African Journal of Biotechnology Vol. 7 (7), pp. 916-922, 3 April, 2008
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2008 Academic Journals

Full Length Research Paper

Technology and physico-chemical characteristics of Bikalga, alkaline fermented seeds of *Hibiscus sabdariffa*

Charles Parkouda[1], Bréhima Diawara[1] and Labia Irène Ivette Ouoba[2]*

[1]Département de Technologie Alimentaire (DTA/IRSAT/CNRST), 03 BP: 7047 Ouagadougou 03, Burkina Faso.
[2]Microbiology Research Unit, Department of Health and Human Sciences, London Metropolitan University, 166-220 Holloway Road, London N7 8DB, United Kingdom.

Accepted 18 January, 2008

Bikalga is a product of traditional alkaline fermentation of *Hibiscus sabdariffa* used as food condiment in African countries including Burkina Faso. Its production process was studied at different production sites of Burkina Faso where the different steps of the technology were recorded, specific diagram of production of each production site was established and a general flow diagram proposed. The changes in pH throughout the fermentation was measured and the proximate composition of unfermented and fermented seeds was determined. The presence of minerals in both unfermented and fermented seeds was also determined by spectrophotometry. The production of Bikalga involves many steps with the most important being cleaning, cooking (with addition of alkalinizing ash leachate), fermentations, steaming and drying. During the process, pH increased with cooking and fermentation (pH 8 - 9), then decreased mainly with steaming and drying (pH 6 in some cases). The studied raw seeds were composed of 28.69 ± 1.15% of crude proteins, 21.93 ± 0.74% of crude lipids, 26.39 ± 1.03 % of carbohydrates and the fermented seed showed 26.47 ± 1.5 for proteins 23.19 ± 1.25 for crude lipids and 13.7 ± 0.62 for carbohydrates. An increase of moisture and ash content as well as total titratable and fat acidity occurred with the fermentation. Variable minerals were detected in the seeds with an outstanding increase (in some cases) of potassium and a pronounced increase of sulphur, sodium and calcium in the final product.

Key words: *Hibiscus sabdariffa*, alkaline fermentation, Bikalga, technology, proximate composition.

INTRODUCTION

Bikalga is a food condiment obtained by a traditional uncontrolled fermentation of *Hibiscus sabdariffa* seeds in African countries, including Burkina Faso, Mali Niger, Nigeria, Cameroon and Sudan among others. It is also known as dawadawa botso (Niger), datou (Mali), Furundu (Soudan), Mbuja (Cameroon). *H. sabdariffa* is an herbaceous plant also known as karkade, roselle, graines d'oseille and guinean sorrel. Seeds of *Hibiscus cannabinus*, *Hibiscus asper*, *Hibiscus esculentus Adansonia digitata* and *Ceiba pentandra* are also sometimes used along with *H. sabdariffa* seeds to produce Bikalga in certain areas of Burkina Faso. Bikalga is mainly produced by women and constitute an economical source for the producers. It is mainly produced in areas of Burkina Faso where *Parkia biglobosa* seeds for production of Soumbala (alkaline food condiment) are not commonly found. Soumbala and Bikalga are the most popular food condiments in Burkina Faso; they are used as meat replacement mainly by low-income population. Bikalga is also used by some ethnic tribes to cure high blood pressure, diarrhoea, and rubella or is used as an antiseptic.

Recent studies showed that the main microorgaminsms involved in the fermentation of *H. sabdariffa* for Bikalga production are *Bacillus* species especially *B. subtilis* (Ouoba et al., 2007). Other species of *Bacillus* such as *B. licheniformis*, *B. pumilus*, *B. cereus, B. badius*, *B. sphaericus* and *B. fusiformis* are also commonly found in

*Corresponding author. Email: I.Ouoba@londonmet.ac.uk or ouobairene@hotmail.com. Tel: +44 020 7133 2155. Fax: +44 020 7133 2571.

the fermented seeds (Ouoba et al., 2007).

