6 a	1.7 ± 1.7 cb	13.9 ± 0.0 a
	cooked	2.8 ± 0.6 cb	6.6 ± 4.5 b	1.6 ± 0.2 cb	0.8 ± 0.2 c	4.3 ± 0.0 cb	2.1 ± 0.0 cb

For each measured parameter, means (± standard deviation) with the same letter are not significantly different ($p < 0.05$).

4.3.5 Ideas for improving mineral/carotenoid availability and suggestions for future studies

Based on the findings of the present study, we suggest that current cooking practices by local populations can be improved for optimal Fe, Zn and carotenoids absorption. Digestibility and availability of iron and zinc can be influenced by several factors. Vitamin C and presence of meat protein are the major factors of importance. Proposed improvement techniques include the use of acidulants (mainly ascorbic acid and citric acid) and meat. One acidulant that may be used in our case is baobab pulp, known for its high ascorbic acid content (about 360 mg/100 g, own data). Ascorbic acid can reduce food ferric iron to the better absorbed ferrous iron thus preventing its reaction with inhibitors such as phytic acid and/or its precipitation as ferric hydroxide (Clydesdale 1983, Hurrell*, et al.* 2006) and consequently improve absorption of non-heme iron present in dietary foods. The bioavailability of non-heme iron is also enhanced by the presence of meat or its consumption together with the dietary food (Cook and Monsen 1976, Hurrell*, et al.* 2006, Sharma 2003). This enhancement is mainly due to the meat factor effect induced by the muscles (Clydesdale 1983, Hurrell*, et al.* 2006). It is good to precise that the meat factor effect is due to the protein components of the muscle that probably form iron-peptide complexes preventing iron to bind to phytate and phenolic compounds (present in the dietary food) that are unavailable for absorption (Hurrell*, et al.* 2006).
It is also well-known that carotenoids bioavailability can be increased by cooking with fat or fat products since these are fat soluble (Chandrika*, et al.* 2006, Hedrén*, et al.* 2002).

In our opinion, future research should focus on in vitro or in-vivo uptake studies to optimize combinations of baobab leaves and enhancing (dietary) factors. Sensory and other acceptability tests should follow in order to ensure compatibility with consumer preferences.

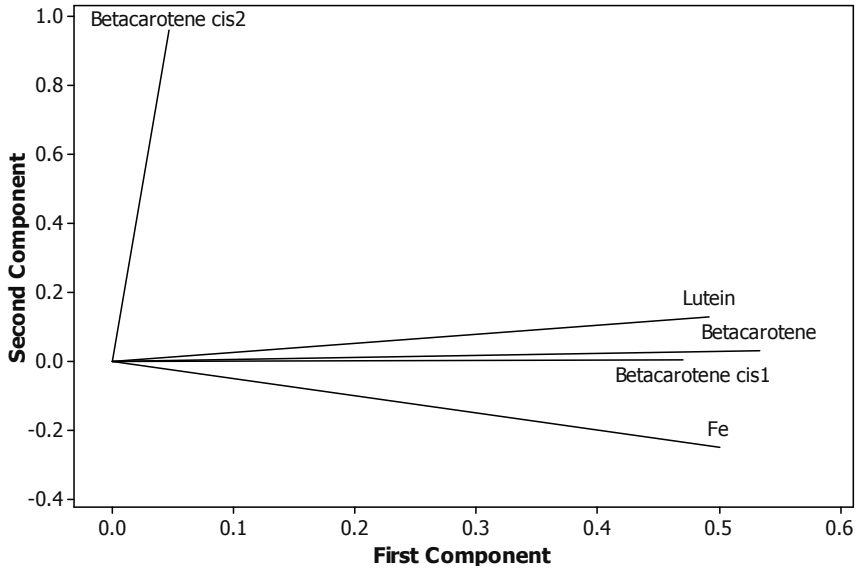

Figure 4.3a Projection of minerals and carotenoids contents in the system axes 1 and 2 of principal component analysis

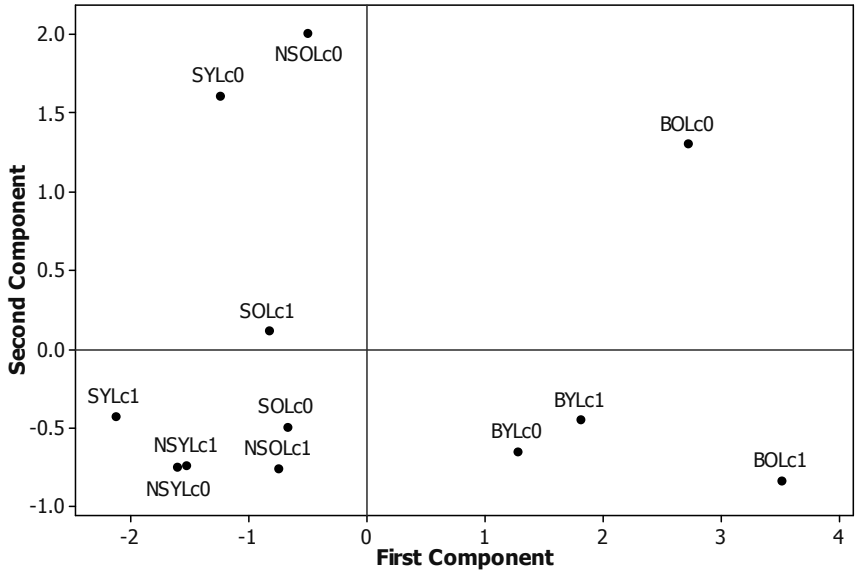

Figure 4.3b Projection of morphotypes of leaves (raw and cooked) in the system axes 1 and 2 of principal component analysis

Symbols: Cooking: c0 = raw; c1=cooked / Morphotypes: BOL = Bitter Old Leaves; BYL = Bitter Young Leaves; NSOL= Non Slimy Old Leaves; NSYL= Non Slimy Young Leaves; SOL= Slimy Old Leaves; SYL = Slimy Young Leaves

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Chapter 5

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

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

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

5.1 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 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.

5.2 Materials and methods

5.2.1 Survey

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

5.2.2 Process and sampling

Baobab fruits were processed according to figure 5.1. 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 5.2.

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.

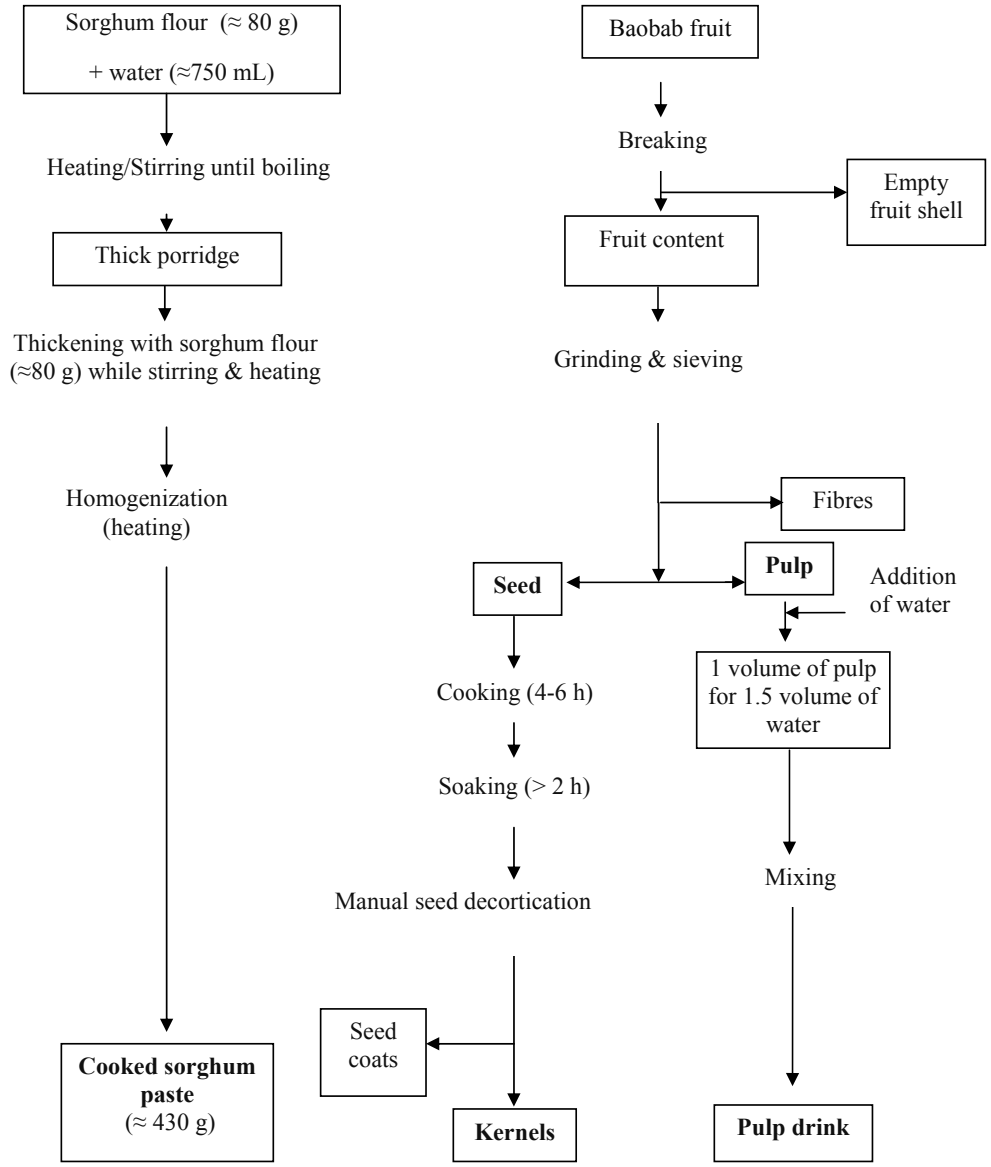

Figure 5.1 Sorghum paste preparation and seeds, kernels and pulp extraction from Baobab fruit

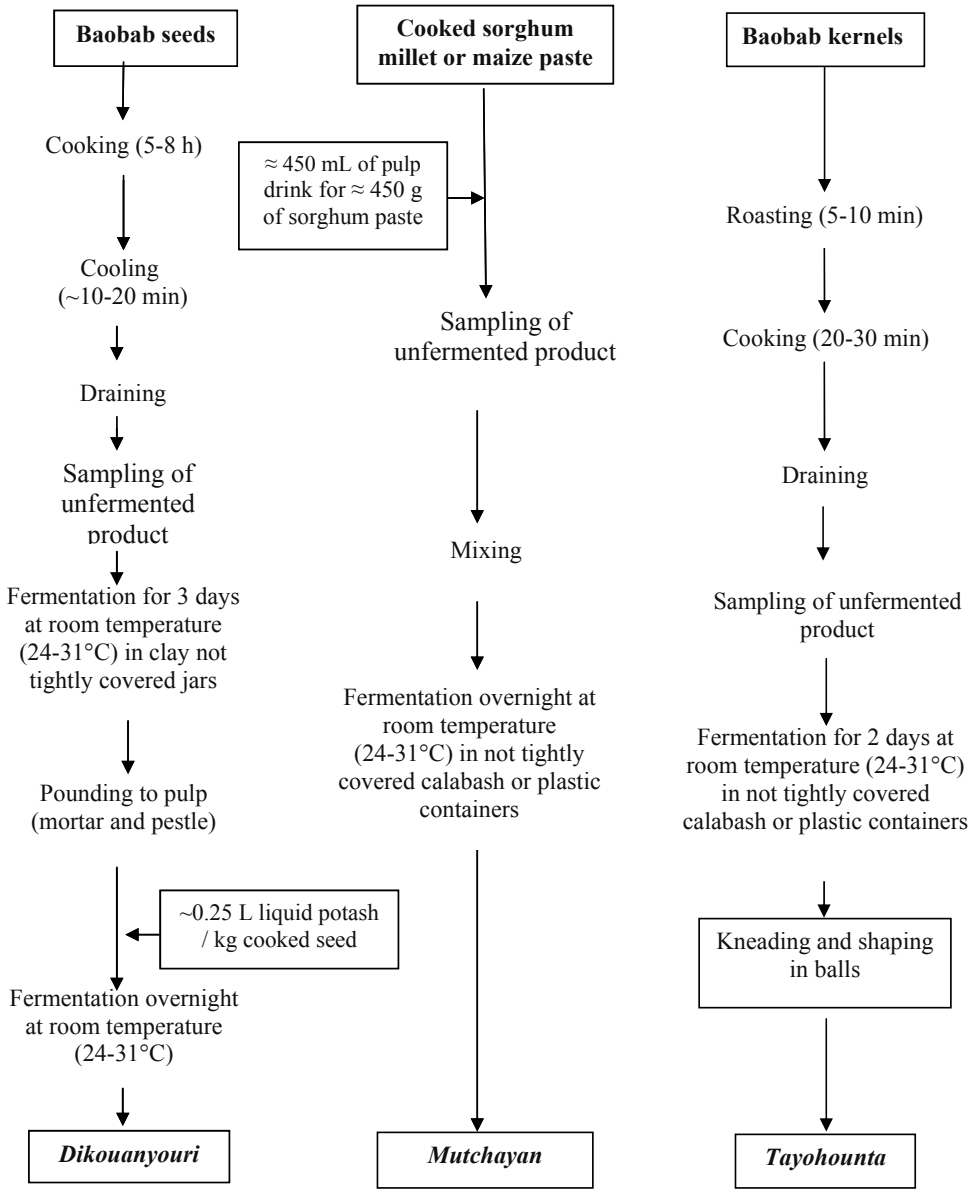

Figure 5.2 Flow diagram for the processing of *Dikouanyouri*, *Mutchayan* and *Tayohounta*

5.2.3 Microbiological analyses

Ten grams of each sample were taken 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 1 min 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. For the drop plate method (Herigstad *et al.*, 2001), the volume of the inoculum was 30 µl 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 (3 g), yeast extract (3 g), Glucose (10 g), Peptone (5 g) and Agar (20 g) 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:

$$X = \frac{AV}{I}$$ with:

X=number of microorganisms per g
A= number of counted colonies on 1/2 or 1/4 petri dish
V = 1/dilution factor
I = Inoculum volume (mL)

5.2.4 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.

5.2.5 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 $p < 0.05$.

5.3 Results

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

The flow diagram for *Dikouanyouri*, *Mutchayan* and *Tayohounta* processing is shown in figure 5.2. *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 5.1. The second important ingredient for *Mutchayan* is cereal (maize, millet or sorghum) flour. In order to obtain the flour, cereal grains are cleaned and sun dried. 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 5.1); 450 mL of baobab pulp drink (made from about 110 g of baobab pulp) for 450 g of sorghum paste (Figure 5.2). 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.

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

Table 5.1 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 5.1). Counts in unfermented products were below the minimum level to be considered of relevance.

Table 5.1 Microorganisms (Log cfu/g) in *Mutchayan, Dikouanyouri* and *Tayohounta*

Microorganisms group	***Mutchayan***	***Dikouanyouri***	***Tayohounta***
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 ($p < 0.05$).

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

Dikouanyouri
As shown in table 5.2, the unfermented product contained 13 g/100 g dm crude lipids, 4.7 g/100 g dm ash, 342.5 mmol total nitrogen/100 g dm and 7.9 mmol/100 g dm free amino nitrogen. After fermentation, crude lipids and ash significantly increased to 17.2 g/100 g dm, and 10.9 g/100 g dm, respectively ($p < 0.05$). Protein degradation allowed an increase of free amino nitrogen to 39.7 mmol/100 g 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/100 g, a crude lipid content of 36.9 g/100 g dm a pH of 6.4; a total nitrogen content of 476.6 mmol N/100 g 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/100 g dm and 105.7 mmol/100 g dm, respectively. The ratio amino nitrogen/total nitrogen increased from 2.3% in the non fermented to 21.3% in the fermented food (Table 5.2).

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/100 g. The lipid content increased from 0.7 in the unfermented to 1.6 g/100 g 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 5.2).

5.3.4 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 5.3). 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 5.3 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 5.3, ANOVA showed that the factor "consumption frequency" ($p < 0.05$) and the interaction "consumption frequency" * "Product" ($p < 0.05$) 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.

Table 5.2 Effect of fermentation on the physico-chemical characteristics of *Mutchayan*, *Dikouanyouri* and *Tayohounta*

Parameters	***Dikouanyouri***		***Tayohounta***		***Mutchayan***	
	Unfermented	Fermented	Unfermented	Fermented	Unfermented	Fermented
Dry matter (g/100 g)	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/100 g 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
Titratable 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 5.3 Consumption frequencies (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

Consumption frequency	***6-7 times/week***		***4-5 times/week***		***2-3 times/week***		***1 time/ week***		***Rarely***	
	A	***S***	***A***	***S***	***A***	***S***	***A***	***S***	***A***	***S***
Natitingou (N=50)										
Mutchayan										
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
Dikouanyouri										
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
Tayohounta										
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)										
Mutchayan										
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
Dikouanyouri										
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
Tayohounta										
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)										
Mutchayan										
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
Dikouanyouri										
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
Tayohounta										
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

5.4 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 proteolyc 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 ≈ 3.6 Log cfu/g after 72 h 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 need to be considered in further studies.
The dry matter content of *Dikouanyouri* is quite stable and increases slightly from 36.6 g/100 g dm to 37.5 g/100 g. 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/100 g 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 48 h 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/100 g 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/100 g). 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 5.2), 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. This will help to 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.

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Chapter 6

Microbiological composition of *Tayohounta*, a fermented baobab flavour food of Benin

This chapter has been submitted as
Microbiological composition of Tayohounta, a fermented baobab flavour food of Benin
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Abstract

The importance of forest foods in general and in particular baobab foods in most African countries is well known. The present work provides data on the microbiology of *Tayohounta*, a product from natural fermentation of baobab seeds kernels from Benin. Samples were produced and collected from 3 different small scale producers from Benin at the end of the fermentation process. Aerobic mesophilic microorganisms, lactic acid bacteria (LAB), Enterobacteriaceae, bacterial spores, micrococci, yeasts and moulds were enumerated and identified using phenotypic and genotypic methods. Strains were further investigated using Denaturing Gradient Gel Electrophoresis (DGGE) and cloning techniques. Isolated microorganisms were tested for their effect on pH, protein degradation and smell as an index of their functionality in baobab seed kernels fermentation.

Whereas *B. subtilis* caused a degradation of protein and developed a lower aroma intensity, Enterobacteria and lactic acid bacteria (LAB) had little impact. On the other hand, a few selected moulds and yeasts caused pH increase, protein degradation and off-flavour. Total viable counts (TVC) were around 9 log cfu/g consisting mainly of *Bacillus* spp., whereas LAB (8 log cfu/g) and yeasts and moulds represented a minor part of the total flora in all samples. Identification of isolated strains showed that samples produced by different producers may have different microbial composition. However, it could be shown that there is a common presence of *Bacillus subtilis, Bacillus licheniformis, Bacillus thermoamylovorans, Lactobacillus fermentum and Streptococcus spp.*, whereas other *Bacillus* spp., *Enterococcus*, *Lactobacillus* and *Pediococcus spp.* were found in products from two of the three producers.

Many other identified species (i.e., *Klebsiella pneumoniae*, *Lactobacillus agilis*, *Staphylococcus aureus*) were found in only one of the 3 *Tayohounta* samples.

Keywords: *Adansonia digitata*, natural fermentation, *Bacillus* spp., DGGE, genotyping, functionality

6.1 Introduction

In many African countries, the baobab (*Adansonia digitata* L.) is a widely used tree for many purposes especially as food and therapeutic product. All parts of the tree (roots, bark, leaves, fruits and seeds) are used as ingredients in the preparation of traditional foods (Nordeide *et al.*, 1996) and some have a therapeutic effect (Codjia *et al.*, 2001). The most common baobab-derived foods are juices from the pulp, powdered leaves used as an ingredient for sauces, and flavouring agents made from the seeds (Sidibe and Williams, 2002). Up till now, data are available on the ethnobotanic, ecological and genetic aspects of the baobab tree, as well as on the chemical composition of baobab parts (Nour *et al.*, 1980; Ajayi *et al.*, 2003; Osman, 2004; Assogbadjo *et al.*, 2005; Assogbadjo *et al.*, 2006). Recent concern about the lack of documentation about traditional and indigenous food cultures, which is important to retain traditional food knowledge and sustainable food systems, has lead towards a focus on African food cultures (Wahlqvist, 2007). Research on traditional African foods can provide possibilities for valorization through improvement of traditional techniques and products leading to production of added value products for a larger market and a better price. This has yielded valuable information (physico-chemical composition and microbiology of the foods) about some West-African traditional foods in general and fermented ones in particular, e.g., fermented cereal products such as *mawe*, *gowe*, *ogi* (Hounhouigan *et al.*, 1993; Agati *et al.*, 1998; Michodjehoun-Mestres *et al.*, 2005), and fermented seed products such as *afitin*, *sonru, iru, kpaye, ogiri, ugba, dawadawa* and *soumbala* (Omafuvbe *et al.*, 1999; Sanni *et al.*, 2000; Dakwa *et al.*, 2005; Azokpota *et al.*, 2006). Some microorganisms present in the above cited fermented products have shown to be functional, i.e. they contribute to desirable outcomes of fermentation. Most of the fermented seed products are used as flavouring agents; therefore, the flavour is desirable and hence functional in those products. Volatile compounds resulting from the metabolic activities of *Bacillus* spp. contribute significantly to flavour during the fermentation of African locust beans to produce *soumbala* (Ouoba *et al.*, 2005) or soya beans to produce *natto* or thai *thua-nao* (Leejeerajumnean *et al.*, 2001). As such, many volatile compounds were found in the Beninese *afitin*, *iru* and *sonru* (Azokpota *et al.*, 2008). Some *Bacillus* strains also showed to be functional in *kinema* and *soumbala* for protein degradation, pH increase and development of desirable stickiness (Sarkar *et al.*, 2002). Such works have, however, never been performed on any of the baobab products.

In relation to baobab, a study was carried out on the possibility to incorporate baobab pulp in tempe, a fungal fermented soybean food (Afolabi and Popoola, 2005). In Benin, thirty five baobab food products have been recorded among which several fermented products which were not yet characterized before (Chadare *et al.*, 2008).

Tayohounta is one of those indigenous fermented baobab foods from Benin, and its production process was described recently (Chadare *et al.*, 2010). It is a product belonging to the category of the alkaline fermented foods (Steinkraus, 1995) and it is used as a source of flavour when making soups. There is some diversity of *Tayohounta* from different producers, possibly because of the uncontrolled natural fermentation taking place, in which high numbers of *Bacillus* spp. were observed. This would give it resemblance to other regional flavour products such as *afitin, iru and soumbala*. In order to better understand and characterize the common microflora and arrive at a basis for the future development of fermentation starter cultures for *Tayohounta*, the present analysis of the product of three individual small-scale producers was carried out.

6.2 Materials and methods

6.2.1 Sampling

Tayohounta production process was followed at 3 representative small-scale production sites in Boukoumbe, North-Benin. The production process was as described by Chadare *et al.* (2010). Briefly, baobab seed kernels were roasted (5-10 min), cooked in water (about 30 min), transferred in a plastic container, covered with leaves (usually leaves of *Annona senegalensis*) and allowed to ferment for 2 days at 24-31°C (night-day ambient temperature). Cover leaves are in contact with the product. Processor 3 cooked for a longer time and used different leaves to cover than processors 1 and 2. Samples were collected just at the end of the fermentation period and stored in refrigerated conditions (± 4°C) for one week prior to analysis. Visual comparison of the samples prior to analysis showed that samples from processor 1 and 2 still had the structure of intact seed kernels with a dry or dull surface while the sample from processor 3 showed more decomposition of the kernel structure and had a moist appearance. Also the flavour of sample from processor 3 seemed much more pungent than the ones of the two other samples.

6.2.2 Enumeration of the groups of microorganisms

Representative sub-samples of 10 g were weighed aseptically and were made into 10^{-1} dilutions and consecutive 10-fold dilutions, using peptone physiological saline (PPS) solution (8.5 g NaCl and 1 g neutralized bacteriological peptone (Oxoid, LP0034) in 1 L demineralised water) in sterile filter Stomacher bags. The samples were homogenized for 60 seconds with a Stomacher (Seward Laboratory Blender Stomacher 400) at normal speed.
Aerobic mesophilic bacteria were enumerated on plate count agar (PCA, Oxoid CM0325) (30°C, 2 days) and reported as Total Viable Count (TVC).
Lactic Acid Bacteria (LAB) were enumerated on de Man, Rogosa and Sharpe Agar (MRSA, 1.5% Technological Agar (Oxoid, LP0013) added to de Man, Rogosa and Sharpe broth (Merck, VM986641, mixed and sterilized) supplemented with natamycin (2 g Delvocid (50% natamycin, DSM) added to 1 L MRSA). After solidification the plates were stored in an air-tight jar under microaerophilic conditions (80% N_2, 10% CO_2 and 10% H_2 gas mixture leaving a final concentration of O_2 of 6%) and incubated at 30°C for two days. Presumptive LAB were confirmed by oxidase and catalase tests, and confirmed counts were reported as Lactic Acid Bacteria (LAB).
Enterobacteriaceae were enumerated on Violet Red Bile Glucose (VRBG) agar (Oxoid, CM0458), in microaerobic conditions (30°C, 24 h).
Bacterial spores were enumerated on PCA (30°C, 2 days), after heating the 10^{-1} dilution at 80°C for 10 min to kill vegetative cells. Micrococci were counted on Mannitol Salt Agar (MSA, Oxoid CM0085) (30°C, 2 days). Yeasts and moulds were enumerated on Oxytetracycline Glucose Yeast Extract Agar (OGYEA, Oxoid CM0545 supplemented with oxytetracycline (25°C, 3 days). Yeast and mould colonies were counted separately (Nout, 1991).

6.2.3 Isolation, purification and confirmation

Distinctive colonies were isolated (5 per group of microorganisms and per sample), from the counted plates to confirm the obtained counts by microscopic observation and biochemical analysis, identification and experimental fermentations. Colonies were purified by streaking

on adequate medium, incubated at the required temperature, and further stored on slants kept at 4-7°C.
Wet-mount preparations of the isolated strains were made and observed under a microscope (Olympus, BX40). A preliminary grouping was based on morpholo ical traits (cell shape and size).

DNA extraction and amplification from isolated bacteria. From the confirmed isolates, DNA was extracted using the 'Isolation Genomic DNA for Gram positive and Gram negative bacteria' kit A1125 from Promega, Southampton, UK, according to the manufacturer's instructions. The DNA was stored at 4°C. The DNA isolated for bacterial isolates was used to amplify the 16S rDNA gene by polymerase chain reaction (PCR), using forward primer 5'-AGA GTT TGA TCC TGG CTC AG-3' and reverse primer 5'- AAG GAG GTG ATC CAG CCG CA-3' (Oomes *et al.*, 2007). The PCR was done using a GeneAmp PCR system 9700 (Applied Biosystems) with PCR conditions as follows: initial denaturation of double-stranded DNA for 5 min at 94°C; 35 cycles each consisting of 30 s at 94°C, 20 s at 56°C, and 1 min at 72°C; and extension of incomplete products for 7 min at 72°C followed by cooling at 4°C. The PCR products were sent to GATC Biotech (http://www.gatc-biotech.com) in Germany for purification and sequencing.

DNA extraction and amplification from isolated yeasts and moulds. Cultures were grown in MEB at 30°C for 1 day for yeasts and for moulds at 25°C for 2 days and then centrifuged at 6000 rpm for 10 minutes at 4°C, followed by washing the pellets with PBS buffer and centrifuged again as mentioned above. After the second centrifugation the pellets were then resuspended in 0.5 mL PBS buffer and transferred into 2 mL eppendorf tubes containing 0.5 g zirconia/silica beads (diameter 0.1 mm; Biospec products, Inc). Furthermore 0.5 mL CTAB and 0.5 mL phenol-chloroform-isoamyl alcohol (25:24:1) were added and tubes were beaten at maximum speed twice per 45 s using Mini beadbeater-8, Biospec, Bartlesville, USA.

Amplification and sequencing of fungal DNA. The DNA isolated from fungal isolated microorganisms was used to amplify the 26S rDNA using forward primer V9G (5'-TTACGTCCCTGCCCTTTGTA-3') and reverse primer LS266 (5'-GCATTCCCAAACAACTCGACTC-3') (van den Ende and de Hoog, 1999). PCR was performed with a total reaction volume of 50 µl containing 26.6 µl ddH_2O, 5 µl PCR buffer, 3 µl $MgCl_2$ (25 mM), 10 µl dNTP (2 mM), 2 µl of each primer (10 µM), 1 µl DNA template, and 0.4 µl Taq DNA polymerase (5 U/µl) (Fermentas, USA). PCR was done using GeneAmp PCR system 9700 (Applied Biosystems) with PCR conditions as follows: initial denaturation for 5 min at 95°C followed by 35 cycles comprising denaturation at 95°C for 60 s, annealing was at 52°C for 45 s, extension at 72°C for 60 s, and final 7 min extension at 72°C followed by cooling to 4°C. The PCR products were sent to GATC Biotech (http://www.gatc-biotech.com) in Germany for purification and sequencing.

DNA extraction directly from Tayohounta samples. Four grams of sample were suspended and DNA was extracted according to Wang et *al.* (2008). From the DNA extracted directly from *Tayohounta* the V6-V8 region of the 16S rDNA gene was amplified using the universal forward primer EUB-968-GC-for (5'-CGC CCG GGG CGC GCC CCG GGC GGG GCG GGG GCA GGG GAA CGC GAA GAA CCT TAC-3') and reverse primer EUBL1401-rev (5'-CGG TGT GTA CAA GAC CC-3 (Engelen, Heuer *et al.,* 1995).

Cloning of PCR products. Identification of microbial strains was obtained by cloning PCR fragments in *E. coli* according to Röling *et al.* (2001) and sequencing 96 out of 200 transformants.

DNA sequence analysis. Obtained sequences were viewed for errors using Chromas Lite V2.01 software (http://www.technelysium.com.au/) and if necessary corrected manually with the IUB/IUPAC nucleic acid code. Afterwards the forward and reverse sequence were assembled to form a contig using the program Seqman II (DNAStar Inc. USA, version 5.08) and exported to FASTA format. These files were used to obtain strain identities by using the nucleotide BLAST program from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). The most probable match with a 16s rDNA sequence was selected based on the percentage of identification (%ID).

Denaturing Gradient Gel Electrophoresis: PCR products were analyzed by DGGE as described by Wang *et al.* (2008). 4-10 µg DNA was applied and a range of 30-60% denaturant was used. The gel was run for 10 min at 200 Volts and 60°C, followed by 16 h at 85 Volts and 60°C. Then, the voltage was switched down to 10 Volts and run until the gel was removed from the buffer. Silver staining was done according to Sanguinetti *et al.* (1994). The dried gel was scanned on a GS 800 calibrated Densitometer (BioRad). The program Bionumerics (Applied Maths) was used to normalise the gel and analyse band positions. Band positions from the pure cultures were compared to the pattern given by mixtures of the pure cultures and of the original samples.

6.2.4 Laboratory-scale fermentation of Baobab seeds

A selection of microorganisms isolated from *Tayohounta* was used as fermentation starters and their effect was measured. A quantity of 1000 g dry seeds was soaked in 2 litres of tap water overnight at 25°C. After pouring off the water, 50 g aliquots of the soaked kernels were transferred into 500 mL glass jars and sterilized by autoclaving at 121°C for 30 min. The seeds were inoculated with pure cultures, pre-grown for 48 h at 30°C in tubes containing 10 mL of nutrient broth (*Bacillus*), MRS broth (LAB) and malt extract broth (MEB; Oxoid CM57) (yeasts and moulds). One mL of a 10^{-1} dilution in PPS was used to inoculate each jar of seeds, and these were incubated at 30°C for 0 h (unfermented control) and 48 h.

Functional properties
Microbial growth. Each sample of fermented kernels was analyzed as described in section 6.2.2.

Terminal amino Nitrogen and pH. Ten grams sample was mixed with 90 g water and made into a suspension with a blender. This suspension (A) was used to measure pH and to perform the formol titration (Han *et al.*, 1999) for terminal amino groups, which were expressed as mM/g sample.

Volatile flavour: Of each fermented or non-fermented sample the flavour was examined by sniffing unfermented as well as fermented seeds. This was done by 3 untrained panelists.

6.3 Results and discussion

6.3.1 Enumeration of groups of microorganisms

Table 6.1 shows that the TVC in all samples was quite high, around 9 log cfu/g. High numbers were expected because there was no process step performed on the samples which would decrease the number of microorganisms after fermentation. The groups of LAB and bacterial spores, with counts around 8 log cfu/g, represented a large part of the TVC, but considering the high number of spores it is probable that the remaining TVC consisted mainly of vegetative cells of *Bacillus* spp. Yeasts and moulds, represented only a minor part of the total flora (Table 6.1).

Table 6.1 Microbiota composition of Tayohounta made by three individual processors

(Log cfu/g)	Tayohounta 1	Tayohounta 2	Tayohounta 3
Total Viable aerobic count	9.0 ± 0.1 a	8.8 ± 0.1 a	8.7 ± 0.1 a
Lactic acid bacteria	7.7 ± 0.0 c	8.5 ± 0.0 a	8.2 ± 0.0 b
Enterobacteriaceae	6.0 ± 0.4 a	7.0 ± 0.0 a	< 1
Bacterial spores	8.0 ± 0.0 b	8.5 ± 0.1 a	8.1 ± 0.0 b
Micrococci	7.2 ± 0.1 a	5.1 ± 0.1 b	5.3 ± 0.2 b
Yeasts	7.2 ± 0.0 a	3.1 ± 0.1 c	5.9 ± 0.0 b
Moulds	2.9 ± 0.1 a	2.4 ± 0.1 b	< 1

For each group of microorganisms, samples with different letters are significantly different ($p < 0.05$)

Substrate depletion by the rapid growth and high numbers of bacteria, as well as formation of metabolites such as ammonia, might retard the growth of yeast and moulds present. Because no starter is added after the seeds are boiled, likely origins of the microorganisms observed include kitchen utensils, cover leaves and other environmental sources (soil, water and air). It can also be possible that some spores were still viable after the long cooking procedure which correspond to moist heat treatment (Melly *et al.*, 2002; Coleman and Setlow, 2009) or that the spores had formed clumps which increase their heat resistance (Furukawa *et al.*, 2005).

6.3.2 Identification of isolated strains

Table 6.2 shows the identification of bacteria in *Tayohounta* made by 3 processors, based on sequencing 16S rDNA of isolated pure cultures, and on cloning DNA products obtained by PCR amplification from DNA extracted directly from *Tayohounta* samples.
The data presented in table 6.2 support the observation that products 1 and 2 show more similarities and that product 3 is different. Cloning techniques were therefore performed only on T1 and T3 (Figure 6.1); however, results from all the used techniques and thus on T1, T2 and T3 are often integrated in the interpretation. The identification of the microorganisms based on clone library of PCR product, DGGE and isolated pure culture (Table 6.1 and Figure 6.1) shows that *Bacillus subtilis, Bacillus licheniformis, Bacillus thermoamylovorans, Lactobacillus fermentum and Streptococcus* spp., were present in T1, T2 and T3 while some strains were found in products from two of the three producers; they are: *Enterococcus casseliflavus* (T1 and T2), *Pediococcus pentosaceus* (T1 and T2) and *Bacillus cereus* (T1 and T3). Many other identified species (i.e., *Bacillus thurengiensis*, *Brevibacillus borstelensis*, *Klebsiella pneumoniae*, *Lactobacillus agilis*, *Staphylococcus aureus*) were found in only one of the 3 *Tayohounta* samples. Strains of *Bacillus subtilis* represent 40% of all strains in product 3 and 27% of the ones in product 1. These two samples are very different while

considering the other strains. Product 1 contains *Enterococcus durans* (22%) and *Streptococcus* sp. (20%) which were less abundant in product 3. On the other hand, *Bacillus thermoamylovorans* is more represented in product 3 (33%) than in product 1 (4%). *Pediococcus pentosaceus* and *Bacillus pumilus* were found in products 1 and 2, confirming their stronger resemblance. All other species identified were only present in one of the three samples. Research done elsewhere on similar products also identified *B. subtilis* as dominating organism within the *Bacillus* group, sometimes even responsible for 50% of the total *Bacillus* spp. count, often accompanied by *B. licheniformis, B. pumilus* and *B. cereus* (Omafuvbe *et al.*, 1999; Dakwa *et al.*, 2005; Azokpota *et al.*, 2006). Heat resistance of *B. subtilis* spores has been investigated by Fox and Eder (1969) and Warth (1978), who found that the decimal reduction time around 100°C (D_{100}) was in the order of magnitude of 10 min. More recently, Oomes *et al.* (2007) reported heat resistance of *B. subtilis* in nutrient broth with added mineral mix to range widely from D_{111} = 0.5 min to D_{121} = 7.9 min. Considering that the boiling step during the process can take up to half an hour it seems quite possible that certain *Bacillus* spores can survive the boiling procedure when present in high numbers. Because there are differences in appearance and smell between the samples it can be stated that next to *B. subtilis* the other organisms play an important role during fermentation of the seed kernels as well. Again a different flora of the environment and leaves or on the skin of the producers and used utensils is suggested as probable causes for variability of microbiota.