Many studies have been focused on the agronomy, the conservation, the culture, the botany and the physico-chemical characteristic of mainly the raw seeds of *H. sabdariffa* (Al Wandawi et al., 1983; El Adawy and Khalil, 1994; Lakshimi et al., 1995; Pu, 1996). However very few published studies were done on the fermented seeds. The aim of this work was to study in detail the technology of Bikalga in different places of Burkina Faso, and then to establish a general flow diagram of production and to determine the biochemical characteristic of the product. This is in view of an optimization of the processing condition leading to a final product with improved nutritional and hygienic quality as well as improved stability. In the present work details on the technology and physico-chemical characteristics of Bikalga are presented. Detailed evolution of the pH throughout the fermentation is fully described and the significant impact of the addition of a traditional alkalinizing product on the minerals content of the final product is also clearly shown.

MATERIAL AND METHODS

Technology of Bikalga

Productions sites were identified in different places of Burkina Faso (Béléhédé, Banfora, Bazoulé, Bobo Dioulasso, Dori, Galcépaté, Gaoua, Gourcy, Hamdalaye, Kargounol, Koupéla, Mansila, Ouagadougou, Pensa, Tamlili and the production process followed through out the fermentation. A flow diagram of each site was established, and then a general diagram for all production sites was proposed.

Sampling

Unfermented and fermented seeds of *H. sabdariffa* were sampled (50 - 100 g) at different production sites and analyzed for their physico-chemical composition. Between two and 12 samples were taken per production site and analysed at least in duplicate.

Determination of the pH

As done for fermented seeds of African locust beans (Ouoba et al., 2005), pH of unfermented seeds of *H. sabdariffa*, fermenting seeds and Bikalga were measured directly in a mixture prepared with 10 g of sample and 30 ml of distilled water previously mixed in a stomacher (Stomacher 400 Lab Blender, London England) at normal speed for 1 min. A glass electrode pH meter (Consort, Thurnhout, Belgium) was used for the measurements.

Proximate composition

The methods of analysis followed were those described by the Association of Official Analytical Chemists (AOAC, 1995). The ash was determined by incineration of known weight of samples at 550°C until as was obtained (usually overnight). Protein (N x 6.25) was determined by the macro-Kjeldahl method. The lipid composition was determined by exhaustively extracting a known weight of sample with hexane using a Soxhlet apparatus. Moisture content was determined by drying a sample at 105°C overnight and then dry matter was calculated. Total titratable acidity and fat acidity were also determined according to AOAC methods (1995). The values were expressed as mg of KOH per 100 g of material.

Minerals contents

Analysis of minerals in unfermented and fermented seeds of *H. sabdariffa* was achieved according to procedures described by Walinga et al. (1989). The investigated minerals included potassium, sodium, phosphorus, iron, zinc, nitrate, ammonium, sulphur and copper. The sample (1 g) was digested with 4 ml of a mixture (ratio 7:1) of perchloric acid ($HClO_4$, 60%)/concentrated sulfuric acid and 15 ml of concentrated nitric acid. After complete digestion, the product was cooled down, filtrated and the volume adjusted to 50 ml. For the determination of calcium, 0.2 ml of the filtrated solution was diluted with 4.8 ml of lanthane (La_2O_3, 1%). For the other minerals the dilutions were made with distilled water. Sodium and potassium were measured using a flame photometer (Corning 400, Essex, England); phosphorus was determined with a Skalar auto analyzer (Skalar, Breda, The Netherlands) and all other minerals with and atomic absorption spectrophotometer (Perkin Elmer Analyst 100).

RESULTS

Technology of production of Bikalga

In Figure 1 the steps of the production of Bikalga are shown.

Preparation of raw material

Raw seeds were pre-processed before the real production step. The pre- processing consists of a prolonged sun - drying followed by a selection by manual sorting. The sorted seeds were then dehulled manually by pounding; manual winnowing eliminated the impurities and the seeds repeatedly washed with water (02 or 03 times). The water cleaning step is in fact a sorting by gravity in the sense that immature and spoiled seeds as well as other light impurities floated while heavy impurities (stones, sand) deposited as sediment. According to producers, this stage confers to the final product a good presentation.

Cooking

After the initial cleaning process the seeds were cooked for 12 to 24 h according to the intensity of fire. Seeds were considered as well cooked when soft and easily crushed with fingers. To accelerate cooking and softening process, ash leachate commonly considered as traditional form of potash was often added, increasing then the pH of the preparation. This alkalinizing product was obtained from the leachate of ash of the stems of sorghum or millet or maize or any wood from variable trees and plants, affecting probably the composition of the leachate that could contain largely potassium compo-

.

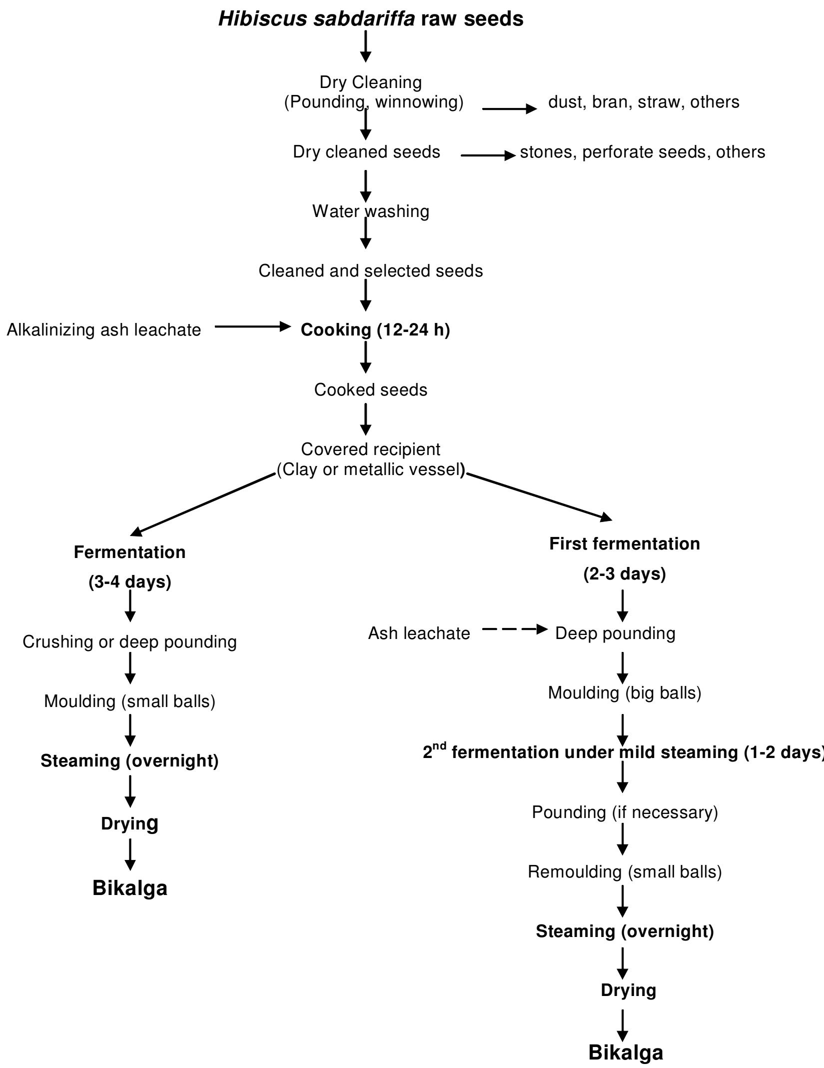

Figure 1. Flow diagram of the technology of Bikalga.

nents or any other alkalinizing compounds as sodium derivate. It was anticipated that the addition of the ash leachate allows a further cleaning by creating an overflow of moss, containing remaining light impurities that could then be easily eliminated. This step is laborious, time and energy consuming.