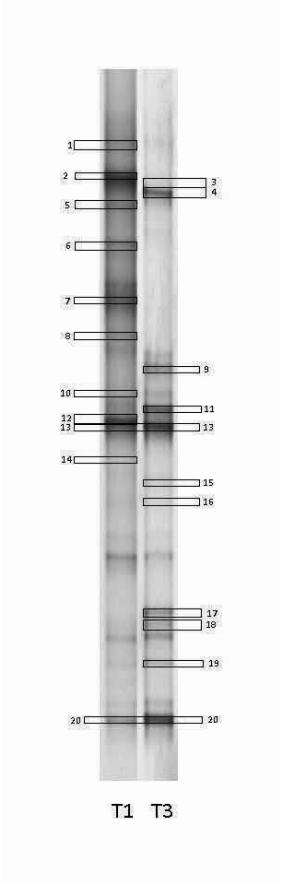
6.3.3 Functionality

Results of functionality test of the microorganisms during the fermentation of baobab seed kernels into *Tayohounta* are presented in table 6.3. All tested strains showed good growth within 48 h of incubation.
Only a few selected isolates for each group of microorganisms were tested for their effect on quality attributes of fermented kernels. *Bacillus subtilis* isolates showed no effect on pH, but were able to degrade protein and caused a typical pungent smell. *Enterobacter* and *Klebsiella* spp. had no effect on pH, protein or smell. *Pediococcus* sp. caused a slight decrease of pH, slight degradation of protein, and minor change of smell. The strongest effects were caused by *Aspergillus* sp. and two unidentified yeast isolates, which caused a rather unpleasant smell.
In the fermentation of soybeans by *Bacillus* strains for preparation of *Soumbala* and *Kinema*, a correlation was observed between increasing pH and level of free amino-N (Sarkar *et al.*, 2002). In the present study, though *Bacillus* strains were able to degrade protein, increase of free amino-N was not correlated with increasing pH in the fermentation of baobab kernels; yeasts and moulds were capable of producing volatile flavour. The reproducibility of the used technique for smell detection might be relatively low and was subjective; it is important to investigate the formation of volatiles more in detail before accepting or rejecting potential fermentation starters.

Table 6.2 Identification of bacteria in *Tayohounta* made by 3 processors, based on sequencing 16S rDNA of isolated pure cultures, and on cloning DNA products obtained by PCR amplification from DNA extracted directly from *Tayohounta* samples

Group	Name	Processor 1	Processor 2	Processor 3
Sporeforming bacteria	*Bacillus sp.*			C 2
	Bacillus cereus	I		C 4
	Bacillus licheniformis	C 4		I; C2
	Bacillus pumilus	I; C5	I	
	Bacillus subtilis	I; C 27	I	I; C 40
	Bacillus thermoamylovorans	C 4		C 33
	Bacillus thuringiensis			C 1
	Brevibacillus borstelensis			C 1
Enterobacteriaceae	*Enterobacter cloacae*		I	
	Klebsiella pneumoniae	I		
Lactic acid bacteria	*Enterococcus casseliflavus*	C 5	I	
	Enterococcus durans	C 22		
	Enterococcus faecium		I	
	Enterococcus italicus			I
	Lactobacillus agilis	C1		
	Lactobacillus equi	C 1		
	Lactobacillus fermentum	C 1		I; C 1
	Pediococcus pentosaceus	I; C 1	I	
	Streptococcus sp.	C 20		C 1
	Weissella confusa	I		
Micrococci	*Jeotgalicoccus halotolerans*			I
	Staphylococcus aureus		I	

I : based on isolated pure culture; C : based on clone library of PCR product from direct sample extraction. Number: percentage of total clones identified (96 clones total).



Legend to marker bands:

1: *Pediococcus pentosaceus*
2: *Streptococcus* sp.
3: *Bacillus thuringiensis*
4: *Streptococcus equinus*
5: *Lactobacillus agilis / Lactobacillus equi*
6: *Bacillus subtilis*
7: *Bacillus pumilus*
8: *Bacillus subtilis*
9: *Bacillus subtilis/Bacillus licheniformis*
10: *Bacillus licheniformis*
11: *Brevibacillus borstelensis*
12: *Enterococcus casseliflavus / Enterococcus durans*
13: *Bacillus subtilis*
14: *Enterococcus durans*
15: *Bacillus subtilis*
16: *Bacillus subtilis / Bacillus licheniformis*
17: *Bacillus subtilis*
18: *Lactobacillus fermentum*
19: *Bacillus licheniformis*
20: *Bacillus thermoamylovorans*

Figure 6.1 Clone library of PCR product from direct extraction of sample made by 2 processors. T1, T3 = sample made by processor 1 and 3, respectively

6.4 Conclusion

There is evidence of considerable microbial and quality diversity of *Tayohounta* made by different traditional processors using different ingredients. Similar to most fermented seed products (*afitin*, *iru*, sonru, *dawadawa*, etc.), *Bacillus subtilis* and other *Bacillus* species are present in *Tayohounta* whatever the process/producer. It also became clear that the culture-dependent analysis (isolation of pure cultures) and culture-independent approaches (direct DNA extraction from *Tayohounta*) resulted in overlapping results. However, cloning of PCR products yielded species that had not been isolated, while some isolated species were not discovered by cloning. This confirms earlier findings (Martin Platero *et al.*, 2008) that a polyphasic approach is the most suitable one for studying the complex microbiota of naturally fermented foods.

Table 6.3 Growth and impact of selected pure isolates from *Tayohounta,* during fermentation of cooked baobab seeds

Species	Code	Log cfu g^{-1}		pH		Terminal Amino Groups (mmol g^{-1})			Smell	
		t = 0 h	t = 48 h	t = 0 h	t = 48 h	t = 0 h	t = 48 h	Increase factor	t = 0 h	t = 48 h
Bacillus subtilis	*Aba*	6.3*	8.8	5.9	5.9	0.03	0.06	2.4	Cooked pasta	Cooked pasta, but older
Bacillus subtilis	*Bba*	6.3	9.2	5.9	5.9	0.02	0.09	3.8	Cooked pasta	less strong than *Aba*
Klebsiella pneumoniae	*16bs*	6.3	8.2	5.9	5.9	0.02	0.02	1.0	Cooked pasta	wet bread
Enterobacter cloacae	*17bs*	6.3	ND	5.8	5.9	0.03	0.03	1.0	Cooked pasta	wet bread
Pediococcus sp.	*3LAB*	6.3	7.5	5.8	5.7	0.02	0.04	1.8	Cooked pasta acidic	beans
Pediococcus sp.	*4LAB*	6.3	7.6	5.8	5.8	0.03	0.04	1.4	Cooked pasta	wet bread
Aspergillus sp.	*9ym*	4.3	7.3	5.8	6.1	0.02	0.05	2.2	Cooked pasta	smoky nuts
Unidentified yeast	*10ym*	5.3	7.3	5.9	6.6	0.03	0.04	2.0	Cooked pasta	less strong than *9ym*
Unidentified yeast	*Dym*	5.3	7.1	5.9	6.4	0.02	0.05	2.1	Cooked pasta	penetrative sour cream

*Approximate values calculated from inoculation rate

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Chapter 7

Colour change and vitamin C degradation of baobab pulp during storage

Abstract

Quality deterioration of baobab pulp during storage was investigated by varying storage conditions, namely temperature and water activity, and measuring vitamin C degradation and colour change as responses. Treatments consisted of three water activities, viz 0.08, 0.45 and 0.74 (measured at 20ºC) and 4 storage temperatures viz 41, 55, 61 and 70°C. Samples were stored for 56, 73, 192 and 240 h. Temperatures were recorded using i-buttons. Vitamin C content was determined by HPLC and colour by L*, a*, b* values. With increasing storage period, pulp colour turned darker with decreasing L* and increasing a* and b* values; vitamin C degraded following approximately first order kinetics. Reaction rates increased with temperature and water activity. These preliminary results are considered to be a good starting point for more detailed studies to build mathematical models that describe quality degradation of baobab pulp during storage.

Keywords: Baobab pulp, vitamin C, colour, storage, temperature, water activity

7.1 Introduction

Baobab pulp is known to be very rich in vitamin C (Sidibe and Williams, 2002) and in that respect it is qualified as the home grown vitamin C for Africa (Scheuring *et al.*, 1999). It is also known to have a high antioxidant activity compared to other fruits and it is believed that the antioxidant capacity of baobab pulp is mainly due to its vitamin C content (Vertuani *et al.*, 2002; Besco *et al.*, 2007). It is consumed by rural communities in Africa as a powder, drink, gruel and as paste. A survey performed in rural Benin allowed identification of several baobab food products, but also of the processing and storage problems (Chadare *et al.*, 2008). Pulp storage turned out to be one of the most important of these problems. In Beninese rural areas, baobab pulp is stored in its fruit until the time it is used or sold. During storage, it happens that the pulp changes colour, making the pulp unsuitable for human consumption. Quantitative information on this phenomenon is lacking and if and how colour change is related to vitamin C in the pulp is not known. The goal of the present work was to assess pulp quality deterioration in terms of pulp colour change and vitamin C content at different water activities and temperatures. The collected data will be used to investigate whether kinetic models can be proposed that describe baobab pulp degradation. With such models, one can predict how long baobab pulp can be stored in known storage conditions and how storage conditions can be improved to preserve pulp quality over time.

7.2 Materials and methods

7.2.1 Sample preparation and storage

Baobab pulp was collected and subjected to different treatments. Normal pulp (as collected freshly from the tree (N), humidified pulp (H) and freeze dried pulp (D) were used for the experiment. Humidified pulp was obtained by putting normal pulp on a thin layer together with water in a desiccator for 72 h to increase its water activity. Normal pulp was freeze dried to obtain pulp with a low water activity. Water activity was measured using AquaLab Series 3 water activity meter (Decagon, Pullman, WA, USA). At 20 ºC, normal pulp had a water activity (a_w) of 0.45, humidified pulp of 0.74 and freeze dried pulp 0.08. The water activities reported below were measured at 20ºC, and don't reflect the water activities at the storage temperatures. Each type of pulp, i.e., normal, humidified and freeze dried was subjected to 4 different storage temperatures viz 41, 55, 61 and 70°C. Samples were stored for 56, 73, 192 and 240 h.

Aliquots of about 4 g of pulp were put into airtight glass tubes, to exclude water exchange. The samples were initially warmed up in a water bath set at a required temperature and as soon as this temperature was reached, they were immediately transferred into an incubator at the required temperature for the remaining duration of the experiment. For each type of pulp, 7 tubes were stored at each temperature, resulting in a total of 28 tubes. Two i-button temperature sensors chips (http://www.maxim-ic.com/products/ibutton/ibuttons/) were put in the last tube containing normal pulp to be removed and recorded at different time interval according to the temperature during the whole experimentation. The temperature changes during storage are presented in figure 7.1. Tubes were removed from the incubator at different times, quickly cooled down in ice, and kept in the freezer until the time they were analysed for colour and vitamin C content.

7.2.2 Colour measurement

Colour was measured using the colour Flex hunterlab (ELSCOLAB, Nederland B.V) and L*, a* and b* values were measured as an expression of colour parameters as follows: L* (+ Light, - dark), a*(+ red, - green) and b*(+ Yellow, - Blue). For each sample, 3 measurements were made and the average value was reported.

7.2.3 Determination of vitamin C content

Vitamin C (ascorbic acid) was measured by HPLC (Thermo Fisher Scientific) (Hernandez *et al.*, 2006). Briefly, about 0.12 g of sample was weighed. 2.5 mL of a solution of 3% metaphosphoric acid (MPA) (Merck, Darmstadt, Germany), 8% acetic acid (Riedel de Haën, Morristown, New Jersey, USA) was added. Next, the mixture was homogenized in the Ultra Turrax at the highest speed for 1 min (in ice and darkness) and centrifuged at 4˚C for 20 min at 10,500 rpm. This procedure was repeated twice and the two supernatants were pooled. The extracts were filtered in a vial through a 0.45 filter prior to injection in the HPLC column. For calibration, a stock standard solution of 10 mg of ascorbic acid per mL of Millipore water was prepared. Subsequently, solutions of various concentrations were prepared by diluting the stock solution in 3% MPA - 8% acetic acid. The solutions were filtered and transferred to HPLC vials. Orthophosphoric acid (Merck, Darmstadt, Germany) 0.2% in milli Q water was used as elution buffer. 20 µl of sample was injected and run at a flow of 1 mL/min and analysed at a wavelength of 245 nm. Samples were analyzed in duplicate.

7.2.4 Estimation of kinetic parameters

Degradation kinetics of vitamin C were found to follow roughly first order kinetics. The first order equation is : $C = C_0 \exp(-kt)$, with C_0 = initial concentration of vitamin C in mg/100 g dm; C = concentration of vitamin C in mg/100 g dm; k = reaction rate constant (h^{-1}) (van Boekel, 2009). This equation was used to calculate/estimate reaction rate constants and initial concentrations for all the temperatures by nonlinear regression using the Solver function in Microsoft Excel 2007.

7.3 Results and discussion

7.3.1 Colour change

The water activity of the product, the temperature and the storage duration appeared to affect pulp quality in terms of colour change. As storage time increased, pulp colour lost its lightness with decreasing L* values and became more brownish with increasing a* value (reddish) and b* value (yellowish). The higher the storage temperature, the faster the colour changed. Whatever the temperature, freeze dried pulp with water activity 0.08 showed a slower browning than normal pulp with water activity 0.45. Browning was more pronounced in humidified pulp with water activity 0.74. Colour change was faster at higher temperatures and at higher water activities (see Figures 7.2a and 7.2b; Figures 7.3a and 7.3b). In fact, initial L*, a*, b* value for untreated baobab pulp were 84.84, 1.78, 16.26 for normal pulp (a_w = 0.45); 84.95, 1.78, 16.03 for freeze dried pulp (a_w = 0.08) and 83.69, 2.63, 17.52 for humidified pulp (a_w = 0.74), respectively. After 56 h of storage at 70°C, the L* value decreased to 55.34 for humidified pulp, 65.86 for normal pulp and 72.94 for freeze dried pulp while a* and b* values increased to 4.18 and 29.28 for freeze dried pulp, 4.89 and 27.6 for

normal pulp and 7.36 and 26.64 for humidified pulp. These results show that for the same temperature, browning is faster at higher water activity. Considering the same water activity, e.g., normal pulp with a_w = 0.45, L* decreased to 65.86 after 56 h of storage at 70°C, 74.89 after 192 h of storage at 55°C, 83.36 after 240 h of storage at 41°C; a* and b* values increased to 4.89 and 27.6 at 70°C, 4.44 and 24.65 at 61°C, 4.12 and 25.34 at 55°C and 2.07 and 19.26 at 41°C. Browning reaction was thus faster at increasing temperature. Baobab pulp contains reducing sugars (Nour *et al.*, 1980) and some protein (Obizoba and Amaechi, 1993; Lockett *et al.*, 2000; Osman, 2004). The observed colour change is, therefore, likely to be due to the Maillard reaction which is in this case non desirable.

7.3.2 Vitamin C degradation

The reported vitamin C content in baobab pulp ranges from 150 to 500 mg/100 g dm (Scheuring *et al.*, 1999). The present data on the unheated samples (397 mg/100 g dm for normal pulp, 383 for humidified pulp and 389 for freeze dried pulp) are within this range.
In general, a fast decrease in vitamin C content occurred at the very first stage of the experiment followed by a slower degradation. This fast decrease at the start of the storage period is an interesting phenomenon. It might be due to an enzymatic reaction, to a phase change of the powder because of a glass transition state change or to an unknown phenomenon during the transfer from water bath to incubator. With the present data, however, further interpretation is not well possible, but future research should take this observation as a starting point.
It has been shown elsewhere that vitamin C degradation during treatment of food material generally follows first order kinetics (Cruz *et al.*, 2008; Gonçalves *et al.*, 2009; Odriozola-Serrano *et al.*, 2009). We excluded the data points at time zero, implying that we did not consider the content of vitamin C of the unheated samples as C_0 but instead the initial concentration of the samples that were put in the incubator. The results are shown in figures 7.4a and 7.4b). Parameter estimation was done via nonlinear regression to avoid statistical artefacts due to logarithmic transformation (van Boekel, 2009). Details about the estimated parameters (according to types of pulp), namely C_0 and k, and their correlation coefficients are given in table 7.1.
Vitamin C content of normal pulp (a_w=0.45) stored at 41°C hardly changed at increasing storage time, as shown by a k value that was virtually zero. In general, whatever the temperature, reaction rate was faster at increasing water activity of baobab pulp. This may be explained by the fact that the available water facilitates several chemical and enzymatic reactions leading to faster quality degradation, or by a faster rate of diffusion (van Boekel, 2009). Moreover, for normal and humidified pulp, the reaction rate constant increased with increasing temperature. For freeze dried pulp, most k-values are rather low, 0.004 at 41°C, 0.002 at 55°C, 0.0013 at 61°C. The correlation coefficient between the two parameters (Table 7.1) was in all cases very acceptable, indicating that they were not strongly correlated. The estimated C_0 values (which correspond to the vitamin C content after the sample had reached the required temperature) are quite variable, ranging from 130.3 to 395.7 mg/100 g dm (Table 7.1). This suggests that vitamin C content was differently affected not only by the temperature, but also by the time it took to reach the required temperature. As mentioned above, this phenomenon requires further investigation. It was also noticed that in general, the darker the pulp, the lower its vitamin C content.
Generally, temperature dependence of rate constants is studied via the Arrhenius equation (van Boekel, 2009):

$$k = k_0 \exp\left(-\frac{E_a}{RT}\right)$$

in which k_0 is the pre-exponential factor, E_a the activation energy, R the gas constant and T absolute temperature. A plot of ln$k$ versus $1/T$ should result in a straight line if the Arrhenius equation holds. However, the temperature range in our study was too small to apply such an analysis in terms of absolute temperature and for this narrow temperature range a plot of ln$k$ versus T should also result in a straight line. That appears not to be the case in the present study. Figure 7.5 shows a non linear temperature dependence for all 3 water activities. Though the first-order plots are certainly not perfect, the 95% confidence intervals obtained for the rate constants were quite acceptable (Table 7.1), and the values were not overlapping each other strongly. Hence, the non-linearity of the temperature plot in figure 7.5 does not seem to be due to imprecision of the estimates of the rate constants. It must be concluded that another factor is interfering in the kinetic analysis that is not yet identified. Most likely, the phenomenon is linked to the observation that the initial vitamin C concentration was very variable. This requires further investigation.

In addition to its vitamin C content, baobab pulp is known to have a high antioxidant activity compared to other fruits and it has been suggested that the antioxidant capacity of baobab pulp is mainly due to its vitamin C content (Vertuani *et al.*, 2002; Besco *et al.*, 2007). Consequently, vitamin C degradation could be positively correlated with loss of antioxidant activity by baobab pulp, hence pulp quality degradation.

In conclusion, it was found that browning occurs (probably via the Maillard reaction) and that vitamin C degradation takes place. More investigations are necessary concerning the initial fast degradation of vitamin C. Also other factors such as the effect of amino acid content (in particular lysine) and sugar content, in relation to the Maillard reaction and brown pigment formation, will be useful. This will give, in addition, more insight in the nutritional loss of lysine in the same framework of quality degradation. The Maillard reaction also lends itself very well to kinetic modelling (Van Boekel, 2009). This chapter provides the initial impetus for the collection of data upon which kinetic models can be built that quantitatively can predict quality changes of baobab pulp during storage. Knowledge of the reactions that lead to quality loss can then also provide the basis for suitable ways of packaging the pulp to preserve its quality. The present work shows that water activity is an important factor, and this can be influenced by choosing the right packaging material.

Table 7.1 Reaction parameters estimated via nonlinear regression at different temperatures and water activities. C_0 in mg/100 g dm, k in h^{-1} ± 95% confidence intervals

Parameters	Humidified pulp	Normal pulp	Freeze dried pulp
Water activity (mean ± standard deviation) at 20ºC (a_w)	0.74 ± 0.002	0.45 ± 0.003	0.08 ± 0.003
70°C			
Initial concentration of vitamin C (C_0)	240.78 ± 55.3	217.2 ± 35.8	163.51 ± 38.3
Reaction rate constant (k)	0.06 ± 0.04	0.05 ± 0.02	0.015 ± 0.01
Correlation coefficients between the parameters C_0 and k	0.52	0.50	0.60
61°C			
Initial concentration of vitamin C (C_0)	380.8 ± 29.2	347.4 ± 30.9	395.7 ± 13.0
Reaction rate constant (k)	0.049 ± 0.008	0.011 ± 0.003	0.0013 ± 0.0008
Correlation coefficients between the parameters C_0 and k	0.51	0.71	0.79
55°C			
Initial concentration of vitamin C (C_0)	203.0 ± 29.4	247.7 ± 28.9	254.7 ± 31.4
Reaction rate constant (k)	0.009 ± 0.003	0.006 ± 0.002	0.002 ± 0.001
Correlation coefficients between the parameters C_0 and k	0.44	0.65	0.75
41°C			
Initial concentration of vitamin C (C_0)	141.05 ± 28.0	130.3 ± 24.0	189.89 ± 58.0
Reaction rate constant (k)	0.008 ± 0.003	0.000 ± 0.001	0.004 ± 0.003
Correlation coefficients between the parameters C_0 and k	0.60	0.81	0.67

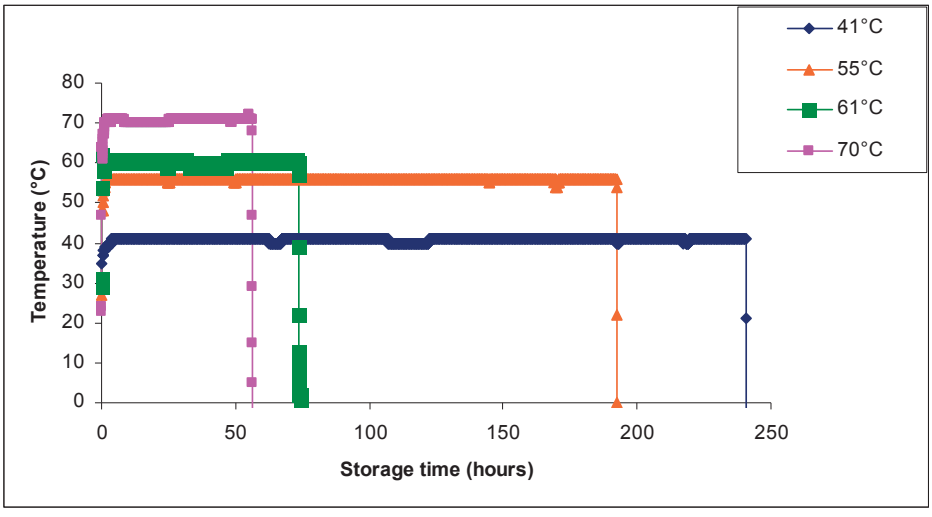

Figure 7.1 Temperatures recorded during storage experiments

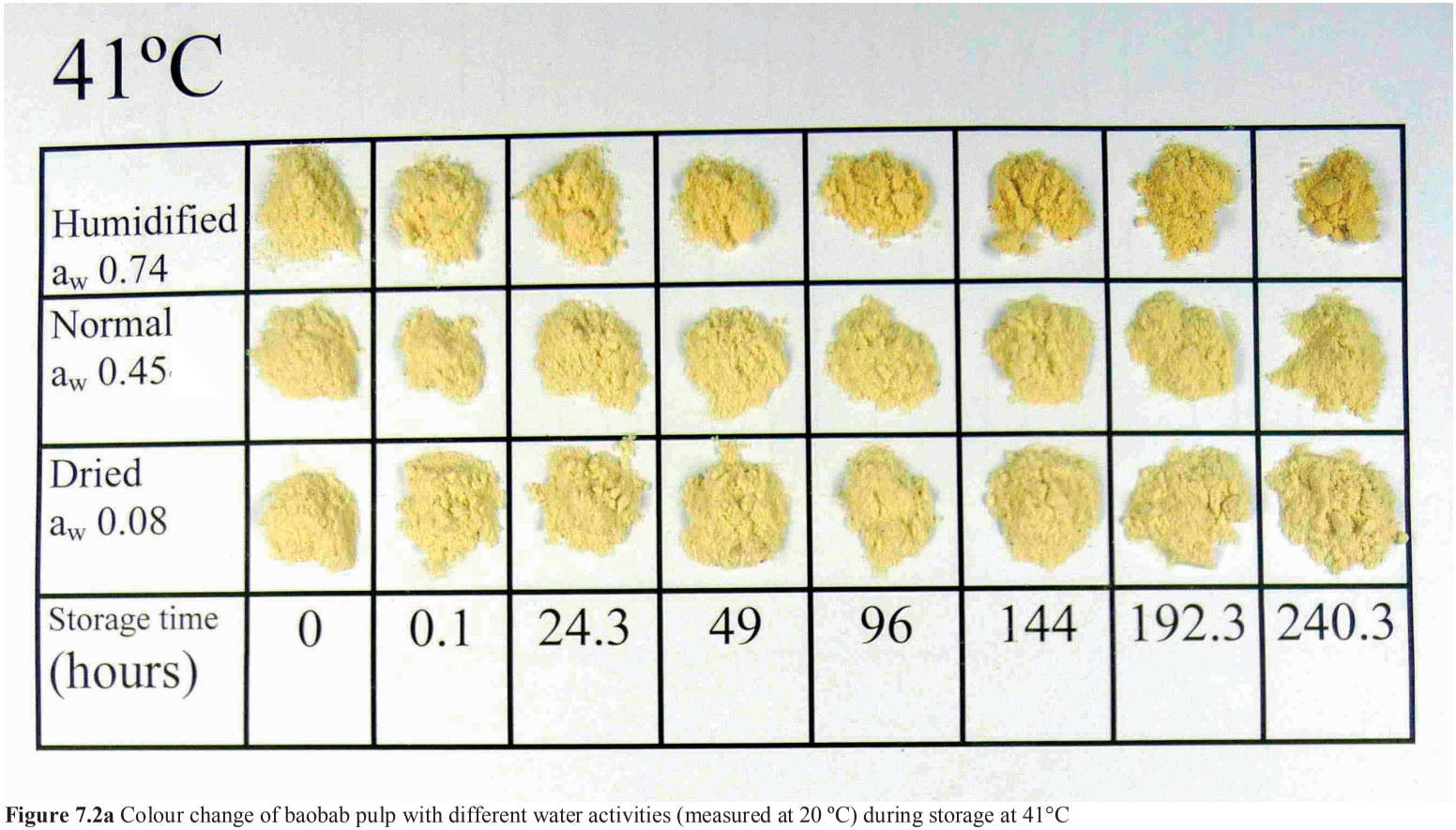

Figure 7.2a Colour change of baobab pulp with different water activities (measured at 20 °C) during storage at 41°C

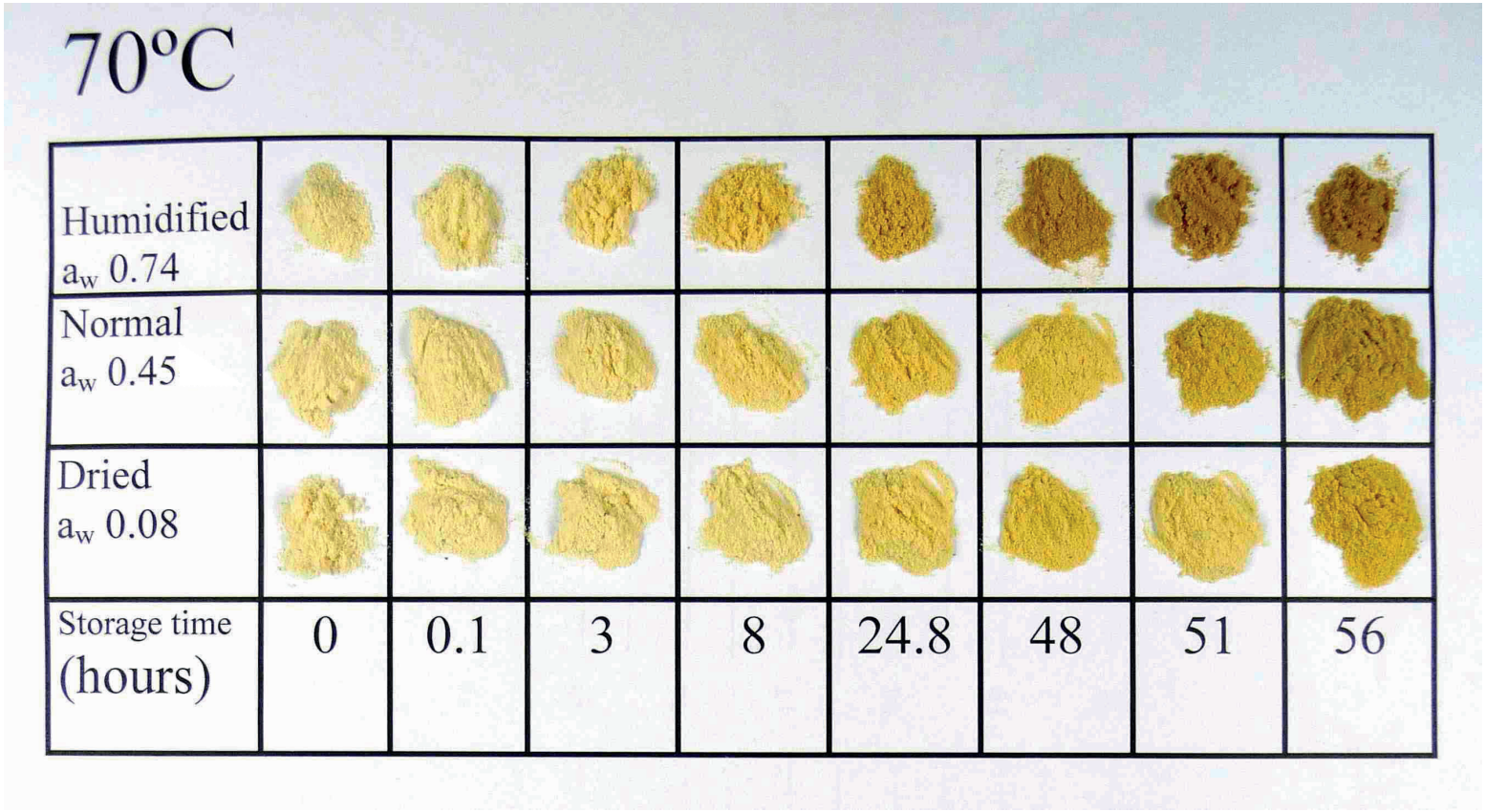

Figure 7.2b Colour change of baobab pulp with different water activities (measured at 20 °C) during storage at 70°C

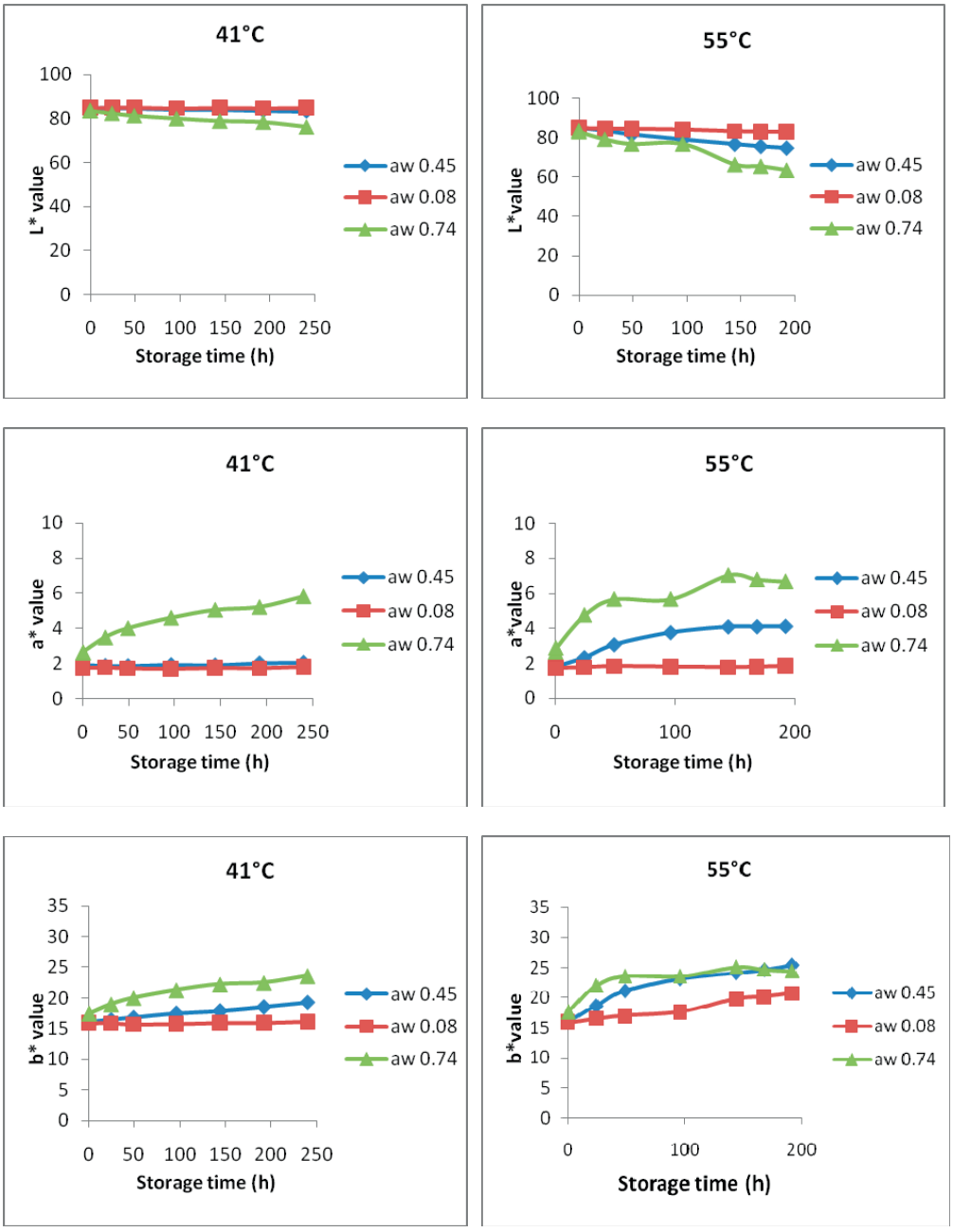

Figure 7.3a L*, a*, b* values showing colour change of baobab pulp with different water activity (measured at 20 ºC) during storage at 41°C and 55°C