Fermentation

After a complete cooking, water was drained in traditional baskets and the seeds stored in covered containers (jars, pots, dishes and other containers) for fermentation. Some producers add green leaves of *Lannea microcarpa* and ash at the bottom of the containers for the fermentation. Depending on the producer the fermentation was done at once or in two steps. In the first case, seeds were let to ferment at once for 3 - 4 days according to the intensity of the desired ammonia-like flavour displayed by the fermented seeds. Then the fermented seeds were crushed or deeply pounded, moulded into small balls and steamed overnight.

However for most producers, fermentation takes place in two phases: during the first phase, seeds were put in baskets and let for fermentation for 2 - 3 days. During this phase, they were stirred repeatedly and acquired a mild ammonia-like odour. They were then pounded nearly to paste in a mortar with sometime addition of ash leachate. The product obtained was divided into big parts, moulded into balls, put in a hermetically closed pot containing a small quantity of water in the bottom (water is covered with any hard material before the addition of the balls on top) and let for a second fermentation for 1 - 2 days under a light wet heating (created by the presence of water in the bottom of the pot). Sometimes, after the first day of this second phase, the balls were removed (usually in the

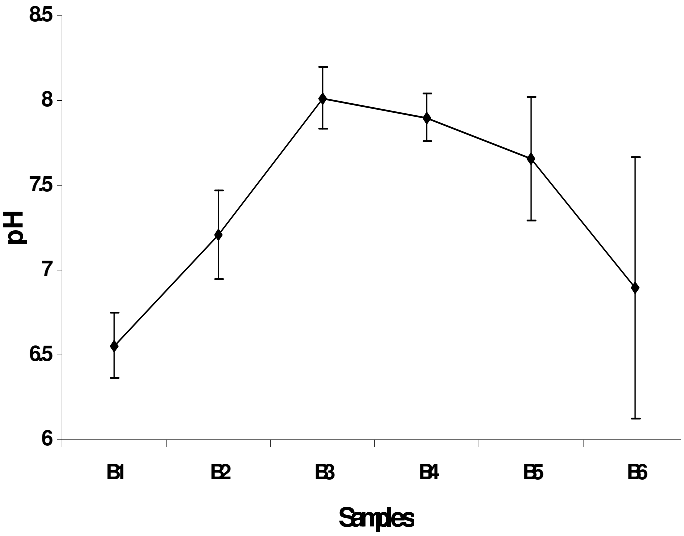

Figure 2. Evolution of pH during the fermentation of *Hibiscus*, *sabdariffa* for Bikalga production. Samples: B1: raw seeds B2: after Cooking, B3: after first fermentation, B4: after second fermentation, B5:

morning), pounded again and put back in the previous pot for fermentation until evening (about 12 h). At the end of the second fermentation the product acquired a very pronounced and stronger ammonia-like odour. The balls were then kneaded together, remoulded into small balls and steamed overnight. A particular attention was given to this steaming stage as producers said it determines both the acceptability and the conservation length of the final product.

Processing of fermented seeds

After fermentations, crushed seeds or moulded dough were always sun dried for one to three days according to the season (intensity of sunshine). Producers said it is a very tiring stage because they must keep changing the position of balls to enable a good drying. In some cases, dried Bikalga was roasted conferring to the final product specific organoleptic characteristics.

Utilisation

Despite Soumbala that is directly added to foods, Bikalga is first steeped in water (preferably warm-like water) for few minutes, then the steeping water is used to prepare stews, soups, sauces and other foods as desired by the consumer.

Evolution of pH during the fermentation

As it can be seen in Figure 2 the change in pH was variable throughout the fermentation. A first increase was observed after cooking followed by a more pronounced increase over pH 8 after the first fermentation. After the second fermentation, the pH rather stayed stable but a slight decrease was sometimes observed. The decrease became much pronounced after the last steaming process but the pH was still alkaline in all cases. With the drying process a continuity of the decreasing process was still observed with however very strong variability according to the sample. Some samples still showed alkaline pH (about 7.7 for the most alkaline) while for other the pH felt sometimes to acidic value (about pH 6.1 for the most acidic sample). Simultaneously to the pH decrease observed, an increase of total titratable acidity (about 78%) and especially fat acidity (about 89%) was noticed in Bikalga compare to the unfermented seeds.