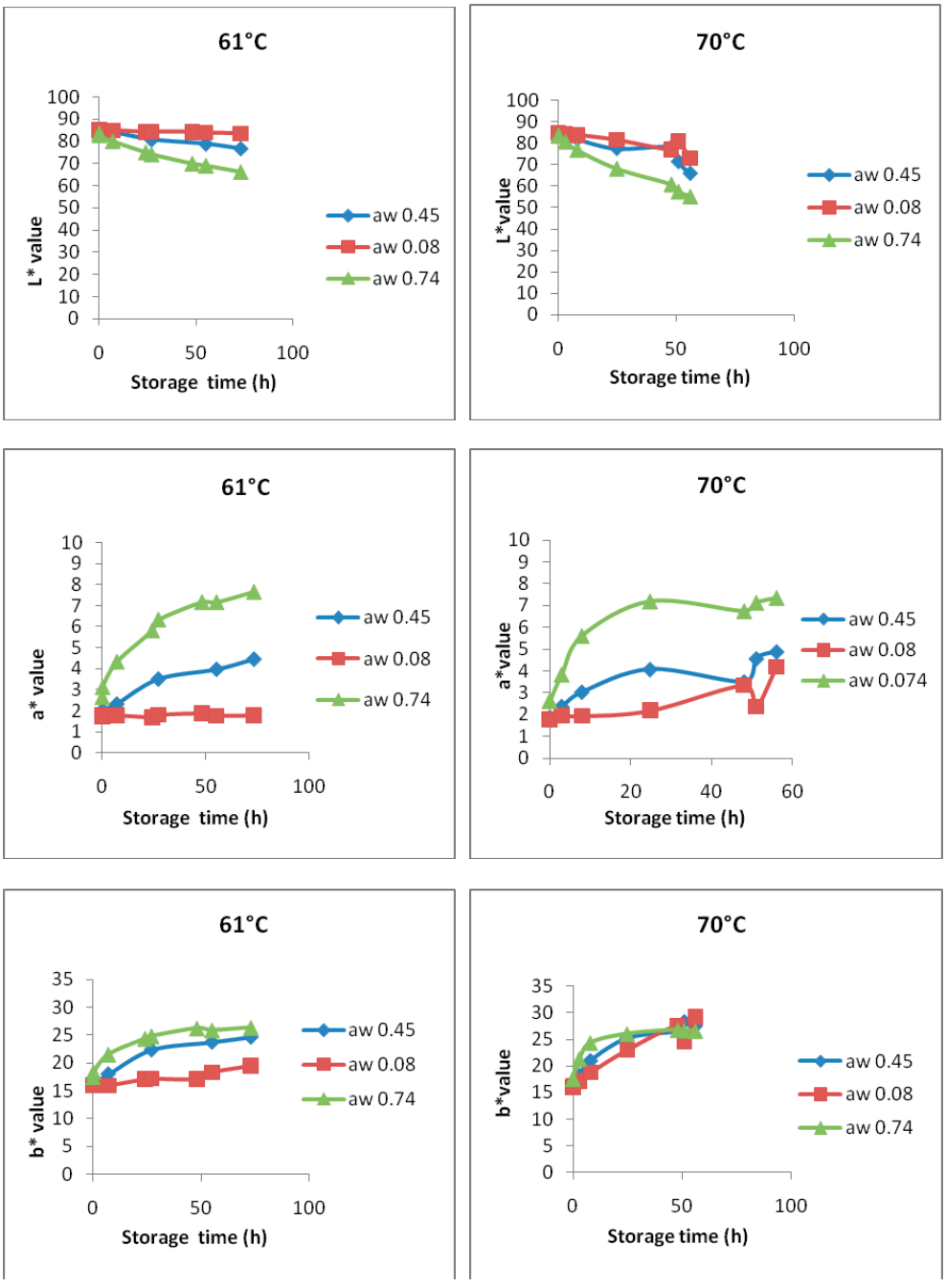

Figure 7.3b L*, a*, b* values showing colour change of baobab pulp with different water activity (measured at 20 ºC) during storage at 61°C and 70°C

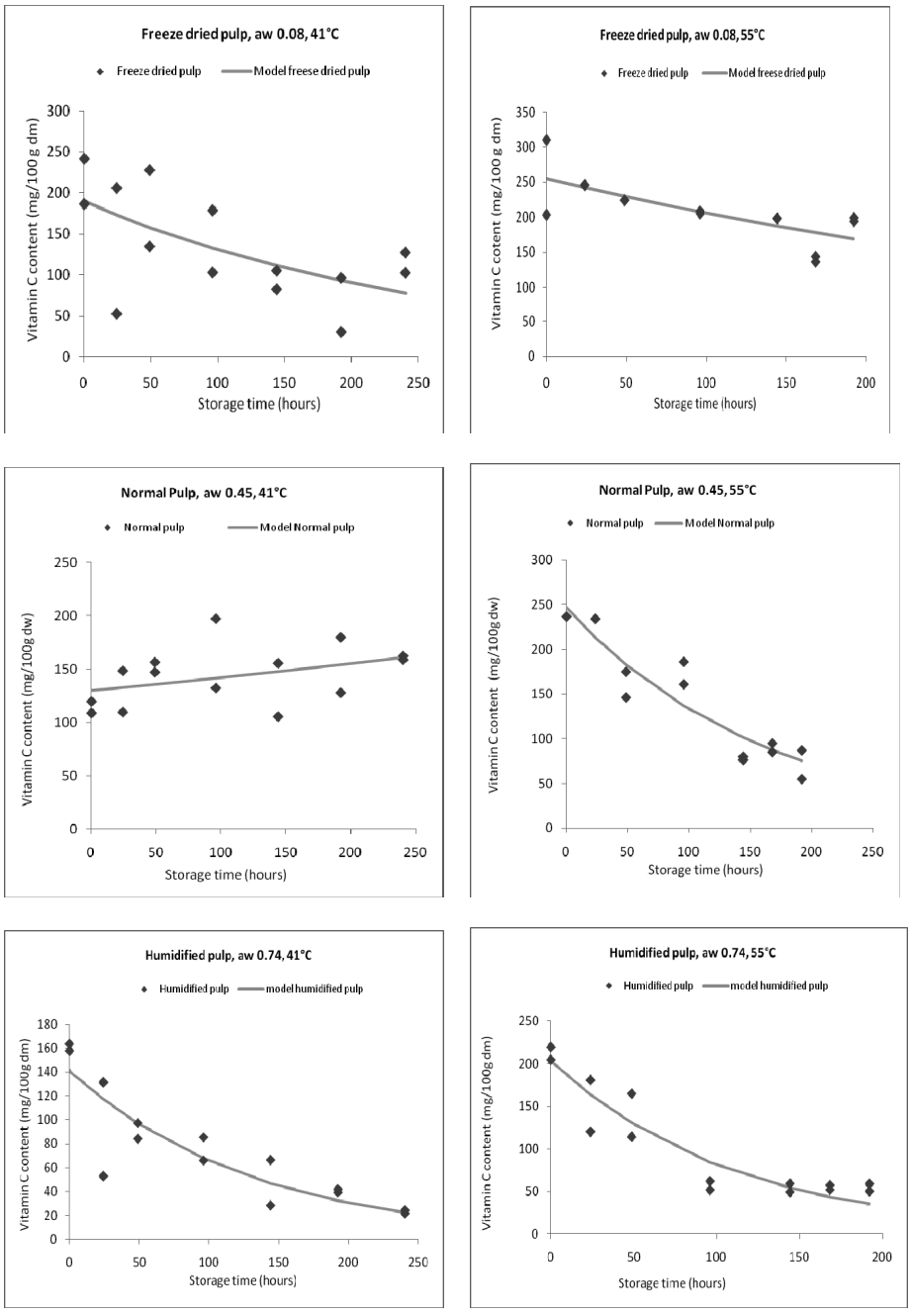

Figure 7.4a Plots of vitamin C degradation during storage of baobab pulp at 41°C and 55°C at different water activities (measured at 20 ºC). Symbols represent experimental data, lines represent the fit of the first-order model

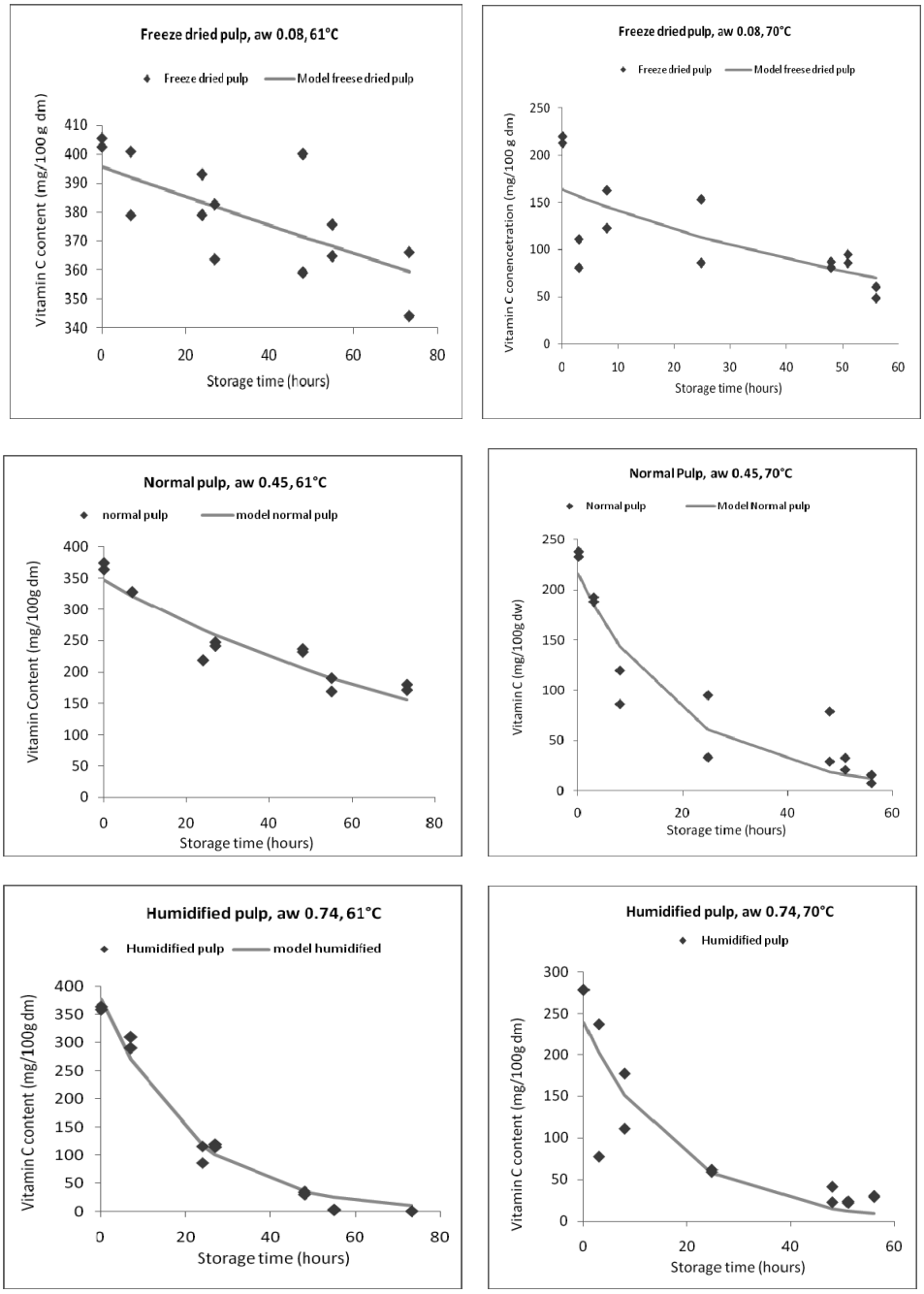

Figure 7.4b Plots of vitamin C degradation during storage of baobab pulp at 61°C and 70°C at different water activities (measured at 20 ºC). Symbols represent experimental data; lines represent the fit of the first-order model

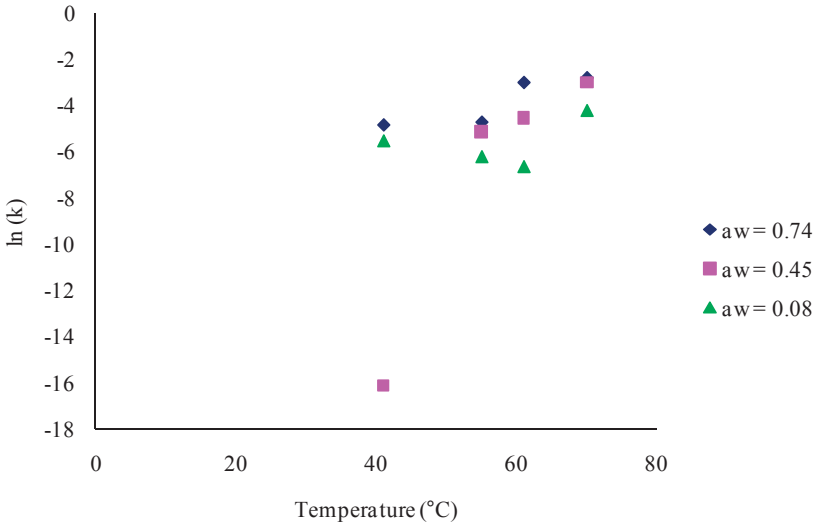

Figure 7.5 Temperature dependence of reaction rate constant *k* at different water activities (measured at 20 ºC)

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Chapter 8

General Discussion

8.1 Introduction

There are still many food and nutrition problems in the developing world, despite the efforts of specialized institutions in the domain. Nutrients deficiencies are partly covered by the picking of natural fruits and vegetables that provide additional food to rural communities especially women and children. It was recognized that Non Timber Forest Products (NTFPs) play an important role in food security, especially in dry years; and that NTFPs play a crucial role in the health and nutrition of people in tropical countries (FAO, 1996). In Benin, among the 3000 identified plants species, 172 are consumed by indigenous people (Codjia *et al.*, 2003). Traditional foods receive remarkably little attention, even though they significantly contribute to the diet of rural people. The baobab is a key forest tree in areas where it occurs naturally and is used daily by rural communities in Africa. Its use for food purposes was studied in this thesis. The baobab parts are claimed to be rich in several nutrients and could in principle help to improve nutritional status among indigenous people of West Africa who frequently consume their derived foods. The present study targeted pulp, seeds, kernels and leaves of the baobab tree and some of their food products with the intention to investigate in how far baobab products can indeed help in alleviating nutritional problems and improve livelihoods of the indigenous populations. In that sense, the following objectives were set in the introduction of this thesis:

i. critically review the nutritional value of baobab parts;
ii. document the ethnofood knowledge related to baobab;
iii. optimize home cooking practices with respect to improve the digestibility and bioavailability of minerals and carotenoids in baobab leaves;
iv. determine the groups of dominant microorganisms in some baobab fermented products as a first step of producing starter cultures by identifying the strains present in *Tayohounta*, fermented decorticated baobab seeds (kernels);
v. build quantitative information on degradation of baobab pulp quality during storage.

To what extent these objectives were reached and what needs to be done next in terms of further research is discussed in this chapter.

As a conclusion from the literature review on the composition and nutritional value of baobab parts, we advise that researchers become more specific with respect to sample collection and analyses of baobab food products. We expect that by doing so the variability in the reported data can be interpreted better. We also recommend to focus nutritional research related to baobab foods on the effect of processing on digestibility and bioavailability for a more meaningful nutritional evaluation of baobab products.
Our study showed that baobab leaf sauce (Figure 8.1) is one of the most important leaf products consumed throughout the year by the village communities in Benin. The end uses of baobab depend on socio-cultural groups and the origin of the latter. However, most of these groups find similar difficulties for baobab processing operations, which means that finding a solution for cumbersome practices will be beneficial for many users.
Leaves are claimed to be rich in several micronutrients, namely Fe, Ca and Zn. Baobab leaves were therefore investigated for in vitro mineral digestibility and bioavailability. However, the slimy nature of the leaves made it impossible to investigate the topic in detail due to experimental difficulties.

Figure 8.1 Baobab leaf sauce

Three hitherto undocumented fermented baobab foods were identified during the survey and subsequently analysed for their nutritional and microbiological properties. The fermented kernels namely *Tayohounta* (Figure 8.2a) and seeds namely *Dikouanyouri* (Figure 8.2b) showed similarities with other seed products. With that respect, they undergo a proteolytic type of fermentation and *Bacillus* spp. is the dominant microorganism. *Mutchayan* (Figure 8.2c) was more acidic.
One important storage problem recorded during the survey was the colour change of baobab pulp during storage leading to a product unsuitable for human consumption. Because baobab pulp is known for its high vitamin C content, both colour change and vitamin C degradation were investigated in different storage conditions. As baobab pulp looses its lightness, its vitamin C content decreases. More accurate data are necessary to build prediction models of baobab pulp quality degradation during storage.

The main outcomes of the present thesis are the following:

- The data reported in literature on the composition of baobab parts vary from one author to another and do not include the effect of processing operations like cooking, drying and fermentation on digestibility and mineral bioavailability (pertains to objective 1).
- Indigenous people from Benin have remarkable knowledge on the food uses of baobab parts. Thirty-five different foods have been recorded. Some difficult processing operations and storage issues (e.g., pulp colour change during storage) were recorded, which need to be improved for a better valorisation of baobab parts (pertains to objective 2).
- The effect of morphotype, cooking and digestion on Ca content of baobab leaves is significant. Fe, Zn and carotenoids content vary significantly from one morphotype to another while the effect of cooking is significant on Fe, Lutein and Betacarotene Cis2.

Ten to thirty per cent of total Ca is soluble while Zn and Fe levels dropped below detection level after in vitro digestion (objective 3).

- The investigation of fermented baobab foods revealed that *Bacillus* spp. were dominant in *Dikouanyouri* (from baobab seeds) and *Tayohounta* (from baobab kernels), while lactic acid bacteria prevailed in *Mutchayan* (from baobab pulp) (objective 4).
- A more detailed characterisation of three samples of *Tayohounta* showed the presence of *Bacillus subtilis, Bacillus licheniformis, Bacillus thermoamylovorans, Lactobacillus fermentum and Streptococcus spp.* in all three samples, whereas other *Bacillus* spp., *Enterococcus*, *Lactobacillus* and *Pediococcus spp.* were found in two of the three samples (objective 4).
- Baobab pulp becomes browner during storage and vitamin C degrades following roughly a first order kinetic reaction. Reactions rates in terms of colour change and vitamin C loss are faster at increasing temperatures and water activity (objective 5).

In the following sections, the outcomes will be discussed from an integrative perspective.

8.2 Reported data on baobab parts and indigenous knowledge

The review on the nutritional value of baobab parts (chapter 2) showed that baobab is a potential source of nutrients that can be provided by its leaves, fruit pulp, whole seeds and decorticated seeds kernels. However, there is a huge variability in the reported data, which may stem from various sources like the origin of the sample (market, field), its purity (mixture of samples, sample from the same tree, contaminated samples, samples from different morphotypes), the storage conditions of the samples during transport to the laboratory and before analysis, the age of the sample, and the respiratory activity of the sample before analysis. The differences in the analytical methods and the degree of precision might also have contributed to the variability in the outcomes. We suggest that care is taken to sample collection and preparation in terms of sample storage and purity, and that more attention is paid to precision during analyses.

Documentation of indigenous knowledge (chapter 3) was done through a survey in January, a period in which the fruit is available but the leaves are scarce. However, leaf powder is available throughout the year. This is positive for the reliability of the answers obtained during the survey because the population can therefore remember the products easily since they are preparing them regularly. The second survey on the consumption of fermented foods (chapter 5) was done in September, a period of shortage for most products, especially pulp. Though we asked respondents to provide answers both on periods of abundance and shortage, it is possible that the collected data reflect the reality less accurately due to memory errors. The documentation of indigenous knowledge on baobab (chapter 3) was also related to processing and preservation of baobab foods. The survey revealed that seed decortication is the most difficult processing operation followed by pulp retrieval. Pulp retrieval as it is practised traditionally is done manually and not in a hygienic manner. The development of appropriate processing devices will be helpful for the processing of baobab fruits in a hygienic way and which could result in better prices on more promising markets.

Figure 8.2a *Tayohounta* balls (fermented baoba kernels)

Figure 8.2b *Dikouanyouri* (fermented baobab seeds)

Figure 8.2c *Mutchayan* (fermented paste)

8.3 Traditional processing practices

8.3.1 Cooking of leaves

Digestibility and bioavailability of hydrophobic compounds such as lutein and β-carotene, can be influenced by the presence of fat. However, due to the sliminess problems mentioned above, it was impossible to find out with the current experimental setup how digestible and bioavailable the lutein and the β-carotene were in baobab leaves via in vitro methods.

Digestibility and availability of iron and zinc can be influenced by several factors. Vitamin C and presence of meat protein are the major factors of importance. Traditional cooking practices can also be determinant depending on the ingredients and their combination. The low digestibility of iron and zinc (chapter 4) was to be expected, as this is the case for most of these nutrients from plant food sources. Proposed improvement techniques include the use of acidulants (mainly ascorbic acid and citric acid) and meat. An investigation about the effect of acidulants on the bioavailability of iron and zinc in foods made from rice (*Oryza sativa*), chickpea (*Cicer arietinum*), green gram (*Phaseolus aureus*) and red gram (*Cajanus cajan*) showed that acidulants such as citric acid (from citrus) and amchur (from *Mangifera indica*) significantly enhanced the bioaccessibility of zinc and iron, both in the raw and cooked form (Hemalatha *et al.*, 2005). One acidulant that may be used in our case is baobab pulp, known for its high ascorbic acid content (chapters 1 and 7). Ascorbic acid can reduce food ferric iron to the better absorbed ferrous iron, thus counteracting its reaction with inhibitors such as

phytic acid and/or its precipitation as ferric hydroxide (Clydesdale, 1983; Hurrell *et al.*, 2006) and consequently improve absorption of non-heme iron present in dietary foods.

The bioavailability of non-heme iron is also enhanced by the presence of meat or its consumption in the diet (Cook and Monsen, 1976; Sharma, 2003; Hurrell *et al.*, 2006). This enhancement is mainly due to the "meat factor" effect induced by the muscles (Clydesdale, 1983; Hurrell *et al.*, 2006). The magnitude of enhancement depends on the type of meat muscle; a 180-200% increase in iron absorption with beef and 100-140% increase with chicken muscle have been reported (Cook and Monsen, 1976; Hurrell *et al.*, 2006). The meat factor effect is due to the protein components of the muscle that probably form iron-peptide complexes preventing iron to bind to phytate and phenolic compounds (present in the food) (Hurrell *et al.*, 2006). However, access to meat protein is getting more and more difficult for the rural poor because of the high meat price on the market and the reduced availability of wild animals in the forests due to intensive hunting. Fortunately, small mammals -mainly rodents- are still easily available and using their meat may help achieving this goal. In addition, most rural populations practice extensive breeding of small animals such as poultry, pork, sheep, and goat. These animals are most of the time sold to overcome periods of hardship. The smallest ones (poultry) can, however, be used occasionally for home consumption.

The choice of cooking practices in rural areas is not determined by their effect on the nutritional value of the food. Cooking practices have been passed on from generation to generation, and have not changed much over the years (Wahlqvist, 2007). Based on our findings (chapter 4), we conclude that the potential of baobab leaves is not fully used in the way that they are prepared at present. The estimated in vitro solubility (IVS) of Ca was used to calculate which quantity of the different leaf powders is necessary to meet the RDI in raw or cooked state (Table 8.1). Assuming that all IVS Ca is bioavailable (it is probably less), it was found that the quantity of leaf powder needed to meet the RDI of Ca cannot be consumed on a single day by a child or a pregnant woman. Thus, these people have to consume other Ca-rich foods in addition to baobab leaf powder sauce to meet their daily requirements. In the villages, people are usually farmers who have the opportunity to produce different types of crops but also have access to various food products from the wild. We suggest that food diversification is used to improve the nutritional status of villagers (Delisle *et al.*, 2003).

Limitations. It will be valuable to proceed with the study on the IVS of minerals and carotenoids and to test various combinations (i.e., addition of different acidulants in different ratios, addition of different types of meat in different quantities, addition of fat in different ratios) to determine the nutritionally advisable practices for baobab leaf consumption. Unfortunately, the constraints of strong viscosity/sliminess of the leaves prevented us to perform more detailed research on the leaves and a solution needs to be found.

In the framework of our research on mineral bioavailability in baobab leaves, we performed an experiment using Caco-2 cells to measure iron uptake in baobab leaves. Bioavailable iron, as assessed by changes in cellular ferritin levels, was determined according to Glahn *et al.* (1996) with minor modifications. Caco-2 cells exposed to digests of raw leaves showed higher levels of cellular ferritin than control treatments without leaf digests (Table 8.2). All ferritin concentrations tended to be higher than the control, suggesting that at least part of the Fe from the leaves was indeed absorbed by the Caco-2 cells, but the considerable standard error from the experiments did not allow statistical significance. The higher the ferritin

Table 8.1 In Vitro Soluble (IVS) Ca and Recommended Daily Intake (RDI) for children (4-8 years) and pregnant women (19-30 years)

Types of leaves	***sweet***				***bitter***	
	slimy		**non-slimy**		**non-slimy**	
	young	old	young	old	young	old
RDI Ca for children (4-8 years) (g/d)[1)]	0.8	0.8	0.8	0.8	0.8	0.8
IVS Ca in raw leaf powder (g Kg^{-1}dm)	1.4	1.9	1.8	2.8	5.8	6.8
Quantity raw leaf powder to cover RDI (g dm)	559.4	423.3	452.0	286.7	137.9	117.6
IVS Ca in cooked leaf powder (g Kg^{-1}dm)	2.0	2.6	2.6	3.6	7.3	9.2
Quantity cooked leaf powder to cover RDI (g dm)	396.0	311.3	303.0	224.1	109.7	86.6
RDI for pregnant women. 19-30 years (g/d)[2)]	1	1	1	1	1	1
IVS Ca in raw leaf powder (g Kg^{-1}dm)	1.4	1.9	1.8	2.8	5.8	6.8
Quantity raw leaf powder to cover RDI (g dm)	699.3	529.1	565.0	358.4	172.4	147.1
IVS Ca in cooked leaf powder (g Kg^{-1}dm)	2.0	2.6	2.6	3.6	7.3	9.2
Quantity cooked leaf powder to cover RDI (g dm)	495.0	389.1	378.8	280.1	137.2	108.2

[1)] Retrieved March 6, 2006 from http://www.fnri.dost.gov.ph/reni/renitable1.htm
[2)] Retrieved March 7, 2006 from http://www.iom.edu/CMS/3788.aspx

content of a product, the more bioavailable the iron. It is unfortunately not easy in our case to make prognoses about the type of leaf powder that would be more suitable for iron bioavailability in the human body. The experiments we performed using Caco-2 cells to measure iron intake were only partly successful because of the viscosity difficulties encountered. The same problem made it impossible to measure IVS of carotenoids because it was impossible to separate the water phase from the organic phase. Moreover, the IVS method used to simulate digestion allowed to measure the nutrients only in the supernatant while both supernatant and pellet material are consumed by the populations.
It is therefore necessary to check whether there are still nutrients, especially iron and zinc, present in the pellet.

8.3.2 Cooking practices related to baobab fermented foods

The production of baobab fermented foods in general, and *Tayohounta* in particular, requires skill and knowledge. Though the steps are known by the producers, the end products are not always identical. The characterisation of products made by 3 different producers of *Tayohounta* (chapter 6) showed considerable variability. The counts of the groups of microorganisms showed that one of the products was significantly different from the two others. The producers used the same processing steps and the same batch of decorticated seeds, but with some differences in the time spent at each processing step. Furthermore, at the end of the preparation process, the product is covered by plant leaves before being stored to ferment. The difference in the cover leaves may induce differences in the microbiological composition. Moreover, during the isolation of microorganisms grown from fermented baobab foods in general, and from *Tayohounta* in particular, some important ones may have been left out. In addition to the above culture-dependent approach, the same *Tayohounta* samples were analyzed by culture-independent molecular methods. Bacterial DNA was extracted and 16s rDNA was amplified by PCR and its amplicon cloned in *E.coli*. Transformants were analyzed for inserted sequences, and this approach resulted in a higher resolution of diversity. Nevertheless, this also confirmed the different microbial composition of the analyzed *Tayohounta* products. A further study of the functionality of dominating microorganisms in the fermentation will be required to select prospective starter strains. Production of starter culture would facilitate large scale production; however, this will be possible only after a certain standardization of the process and the product. We consequently suggest that in the future the production is standardized to facilitate production at a larger scale using starter cultures.

Limitations. The *Tayohounta* samples used to perform microbiological analysis (chapter 6) were one week old and had been freshly collected just at the end of the fermentation process and kept at 4°C. The general rule for laboratory analyses is that the fresher the samples the better it is to prevent possible microbiological and chemical changes. In practice, rural populations are accustomed to dry the product at the end of the fermentation to extend its shelf life. We expect that microbiological changes may occur during the drying process (i.e., other microorganisms might co-predominate together with the *Bacillus* spp.). We suggest that in the future this step is also considered for the analysis to have a better view of the microbiological succession (i.e., the different groups of microorganisms that predominate in the product from the beginning of the production to end of the process i.e. drying step). This will help to understand the process and how the product can be standardised best.

8.4 Consumer preferences in relation to baobab food quality

8.4.1 Consumer preferences for baobab leaves

For consumers, sensory food characteristics are most important. Though the baobab leaves characterised as bitter are nutritionally better than sweet ones (chapter 4), consumers only eat the bitter leaves when the sweet ones are not available. One needs, however, to check whether bitter leaves do not contain toxic compounds at an unacceptable level. The presence of antinutritional factors in baobab leaves has indeed been reported in literature (Andy and Eka, 1985), though the concerned morphotype was not described. Phytic acid content is about 0.04 mg/100 g both in market and field samples. Total oxalic acid ranges from 4.4 mg/100 g in field samples (with 40% soluble oxalate) to 5.3 mg/100 g in market samples (with 37% soluble oxalate). Baobab leaves contain also 37.2 g/100 g (market samples) to 40.4 mg/100 g (field samples) hydrocyanic acid. Tannin levels are 17.8 mg/100 g and 19.8 mg/100 g in market and field samples, respectively. These compounds, though at a non toxic level in baobab leaves, may interfere with the nutrients and possibly decrease their digestibility and availability (Andy and Eka, 1985).

Moreover, sliminess constitutes an important quality criterion for consumers of dishes from baobab leaves. Looking at the experiment with Caco-cells, which mimic human absorption, the results on bitter leaves, which are the least slimy ones, showed that old leaves release more iron than young leaves, either raw or cooked (Table 8.2). Such a conclusion cannot be drawn for other types of leaves, especially the slimy ones which are preferred by the populations.

8.4.2 Consumer preferences for baobab fruit pulp

Pulp colour is an important quality criterion. Consumers prefer pulp with a light whitish colour and dislike pulp with brown colour. However, pulp changes colour during storage and will turn brown. The storage experiment we performed showed that browning reaction goes along with a degradation of the vitamin C present in the pulp. The higher the water activity and the storage temperature of the pulp, the faster the reaction rates in terms of colour change and vitamin C degradation. Moreover, the high vitamin C content of baobab pulp is an explanation to its high antioxidant activity (Vertuani *et al.*, 2002; Besco *et al.*, 2007). Vitamin C degradation could consequently be the reason for the loss of antioxidant capacity by baobab pulp, hence pulp quality degradation.

The consumer's preference in this context coincides with respect to optimal vitamin C and antioxidant intake. Adequate packaging is necessary for pulp storage, preferably in dry and cool environment.

8.5 Implications of the thesis

The implications of the present thesis are the following:

- Seed decortication is the most difficult processing operation mentioned by the population (chapter 3). We suggest that the development of a device that can facilitate such an activity becomes a priority for Benin research institutes for the benefit of poor populations especially women who mostly take care of processing activities.
- Future research on cooking practices of baobab leaves (chapter 4) requires a method to handle the sliminess of baobab leaves in the in vitro determination, so that it can be

Table 8.2 Bioavailability measured as Fe incorporated as ferritin in Caco-2 cells

	Control (without leaf digest)	Sweet, slimy leaves		Sweet, non slimy leaves		Bitter leaves	
		young	old	young	old	young	old
Ferritin content in raw baobab leaves (ng ferritin/µg protein ± Standard Error)	5.1 ± 12.9c	36.3 ± 10.6 abc	19.0 ± 10.6 abc	16.3 ± 12.9 abc	50.2 ± 12.9 abc	14.6 ± 12.9 abc	66.9 ± 10.6 a
Ferritin content in cooked baobab leaves (ng ferritin/µg protein ± Standard Error)		16.9 ± 9.2 abc	16.1 ± 9.2 abc	61.2 ± 9.2 ab	41.2 ± 9.2 abc	9.8 ± 9.2 bc	19.2 ± 9.2 abc

Data represent ng ferritin / µg protein, and are means of duplicate measurements in three replications, with standard error.
Means with the same letter are not significantly different ($p < 0.05$)

better estimated how cooking practices affect mineral and carotenoid availability. Investigations of adding other food ingredients, such as meat, lemon and fat, to baobab leaf sauce are recommended to determine how the best mineral and carotenoid solubility and bioavailability can be obtained to combat malnourishment in vulnerable populations. Once the best practice is found, sensorial tests should follow to fit the recommended combination to the desired taste of the consumers.

- More research on bitter leaves (richer in nutrients than the others) is necessary for technological and nutritional opportunities: wider exploration of their nutrients (other than Ca, Fe, Zn, Carotenoids) and antinutrients content; combination of bitter leaves at different levels with sweet leaves for optimal nutrients uptake, use of bitter leaf powder in other dishes.
- As already started on *Tayohounta* (chapter 6), detailed microbiological studies are necessary for a full characterisation of the *Mutchayan* and *Dikouanyouri* foods as well. These are prerequisite for the development of starter cultures that allow the production of more standardised products in a more hygienic way and with an optimal nutritional composition. Moreover, additional studies should be performed during the drying process of *Tayohounta* and *Dikouanyouri*.
- A repetition of the pulp storage experiment (chapter 7) would enable to build kinetic models that can quantitatively predict how long pulp can be stored in known storage conditions and how storage conditions can be improved to preserve baobab pulp quality.

In conclusion, this research has shown that baobab food products are important for rural populations in that they contribute substantially in required nutrients. The research also showed that there are still many ways in which the nutritional value can be improved. In general, there is a diversity of forest food resources that are integrated in the eating habits of villagers and that can help to improve their nutritional status if used in the right way. Improving traditional processing and preservation techniques will add value to the end products, which can consequently be more beneficial from a nutritional point of view and, in addition, get a better price on the market.

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Summary

In the developing world, many people do not have enough to eat. The number of people who rely for their livelihood on the collection of fruits, leaves and other foods from the wild is high. Among the frequently harvested trees, the baobab tree (*Adansonia digitata* L.) is a key economic tree, a multipurpose, widely-used species, rich in nutrients in its leaves and fruits, used daily by local populations in Africa for food, medicines and rope production. The tree contributes significantly to nutrient supply in those areas where it grows.

However, very few studies have been undertaken on the food use of the tree products. The present thesis focused therefore on indigenous knowledge on baobab foods and their nutritional aspects, because such knowledge is prerequisite for any valorization and improvement of traditional baobab food handling practices. The project aimed at studying the potential of baobab food products to improve food security in rural Benin. The specific objectives of the study were to (i) review the nutritional value of baobab parts, (ii) document ethno-food knowledge and processing of baobab food products in rural areas in Benin, (iii) assess the effect of traditional cooking on in vitro digestibility and bioavailability of minerals and carotenoids in sauce made of baobab leaves, and (iv) characterise the microbiological flora of baobab fermented food products, in particular a flavouring agent called *Tayohounta* (fermented seed kernels).

In chapter 2, a critical literature review showed that pulp from baobab fruits is particularly rich in vitamin C (150 to 360 mg/100 g dw); the leaves are particularly rich in calcium (307 to 2640 mg/100 g dw); the whole seeds and the kernels have a relatively high lipid content, viz. 12 to 33 g/100 g dw and 19 to 35 g/100 g dw, respectively. The pulp and leaves exhibit antioxidant properties with a higher activity in the pulp than in the leaves. The reported data on the composition of baobab parts are, however, very variable. This variability with respect to the nutritional value of baobab parts may have arisen from various factors: the type of sample (e.g. degree of maturity and homogeneity with respect to the origin of the sample, like whether or not obtained from individual trees), the age and the storage conditions of the sample, the treatment before analysis, the analytical methods, a genetic variation and the growing conditions (soil type, chemical composition of the soil, available water, sunlight intensity). The review showed that more attention must be paid to all stages involved in performing analyses before reliable statements can be made on the variation in composition of baobab food products. The review concludes with priorities for further research, namely: 1. accuracy in the application of analytical methods need to be improved, 2. research about digestibility and bioavailability of nutrients in baobab products is needed, 3. the effect of storage and processing on the nutritional value of baobab food products needs to be assessed.

The field survey, described in chapter 3, showed that several baobab foods are available and used; different types of baobab parts are processed, and some difficulties in processing operations and preservation problems were identified. Local populations reported up to 35 baobab food products processed from the leaves (among the most important ones), the pulp, the seeds and the kernels, some fermented foods, most of which have never been described or characterized. Multivariate analysis showed that the types of foods processed from baobab differed among socio-cultural groups. Over the centuries, baobab products have been used for a variety of purposes and local populations have gained rich experience and knowledge in processing techniques, mostly with reference to their locality of origin. Most socio- cultural groups have similar opinions about the difficulty of certain processing operations, in

particular seed decortication, grinding and sieving operations. The survey revealed that seed decortication is considered to be the most laborious processing operation and that the preservation of pulp and kernels is difficult. Seed decortication is a tedious operation and is mainly handled by women in several communities. Because many seeds are very hard to decorticate, a large amount of seeds is thrown away.