Proximate composition and minerals profile

The proximate compositions of unfermented and fermented seeds (Bikalga) of *Hibiscus sabdariffa* are shown in Table 1. Quantitatively the most important components were proteins, carbohydrates and lipids. Moisture content increased in the fermented seeds. The content of crude protein decreased only slightly if at all with the fermentation while as slight increase of crude lipid content was observed. A very significant decreased (about 50%) for total carbohydrates were observed in the final product. In the other hand, ash content showed a substantial increase in fermented seeds. Table 2 gives and idea of some minerals found in the studied seeds. The values of those minerals were significantly variable especially for fermented seeds. In the various raw seeds studied, iron, ammonium, nitrate followed by zinc and sodium were quantitatively the major minerals. In the final product the highest increase was observed for potassium (more than 2500 mg/kg was brought by the process). A substantial increase was also observed in some case for sulphur, sodium and calcium.

DISCUSSION

The technology of bikalga like most of the Africa fermented food is a laborious, energy and time consuming process. The process is traditional, uncontrolled with no standards, leading to the variability of the nutritional and hygienic qualities as well as the stability of the final product (Sanni, 1993). The long cooking time observed during the production of Bikalga was also observed during the fermentation of *Parkia biglobosa* for Soumbala production (Diawara et al., 1998). This constitutes a first step of selection for heat and alkali resistant bacteria as *Bacillus* species (Ouoba et al., 2007). The second fermentation under steaming as well as the final steaming constitutes a further selection of the pre-cited bacteria. During the production of Furundu, and Mbuja fermented seeds of *Hibiscus sabdariffa* respectively from Sudan and

Table 1. Composition of unfermented and fermented seeds of *H. sabdariffa*.

Composition	Unfermented seeds	Fermented seeds
% Moisture	7.05 ± 1.5	8.24 ± 2.7
% Total proteins	28.69 ± 1.15	26.47 ± 1.5
% Crude lipids	21.93 ± 0.74	23.19 ± 1.25
% Total carbohydrates	26.39 ± 1.03	13.7 ± 0.62
% Ash	6.32 ± 1.44	9.03 ± 1.90
Total titratable acidity (mg of KOH/100g of sample)	0.33 ± 0.12	1.52 ± 0.13
Fat acidity (mg of KOH/100 g of sample)	2.03 ± 021	19.43 ± 2.14

Table 2. Minerals content of unfermented and fermented seeds of *H. sabdariffa*.

Minerals	Unfermented seeds (mg/kg)	Fermented seeds (mg/kg)
Calcium	2.79 - 2.95	3.45 - 286
Phosphorus	1.26 -1.49	1.49 -17.00
Iron	116.34 - 438.81	11.4 -175.28
Potassium	1.65 - 1.88	4.81 - 2800
Sodium	41.90 - 52.19	109.53 - 336
Zinc	83.41 - 85.09	0.97 - 90.79
Nitrate	91.97 - 245.24	34.9 - 61.31
Ammonium	148.27 - 349.58	15.4 -141.40
Sulphur	1.25 - 1.30	1.71 - 364.30
Copper	15.47 - 19.64	1.5 - 18.96

Cameroon, the seeds are cooked for a much shorter time (about 3 h) (Yagoub et al. 2004; Mohamadou et al., 2007) making them less accessible for the action of degrading enzymes (Harper and Collin, 1992). Differences also appears in the fermentation process of Bikalga compare to the precited similar products. For Furundu, fermentation is done at once and lasts longer (7 - 10 days) (Harper and Collin, 1992; Yagoub et al., 2004). For Mbuja, fermentation takes place in two steps: seven days for the first fermentation, then the seeds are pounded and let to ferment again for three days (Mohamadou et al., 2007). In both cases, no steaming step occurs during the process.