In chapter 4, the effect of home cooking on the quality of traditionally processed baobab leaves was evaluated. The different morphotypes of baobab leaves mentioned and recognized by local dwellers were considered. Three major criteria were used to distinguish different types of baobab leaves, namely taste (bitter, vs. sweet), age (young, vs. old) and sliminess (slimy, vs. non-slimy). Assessment of in vitro digestibility and bioavailability of Ca, Fe, and Zn in non-processed and processed different morphotypes of baobab leaves showed that the in vitro solubility of Ca ranged from 10-24% and 13-30% of total Ca in raw and cooked leaves, respectively. Fe (9.2-15.7 mg/100 g dm) and Zn (2.5-4.8 mg/100 g dm) in leaves were much lower than the levels of Ca (1371–3655 mg /100 g dm), and their solubility after in vitro digestion was below detection level. Lutein and beta-carotene were the most important carotenoids. Bitter leaves were the richest in Ca, Fe, lutein, and β-carotene (compared to the sweet ones which are preferred by the consumers) and therefore offer the best opportunities for technological improvements to obtain nutritional benefits. Current cooking practises by local populations need to be improved for optimal Fe, Zn and carotenoids absorption. Though it is known from the literature which treatments can probably improve Fe and Zn bioavailability (e.g., use of acidulants, presence of heme iron) and carotenoids bioavailability (e.g., cooking with fat or fatty products since the carotenoids present in baobab leaves are fat soluble vitamins), it was difficult to proceed with experiments due to the slimy nature of the leaves which make them hard to analyse.

Chapter 5 focused on the characterisation and consumption of fermented baobab products recorded during the survey: *Tayohounta* (fermented kernels, pH = 7), *Dikouanyouri* (fermented seeds; pH = 6.5), *Mutchayan* (sorghum paste with baobab pulp, pH = 4.2). The main groups of microorganisms encountered in *Dikouanyouri* and *Tayohounta* were mesophilic bacteria in equally high numbers in both products (9.5 Log cfu/g), sporeforming bacteria (8.5 and 9.1 Log cfu/g respectively) and lactic acid bacteria (8.9 and 8.4 Log cfu/ g respectively). *Mutchayan* contained mainly lactic acid bacteria (7.6 Log cfu/ g) with a substantial number of yeasts (7.2 Log cfu/ g). Counts in unfermented products were below the minimum level to be considered of relevance. The arbitrary index of protein cleavage, which is an expression of protein degradation due to fermentation (free amino nitrogen / total nitrogen), increased from 2.3% in the unfermented products to 13.7% in *Dikouanyouri* and 21.3% in *Tayohounta*, indicating significant protein degradation. No protein degradation was noticed in *Mutchayan*. The latter is the most frequently consumed baobab fermented product followed by *Tayohounta*, while *Dikouanyouri* is the least frequently consumed one.

In chapter 6, detailed microbiological studies of three different samples of *Tayohounta* showed that total viable counts were around 9 Log cfu/g, made up mainly of vegetative cells of *Bacillus* spp., *Bacillus* spores and lactic acid bacteria (8 Log cfu/g) while yeasts and moulds represented a minor part of the total flora in all samples. Because no starter is added after the product is boiled, the encountered organisms probably originated from the kitchen utensils, cover leaves and from the environment (soil, water and air). Identification of isolated strains showed that there were large differences between the samples made by different producers. *Bacillus subtilis, Bacillus licheniformis, Bacillus thermoamylovorans, Lactobacillus fermentum* and *Streptococcus* spp. were found in all three samples, whereas other *Bacillus* spp., *Enterococcus*, *Lactobacillus* and *Pediococcus spp.* were found in two of

the three. In general, culture-dependent data (i.e. obtained after enumeration and characterization of microorganisms grown on a culture medium) were confirmed by denaturing gradient gel electrophoresis (DGGE) and cloning of DNA amplicons obtained by PCR (molecular techniques). In fact, DGGE patterns of the strains isolated from the samples and mixtures of isolates, and of DNA extracted directly from the samples indicated that *Bacillus* spp. dominate the microbiota of all samples; they also showed that several bands present in one sample were absent in another sample, which confirms that a polyphasic approach, i.e. a combination of culture-dependent and culture-independent techniques is desirable to obtain a good representation of microbiota in *Tayohounta*.

In chapter 7, some additional experiments were reported and discussed on the kinetics of quality degradation of baobab pulp during storage. The water activity of the product, the temperature and the storage duration affected pulp quality in terms of colour change and vitamin C content degradation. Vitamin C degradation followed roughly a first order kinetic reaction with reaction rates faster at higher temperatures and water activity. More kinetic investigations are necessary to build a prediction model that quantifies quality changes during baobab pulp storage. Suggestions are made for future research on baobab foods products.

The results obtained are evaluated and integrated in the general discussion in Chapter 8. The central point of discussion is the role of baobab foods in relation to the nutrition and preferences of rural populations in Benin. Local populations living in areas where baobab occurs, have outstanding knowledge on different morphotypes and food uses of the species. An upgrading of the traditional processing and preservation techniques is required for an optimal bioavailability of nutrients and for an easier and more beneficial processing that can save time, protect the environment and improve income generation to poor populations. Development of starter culture is necessary for controlled fermentation and intensive production of baobab fermented products. The work presented in this thesis gives directions for further research on the potential of baobab foods to improve food shortage and nutritional problems in Benin and more generally in Africa.

Samenvatting

In de derde wereld hebben veel mensen onvoldoende te eten. Het aantal mensen dat voor hun levensonderhoud afhankelijk is van het verzamelen van vruchten, bladeren en andere producten uit de natuur, is hoog. Een boom waarvan regelmatig producten worden verzameld, is de baobab (*Adansonia digitata* L.). Deze boom is belangrijk voor de locale economie en dient meerdere gebruiksdoelen. De bladeren en vruchten van de baobab zijn rijk aan voedingsstoffen, en worden dagelijks gebruikt door lokale bevolkingsgroepen in Afrika als bron van voeding, medicijnen en voor het maken van touw. De boom levert een belangrijke bijdrage aan de voorziening van voedingsstoffen in de gebieden waar hij voorkomt.

Er is echter slechts weinig onderzoek gedaan naar het gebruik van de producten van de baobab als voedingsmiddel. Dit proefschrift richt zich daarom op de lokale kennis van voedingsmiddelen van de baobab en hun voedingskundige aspecten. Dergelijke kennis is een voorvereiste voor valorisatie en verbetering van traditionele bereidingstechnieken. Het project had ten doel om het potentieel te bepalen van voedingsmiddelen van de baobab ter verbetering van voedselzekerheid in de rurale gebieden van Benin. De specifieke doelen van deze studie waren om (i) een overzicht te geven over de voedingswaarde van de producten van de baobab, (ii) de lokale kennis over de voedingsmiddelen van de baobab en hun bereidingswijzen in de rurale gebieden van Benin in kaart te brengen, (iii) de invloed van traditionele bereidingswijzen op de in vitro verteerbaarheid en biobeschikbaarheid van mineralen en carotenoïden in saus gemaakt van bladeren van de baobab te bepalen, en (iv) de microbiologische flora te karakteriseren van gefermenteerde producten van de baobab, met name *Tayohounta*, dat wordt gemaakt door fermentatie van gepelde zaden (nootjes) en dient om gerechten op smaak te brengen.

Uit het literatuuroverzicht in hoofdstuk 2 komt naar voren dat de pulp van baobab vruchten bijzonder rijk is aan vitamine C (150 tot 360 mg/100 g ds), dat de bladeren bijzonder rijk zijn aan calcium (307 to 2640 mg/100 g ds) en de hele zaden en de nootjes een relatief hoog vetgehalte hebben, namelijk respectievelijk 12 tot 33 en 19 tot 35 g/100 g ds. De pulp en de bladeren hebben antioxidatieve eigenschappen met een hogere activiteit in de pulp dan in de bladeren. Echter, de gegevens over de samenstelling van de producten van de baobab zijn zeer divers. Deze uiteenlopende gegevens ten aanzien van de voedingswaarde van de producten van de baobab kunnen door verschillende factoren zijn veroorzaakt: het soort monster (denk hierbij aan rijpheid en homogeniteit qua herkomst van het materiaal, zoals al dan niet afkomstig van individuele bomen), versheid en bewaaromstandigheden van het monster, opwerkingsmethode, analysemethode, genetische variatie en omgevingsfactoren tijdens de groei in het veld (grondsoort en -samenstelling, hoeveelheid water en zonlicht). Het overzicht toont aan dat er veel meer aandacht moet worden besteed aan alle stappen van het onderzoeksprotocol voordat er betrouwbare uitspraken kunnen worden gedaan over de variatie in de samenstelling van producten van de baobab. Het literatuuronderzoek eindigt met prioriteiten voor verder onderzoek, te weten 1. De nauwkeurigheid en precisie van de analysemethoden dienen te worden verbeterd, 2. De verteerbaarheid en biobeschikbaarheid van voedingsstoffen in producten van de baobab moeten worden onderzocht, 3. De invloed van bewaring en bereiding op de voedingswaarde van producten van de baobab moeten worden bepaald.

Het veldonderzoek, dat wordt beschreven in hoofdstuk 3, liet zien dat diverse voedingsmiddelen van de baobab beschikbaar zijn en worden gebruikt; dat verschillende delen van de baobab worden verwerkt, en identificeerde enkele problemen in de verwerking

en bewaring. Lokale bevolkingsgroepen beschreven 35 voedingsmiddelen op basis van baobab bladeren, de pulp, de zaden en de nootjes, en enkele gefermenteerde producten, waarvan de meeste niet eerder zijn beschreven of gekarakteriseerd. Multivariate analyse toonde aan dat het soort voedingsmiddel dat wordt gemaakt van de baobab verschilt per sociaal-culturele bevolkingsgroep. Door de eeuwen heen zijn producten van de baobab gebruikt voor verschillende doeleinden en hebben lokale bevolkingsgroepen een rijke kennis en ervaring opgedaan in bereidingswijzen, meestal gerelateerd aan hun plaats van origine. De meeste sociaal-culturele bevolkingsgroepen hebben overeenkomstige meningen over de problemen met bepaalde verwerkingsstappen, met name het pellen van de zaden, het malen en het zeven. Het veldonderzoek maakte duidelijk dat het pellen van de zaden wordt beschouwd als de meest arbeidsintensieve verwerkingsstap en dat de bewaring van pulp en nootjes moeilijk is. Het pellen van de zaden is een langdurig karwei en wordt voornamelijk door vrouwen gedaan in diverse gemeenschappen. Omdat veel zaden bijzonder moeilijk te pellen zijn, wordt een groot gedeelte weggegooid.

In hoofdstuk 4 wordt de invloed van thuisbereiding op de kwaliteit van traditioneel verwerkte baobab bladeren geëvalueerd. De verschillende typen baobab bladeren, zoals genoemd en onderkend door de plaatselijke bevolking, werden hierbij in acht genomen. Drie belangrijke kenmerken om de verschillende typen bladeren te onderscheiden, zijn smaak (bitter, vs. zoet), leeftijd (jong, vs. oud) en slijmerigheid (slijmerig, vs. niet slijmerig). Bepaling van de in vitro verteerbaarheid en biobeschikbaarheid van Ca, Fe, and Zn in al dan niet verwerkte, verschillende typen bladeren toonde aan dat de in vitro oplosbaarheid van Ca varieerde van 10-24% and 13-30% van de totale hoeveelheid Ca in respectievelijk ongekookte en gekookte bladeren. Gehalten aan Fe (9.2-15.7 mg/100 g ds) en Zn (2.5-4.8 mg/100 g ds) in de bladeren waren veel lager dan de gehalten aan Ca (1371–3655 mg /100 g ds), en hun oplosbaarheid na in vitro vertering was beneden de detectiegrens. Luteïne en beta-caroteen waren de voornaamste carotenoïden. Bittere bladeren bevatten de hoogste gehalten aan Ca, Fe, luteïne, en β-caroteen (vergeleken met de zoete bladeren, die de voorkeur genieten van de consumenten) en bieden daarom de beste kansen voor technologische verbeteringen voor het verkrijgen van voedingskundige voordelen. De huidige bereidingswijzen, zoals toegepast door lokale bevolkingsgroepen, moeten worden verbeterd voor een optimale absorptie van Fe, Zn en carotenoïden. Alhoewel in de literatuur opties worden beschreven voor het verbeteren van de biobeschikbaarheid van Fe en Zn (bijv. door aanzuren en de aanwezigheid van heem ijzer) en de biobeschikbaarheid van carotenoïden (bijv. door te koken met vet of vetrijke producten aangezien de carotenoïden in de baobab bladeren vetoplosbare vitaminen zijn), belemmerde het slijmerige karakter van de bladeren verder onderzoek vanwege het feit dat het materiaal ongeschikt was voor analyse.

Hoofdstuk 5 geeft een karakterisering van de bereiding en consumptie van gefermenteerde baobab producten die tijdens het veldonderzoek werden waargenomen, namelijk *Tayohounta* (gefermenteerde nootjes, pH = 7), *Dikouanyouri* (gefermenteerde zaden, pH = 6,5) en *Mutchayan* (een sorghumpap gemengd met baobabpulp, pH = 4,2). De belangrijkste groepen micro-organismen aangetroffen in *Dikouanyouri* en *Tayohounta* waren mesofiele bacteriën, in gelijke, grote aantallen in beide producten (9,5 Log kve/g), waarin bacteriesporen een belangrijk aandeel hadden (respectievelijk 8,5 en 9,1 Log kve/g), en melkzuurbacteriën (respectievelijk 8,9 en 8,4 Log kve/g). *Mutchayan* bevatte vooral melkzuurbacteriën (7,6 Log kve/g) en een aanzienlijke gistenpopulatie (7,2 Log kve/g). Alle drie producten ondergaan een natuurlijke fermentatie zonder toevoeging van starter; aantallen micro-organismen in ongefermenteerde ingrediënten waren zeer laag. Het gehalte eindstandige aminogroepen geeft een indruk van eiwitafbraak tot kleinere peptiden. De verhouding eindstandige

aminogroepen : totaal stikstof nam toe van 2,3% in ongefermenteerde ingrediënten tot 13,7% in *Dikouanyouri* en 21,3% in *Tayohounta*, hetgeen duidt op een aanzienlijke afbraak van eiwit. Dit gebeurde niet in *Mutchayan*. Laatstgenoemd product wordt het vaakst en in de grootste hoeveelheden geconsumeerd, gevolgd door *Tayohounta*, terwijl *Dikouanyouri* het minst vaak wordt gegeten.

In hoofdstuk 6 wordt nader ingegaan op de microbiologische samenstelling van *Tayohounta*, geproduceerd door drie verschillende kleinschalige producenten. Het totaal aantal mesofiele aerobe bacteriën was ongeveer 9 Log kve/g, voornamelijk bestaande uit vegetatieve cellen van *Bacillus* soorten, terwijl verder aanzienlijke hoeveelheden melkzuurbacteriën werden aangetroffen (8 Log kve/g). Gisten en schimmels waren tevens in kleinere aantallen aanwezig. Aangezien de nootjes eerst worden gekookt en er geen fermentatiestarter wordt toegevoegd, zijn veel van de aangetroffen micro-organismen waarschijnlijk afkomstig van hitteresistente sporen die het kookproces hebben overleefd, en besmetting door keukengerei, handen, enz. Zoals verwacht mag worden, waren er verschillen in microbiota tussen de producten van de verschillende producenten. Echter, in alle producten werden *Bacillus subtilis, Bacillus licheniformis, Bacillus thermoamylovorans, Lactobacillus fermentum* and *Streptococcus* spp. gevonden. Andere *Bacillus* soorten en *Enterococcus*, *Lactobacillus* and *Pediococcus* spp. werden in 2 van de 3 producten gevonden. Het bleek dat zowel kweekmethoden als directe extractie van DNA uit producten de dominante microflora aantoonden, maar ook dat beide methoden soorten opspoorden die door de andere methode niet gevonden werden. Hiermee is eens te meer aangetoond dat de zgn polyfasische onderzoeksaanpak de voorkeur heeft. Er zijn ook een aantal reincultuur experimenten met geïsoleerde stammen uitgevoerd, waaruit voorlopig geconcludeerd werd dat *Bacillus* soorten essentieel zijn in dit product en zorgen voor een zekere eiwitafbraak en vorming van aromastoffen. Andere stammen hadden geen merkbare invloed op de kwaliteit, terwijl sommige gisten en schimmels een mogelijk negatieve invloed hebben, voornamelijk op de geur.

In hoofdstuk 7 wordt nog een aantal experimenten beschreven met betrekking tot kwaliteitsverliezen die tijdens de bewaring van baobabpulp optreden. De kwaliteit, m.n. de kleur en het vitamine C gehalte, van de pulp wordt tijdens bewaring beïnvloed door de wateractiviteit, de bewaartemperatuur en de periode van bewaring. Het verlies van vitamine C verliep ruwweg volgens 1e orde kinetiek en sneller naarmate temperatuur en wateractiviteit hoger werden. Eén en ander zou in voorspellende mathematische modellen kunnen worden beschreven als er meer meetpunten beschikbaar zijn. Aanbevelingen voor verder onderzoek aan baobab voedsel worden gegeven.

De verkregen resultaten worden geëvalueerd en geïntegreerd in de algemene discussie in Hoofdstuk 8. Het centrale discussiepunt is de potentie van baobab als voedingsmiddelenbron in relatie tot de voeding van de rurale bevolking in Benin. Het is gebleken dat de plaatselijke bevolking uitstekend weet welke baobab bomen geschikt zijn voor welke toepassingen. Er is echter behoefte aan een verbetering van de traditionele verwerkings- en bewaringsmethoden teneinde een betere voedingswaarde te krijgen, en tijdrovende methoden kunnen worden verkort waarbij hopelijk het milieu beter wordt gespaard en meer inkomsten kunnen worden gegenereerd door arme plattelandsbewoners. Eén van vele aspecten is dat de kwaliteit van gefermenteerde producten beter kan worden beheerst en gestandaardiseerd door het gebruik van verbeterde startertechnologie. Tenslotte worden aanbevelingen gedaan voor verder onderzoek dat moet leiden tot een vermindering van voedseltekorten en slechte voedingstoestand in Afrika en Benin in het bijzonder.

Résumé

Dans le monde en développement, nombreuses sont les personnes qui n'ont pas suffisamment à manger. Le nombre de celles dépendant de la collecte/cueillette des fruits, feuilles et autres aliments de la nature pour leur bien-être reste élevé. Au nombre des arbres les plus fréquemment exploités en Afrique, le baobab (*Adansonia digitata* L.) occupe une place de choix en raison de son importance économique certaine, de son usage multiple, avec des feuilles et des fruits riches en nutriments. Il est également utilisé quotidiennement par les populations rurales à des fins alimentaires, médicinales et pour la production de matériels d'usage courant comme les cordes.
Le baobab contribue de façon significative à l'apport en nutriments dans les milieux où il pousse. Toutefois, très peu d'études ont été entreprises sur les utilisations alimentaires de ses parties. La présente thèse est par conséquent orientée vers les connaissances endogènes liées aux produits alimentaires du baobab y compris leurs aspects nutritionnels. Ces connaissances constituent en effet un pré requis pour toute valorisation et amélioration des techniques traditionnelles de traitement des aliments issus du baobab. Les objectifs du projet sont de: (i) effectuer une revue de la valeur nutritionnelle des produits issus du baobab ; (ii) capitaliser les connaissances ethno alimentaires et sur la transformation des produits de baobab en milieu rural Béninois ; (iii) évaluer l'effet des techniques culinaires traditionnelles sur la digestibilité in vitro et la biodisponibilité des minéraux et des caroténoïdes dans la sauce à base de feuilles de baobab et (iv) caractériser la flore microbienne des aliments fermentés de baobab et en particulier le *Tayohounta* (amandes fermentés).
Dans le chapitre 2, une revue critique de la littérature a montré que la pulpe de baobab est particulièrement riche en vitamine C (150 - 360 mg/100 g bs) ; les feuilles sont particulièrement riches en calcium (307 - 2640 mg/ 100 g bs) ; les graines entières et les amandes ont une teneur en lipide relativement élevée, respectivement 11,6 - 33,3 g/100 g bs et 18,9-34,7g/100 g bs. La pulpe et les feuilles ont des propriétés antioxydantes avec une activité plus élevée dans la pulpe que dans les feuilles. Les données reportées sur la composition des parties du baobab sont toutefois très variables. Cette variabilité de la valeur nutritionnelle des produits issus du baobab serait due à plusieurs facteurs: le type d'échantillons (degré de maturité et homogénéité de l'échantillon en relation avec son origine c'est-à-dire du marché ou d'arbres individuels); l'âge et les conditions de stockage de l'échantillon; traitements avant analyse; les méthodes analytiques; une variation génétique et l'environnement (type de sol, composition chimique du sol, eau, intensité de la lumière solaire). La revue a montré qu'il faudrait accorder plus d'attention à toutes les étapes des analyses avant de pouvoir conclure sur les variations de la composition des produits de baobab. En plus de la variabilité observée sur les données reportées, la revue a mis en exergue les axes de recherche. Trois recommandations sont formulées. Il est nécessaire : 1. d'améliorer la précision dans l'application des méthodes analytiques, 2. d'entreprendre des recherches sur la digestibilité et la biodisponibilité des nutriments dans les produits de baobab, 3. d'évaluer l'effet du stockage et de la transformation sur la valeur nutritionnelle des produits de baobab.

Dans le chapitre 3, l'enquête de terrain a montré que plusieurs aliments issus du baobab sont disponibles et utilisés; différents types des parties de baobab sont transformés, et les opérations de transformation et de stockage ainsi que leurs difficultés ont été identifiées. Les populations locales ont signalé jusqu'à 35 aliments de baobab transformés à partir des feuilles (qui sont les plus importantes), la pulpe, les graines et les amandes. Quelques produits fermentés dont la plupart n'ont jamais fait l'objet d'études ont également été identifiés.

L'analyse multivariée a montré que les types d'aliments produits à partir du baobab varient d'un groupe socio-culturel à un autre. Pendant des siècles, les aliments du baobab ont été utilisés à de fins diverses et les populations locales ont accumulé de l'expérience et de la connaissance sur les techniques de transformation, qui, en général, varient en fonction de leur localité d'origine. La plupart des groupes socioculturels ont une opinion semblable en ce qui concerne la difficulté de certaines opérations de transformation, en particulier le décorticage des graines, les opérations de mouture et de tamisage. L'enquête a révélé que le décorticage des graines est considéré comme l'opération de transformation la plus difficile et est essentiellement effectué par les femmes dans plusieurs communautés. Une grande quantité de graines est parfois jetée à cause de cette difficulté.

Dans le chapitre 4, il a été évalué l'effet de la préparation domestique sur la qualité des feuilles de baobab traditionnellement transformées. Les différents types morphologiques des feuilles de baobab mentionnés et reconnus par les populations locales ont été considérés. Trois critères majeurs sont utilisés pour distinguer différents types de feuilles de baobab notamment le goût (feuille amère vs. douce), âge (feuille jeune vs. vieille) et viscosité (feuille gluante, vs. non gluante). L'évaluation de la digestibilité in vitro et de la biodisponibilité du Ca, Fe, et du Zn dans différents types de feuilles de baobab non transformées et transformées a montré que la solubilité in vitro du Ca varie de 10 à 24% et de 13 à 30% du Ca total respectivement dans les feuilles crues et cuites. Les teneurs en Fe (9,2-15,7 mg/100 g bs) et en Zn (2,5-4,8 mg/100 g bs) dans les feuilles sont inférieures à celles du Ca (1371-3655 mg/100 g bs) et leur solubilité après la digestion in vitro était en dessous du niveau de détection. La lutéine et le beta-carotène sont les caroténoïdes les plus importants. Les feuilles amères sont les plus riches en Ca, lutéine et beta-carotène (comparées aux feuilles douces qui sont préférées par les consommateurs) et offrent en conséquence les meilleures opportunités d'améliorations technologiques à des fins nutritionnelles. Il est nécessaire d'améliorer les pratiques de préparation actuellement utilisées par les populations locales pour une absorption optimale du Fe, Zn et des caroténoïdes. Bien que la littérature fournisse des informations sur les traitements probables pouvant permettre d'améliorer la biodisponibilité du Fer et du zinc (utilisation des acidulants, présence du fer hémique) et celle des caroténoïdes (préparation avec des matières grasses puisqu'elles sont des vitamines liposolubles), il a été difficile de procéder à des expérimentations en raison de la nature gluante des feuilles qui rendait pénible leur manipulation.

Le chapitre 5 est centré sur la caractérisation et la consommation des produits fermentés de baobab identifiés au cours de l'enquête. Le *Tayohounta* (amandes fermentées, pH =7), le *Dikouanyouri* (graines fermentées ; pH = 6.5), le *Mutchayan* (pâte de sorgho et pulpe de baobab fermentée ; pH = 4.2). Les groupes de microorganismes rencontrés dans le *Dikouanyouri* et le *Tayohounta* sont les bactéries mésophiles en nombre également élevé dans les deux produits (9,5 Log ufc/g), essentiellement des sporulés (respectivement 8,5 et 9,1 Log ufc/g) et les bactéries lactiques (respectivement 8,9 et 8,4 Log ufc/g). Le *Mutchayan* contenait essentiellement des bactéries lactiques (7,6 Log ufc/g) avec un nombre substantiel de levures (7,2 Log ufc/g). Le nombre de microorganismes dans les produits non fermentés était en dessous du niveau minimum considéré. L'index arbitraire de clivage qui est une expression de la dégradation des protéines due à la fermentation (amino azotes libres/ azotes totales) a augmenté de 2,3% (produits non fermentés) à 13,7% dans le *Dikouanyouri* et 21,3% dans le *Tayohounta* indiquant une dégradation significative des protéines. Aucune dégradation des protéines n'a été observée dans le *Mutchayan*.

Dans le chapitre 6, des études microbiologiques détaillées de trois échantillons de *Tayohounta* ont montré que le nombre total de microorganismes viable était d'environ 9 Log ufc/g constitué essentiellement de bactéries lactiques (8 Log ufc/g) pendant que les levures et moisissures représentaient une infime partie de la flore totale dans tous les échantillons.

Puisque qu'aucun ferment n'était ajouté après la cuisson des amandes, les microorganismes rencontrés proviendraient probablement des ustensiles de cuisine, des feuilles de couverture et de l'environnement (sol, eau et air). L'identification des souches de microorganismes isolées a montré qu'il existe une grande différence entre les échantillons, l'un étant très différent des deux autres. *Bacillus subtilis, Bacillus licheniformis, Bacillus thermoamylovorans, Lactobacillus fermentum and Streptococcus spp.* ont été retrouvés dans tous les 3 échantillons, pendant que d'autres (*Bacillus* spp., *Enterococcus*, *Lactobacillus* et *Pediococcus spp).* ont été retrouvés dans deux des trois échantillons. Les différences observées entre les échantillons pourraient être dues aux différences dans l'hygiène, la flore de la peau, la flore présente dans les ustensiles de cuisine utilisées ou sur les feuilles utilisées pour couvrir le produit par les petits producteurs pendant la fermentation. En général, les résultats des techniques utilisant les milieux (comptage, énumération et caractérisation des microorganismes après culture sur un milieu) ont été confirmés par des techniques moléculaires notamment l'électrophorèse sur gel en gradient dénaturant (DGGE) et les techniques de clonage. En effet, les empreintes données par les souches isolées des échantillons et les mélanges des isolats et des empreintes des échantillons ont montré que *Bacillus* spp. domine la flore microbienne totale de tous les échantillons ; ils ont aussi montré que plusieurs bandes présentes dans un échantillon ne le sont pas dans d'autres, ce qui confirme la différence observée utilisant les techniques de comptage et confirme qu'il est nécessaire d'isoler un nombre représentatif de microorganismes des échantillons.

Dans le chapitre 7, des expérimentations additionnelles ont été rapportées et discutées. L'activité de l'eau du produit, la température et la durée du stockage affectent la qualité de la pulpe en termes de changement de couleur et de dégradation de la vitamine C. La dégradation de la vitamine C suit approximativement une cinétique de premier ordre et la vitesse de la réaction est plus élevée au fur et à mesure que la température et l'activité de l'eau augmentent. Des investigations supplémentaires sont nécessaires pour construire un modèle de prédiction sur la cinétique de dégradation de la qualité de la pulpe de baobab au cours du stockage. Des suggestions ont été formulées pour les recherches futures sur les produits alimentaires de baobab.

Les différents résultats ont été évalués et intégrés dans le chapitre 8. Au vu des résultats obtenus, le potentiel des aliments de baobab a été discuté en relation avec la nutrition et des préférences alimentaires des populations rurales.

Les populations locales vivant dans les régions où pousse le baobab ont une connaissance approfondie sur les différents types morphologiques et les utilisations alimentaires de l'espèce. Une amélioration des techniques traditionnelles de transformation et de stockage est nécessaire pour une biodisponibilité optimale des nutriments et pour une transformation plus facile et plus bénéfique qui fera gagner du temps, protéger l'environnement et améliorer les revenus des populations pauvres. Le développement des ferments (starter culture) est nécessaire pour la fermentation contrôlée et une production intensive des produits fermentés de baobab.

Le travail présenté dans cette thèse, donne des directives pour les recherches futures sur le potentiel des aliments issus du baobab à réduire l'insécurité alimentaire et nutritionnelle au Bénin et plus généralement en Afrique.

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Curriculum Vitae

Flora Josiane Chadare was born on July 4th 1976 in Porto-Novo, Republic of Benin. She attended primary and secondary school in Benin and graduated from the secondary school in 1995. In October of the same year, she started her training at the Faculty of Agronomic Sciences of the University of Abomey-Calavi (FSA/UAC) in Benin and graduated in December 2000 as "Ingénieur Agronome" with a specialization in Nutrition and Food Science. From 2003 to 2005, she followed a Master of Science programme at the University of Gent and the Catholic University of Leuven in Belgium. Upon completion, she was granted a PhD fellowship under the Netherlands Fellowship Programme (NFP) to undertake PhD research at Wageningen University, The Netherlands. She carried out the research presented in this thesis from January 2006 to December 2009, alternately in Benin (3 years) and at Wageningen University (1 year). She was awarded a Storm van der Chijs stipend from Wageningen University in 2008; a Dr. Zwartz foundation in 2009 and LEB fonds in 2010.

Publications

Full papers

1. Assogbadjo A.E., Glèlè Kakaï R., **Chadare F.J.**, Thomson L., Kyndt T., Sinsin B. and van Damme P. (2008), Folk Classification, Perception, and Preferences of Baobab Products in West Africa: Consequences for Species Conservation and Improvement, *Economic Botany,* 62, 1, 74-84.

2. Assogbadjo A., Kyndt T., **Chadare F.J.**, Sinsin B., Gheysen G., Eyog-Matig O. and van Damme P. (2009), Genetic fingerprinting using AFLP cannot distinguish traditionally classified baobab morphotypes, *Agroforestry Systems,* 75, 2, 157-165.

3. **Chadare F.J.**, Gayet D.P., Azokpota P., Nout M.J.R., Linnemann A.R., Hounhouigan J. and 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, 1-19.

4. **Chadare F.J.**, Hounhouigan J.D., Linnemann A.R., Nout M.J.R. and van Boekel M.A.J.S. (2008), Indigenous knowledge and processing of *Adansonia digitata* L. food products in Benin, *Ecology of Food and Nutrition,* 47, 4, 338-362.

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

Papers submitted or in preparation

1. **Chadare F.J.,** Hooiveld G.J.E.J., Linnemann A.R., Nout M.J.R., Hounhouigan J.D. and van Boekel M.A.J.S. Carotenoid content and mineral solubility in *Adansonia digitata* (baobab) leaves (submitted)

2. **Chadare F.J.,** Jonkman J., Wolkers-Rooijackers J., Nout M.J.R., Hounhouigan J.D. and Zwietering M. Microbiological composition of Tayohounta, a fermented baobab flavour food of Benin (submitted).

Proceedings

1. **Chadare F.J.**, Gayet D.P., Azokpota P., Nout M.J.R., Linnemann A.R., Hounhouigan J.D. and van Boekel M.A.J.S. (2009), *Mutchayan*, *Dikouanyouri* and *Tayohounta*: three baobab fermented foods from Benin, *Actes du deuxième coloque de l'Université d'Abomey-Calavi.* May 2009, Abomey-Calavi, Benin.

2. **Chadare F.J.**, Linnemann A.R., Hounhouigan J., Nout M.J.R. and van Boekel M.A.J.S. (2007), Baobab pulp: a review of its chemical composition and nutritional value, *Actes des 10ièmes journées scientifiques de la SOACHIM*. August 2007, Cotonou, Benin.

Abstracts

1. **Chadare F.J.**, Gayet D.P., Azokpota P., Nout M.J.R., Linnemann A.R., Hounhouigan J. and van Boekel M.A.J.S. (2009), Three hitherto undocumented fermented baobab foods from Benin: *Mutchayan*, *Dikouanyouri* and *Tayohounta*. Preparation, properties and consumption, *Book of abstracts of the EFFoST 2009 conference "New Challenges in Food Preservation: Processing – Safety – Sustainability"*, page 298. 11-13 November 2009, Budapest, Hungary.

2. **Chadare F.J.**, Hounhouigan J.D., Linnemann A.R., Nout M.J.R. and van Boekel M.A.J.S. (2009), Indigenous knowledge and processing of *Adansonia digitata* L. food products in Benin, *Book of abstracts of the International Conference on Human Ecology*, page 37. 29th June – 3rd July 2009, Manchester, UK.

Posters

1. **Chadare F.J.**, Gayet D.P., Azokpota P., Nout M.J.R., Linnemann A.R., Hounhouigan J.D. and van Boekel M.A.J.S. (2009), *Mutchayan*, *Dikouanyouri* and *Tayohounta*: three baobab fermented foods from Benin. *Deuxième coloque de l'Université d'Abomey-Calavi.* Mai 2009, Abomey-Calavi, Bénin.

2. **Chadare F.J.**, Gayet D.P., Azokpota P., Nout M.J.R., Linnemann A.R., Hounhouigan J.D. and van Boekel M.A.J.S. (2009), Three hitherto undocumented fermented baobab foods from Benin : *Mutchayan*, *Dikouanyouri* and *Tayohounta*. Preparation, properties and consumption. *EFFoST 2009 conference "New Challenges in Food Preservation: Processing – Safety – Sustainability"*. November 2009, Budapest, Hungary.

3. **Chadare F.J.,** Jonkman J., Wolkers-Rooijackers J., Nout M.J.R., Hounhouigan J.D. and Zwietering M. Microbiological composition of Tayohounta, a fermented baobab flavour food of Benin. *22nd International ICFMH Symposium, Food Micro 2010*. August-September 2010, Copenhagen, Denmark.

Overview of completed training activities

Courses

Course name	Organization, place	Period
People and plants	MG3S, Wageningen	February 2006
Food and nutrition interventions	Ghent University, Ghent	April-June 2006
Formation Internationale en Nutrition et Sciences Alimentaires (FINSA). Topic : «Planification des programmes communautaires de nutrition »	Faculty of Agronomic Sciences, University of Abomey-Calavi, Abomey-Calavi	August–September 2007
Food perception and food preference	VLAG, Wageningen	November 2007
Genetics and physiology of food-associated microorganisms	VLAG, Wageningen	December 2007
Food Fermentation	VLAG, Wageningen	April 2008
Reaction kinetics in food science	VLAG, Wageningen	October 2009

Meetings

Meeting type	Organization, place	Period
Africa Symposium. Topic: "Does the export of agricultural products bring more development to Africa?"	OXFAM-Belgium, Brussels	April 2006
Xième Journées scientifiques de la SOACHIM	SOACHIM-Benin , Cotonou	August 2007
Deuxième Colloque de l'Université d'Abomey-Calavi	University of Abomey-Calavi, Abomey-Calavi	May 2009
EFFoST conference 2009	EFFoST, Budapest	November 2009
22nd International ICFMH Symposium, Food Micro 2010	Food micro, Copenhagen	August to September 2010

General courses

Course name	Organization, place	Period
Information literacy	WGS, Wageningen	February 2006
Philosophy and ethics of food Science & technology	VLAG, Wageningen	February-March 2006
VLAG PhD week 2006	VLAG, Wageningen	March 2006
Writing a research project	University of Ghent, Ghent	May-June 2006
Introduction to Wageningen UR digital library	WUR library, Wageningen	October 2007
Career orientation	WGS, Wageningen	May-June 09

Optional

Activity	Nature of activity/place	Period
Preparation PhD research proposal	Research planning / Wageningen	January-March 2006
Preparation Codesria grant proposal	Fundraising / Abomey-Calavi	March 2007
PhD study Trip	Networking / USA	April 2007
Preparation application Storm van der Chijs stipend	Fundraising / Wageningen	2008