So far, to our knowledge this is the first work describing in details the variation of pH during the fermentation of *H. sabdariffa* for Bikalga production. The first increase after cooking was probably related to the addition of the alkalinizing leachate but also to the leaching of acidic component in the cooking water according to Yagoub et al. (2004). The further increase after the first fermentation might be due to the proteolytic activity of the *Bacillus* isolate responsible of the fermentation (Ouoba et al., 2007) which degraded proteins into amino acids, a part of which was further degraded leading to production of components as ammonia responsible of the pH increase and the ammonia like-flavour. It is a common scenario observed during *Bacillus* fermentation (Odunfa, 1985; Aderibigbe and Odunfa, 1990; Allangheny et al., 1996; Ouoba et al., 2003). The stabilization of pH during the second fermentation could be explained by a deceleration of the proteins metabolism. This deceleration was simultaneous or followed by an increased degradation of carbohydrates and lipids leading to a high production of acidic compounds responsible of the pH decrease during the final steaming and the drying processes. Enzymatic activities likely continued after fermentation. An evaporation of ammonia during the steaming and drying processes might have also highly contributed to the pH decrease. Harper and Collin (1992) reported an increasing production of acids such as lactic acid and especially acetic acid accompanied with a decrease of pH during the production of Furundu. Volatiles fatty acids as well as traces of propionic acids were also detected. Moreover Odunfa (1985) and Ouoba et al. (2003) reported that *Bacillus* proteolytic activity decreased after 36 – 48 h of fermentation for Soumbala production.

The values of all proximate components were in agreement with earlier studies especially the raw material (Harper and Collin, 1992; Abou-tarboush et al., 1997; Omobuwajo et al., 2000; Yagoub et al., 2004; Bengaly et al., 2006). The moisture content increased in the fermented seeds probably related to the long cooking period. The slight decrease in the content of crude protein can be attributed to a little loss of nitrogen according to Harper and Collin (1992) whose results showed similar change during the fermentation of Furundu. However Bengaly et al. (2006) reported an increase of 5% of crude protein during fermentation of *H. sabdarrifa* to produce Bikalga. The slight increase of crude lipid content has been also observed in Furundu even though it was reported as not significant (Harper and Collin, 1992; Abou-tarboush et al., 1997). According to Ikenebomeh et al. (1986) and Ibrahim and Antai (1986), who reported an increase of lipid content during the fermentation of African locust beans for Soumbala production, this is due to a selective utilization of carbohydrate by the microflora during the fermentation. In fact, a very significant decreased (about 50%) for total carbohydrates were observed after fermentation. This decrease that has been observed for Furundu (Yagoub et al., 2004) as well as Soumbala (Ibrahim and Antai, 1986), might be mainly linked to two factors: the long cooking where a certain amount of soluble carbohydrates was lost in the cooking water and the fermentation where carbohydrates were hydrolyzed into reducing sugars easily utilizable by the microorganisms as source of energy (Odunfa, 1985; Yagoub et al., 2004). Ash content showed a substantial increase in fermented seeds which reflects mainly the mineral contribution made by liberal add of ash-leachate. The significant variation in minerals content can be related to the type of soil from which the seed were harvested in the case of unfermented seeds and mainly to the addition of ash-leachate for the fermented seeds. In fact the amount and type of the alkalinizing leachate to be added as well as the precise step during the process where it should be added varied significantly from one producer to another according to the organoleptic characteristics expected. Harper and Collin (1992) reported that dried leachate of ash from sorghum is largely composed of potassium bicarbonate with smaller quantities of potassium chloride, silicate and sulphates, explaining then the increase and the large variation for some minerals especially potassium. Leachate from other plants might be composed differently by large amount of other alkalinizing compounds as sodium, calcium, iron derivates leading then to different mineral content and balance. Another origin of minerals could be attributed to the fermentation recipients (Harper and Collin, 1992).

In conclusion, the fermentation of *H. sabdariffa* for Bikalga production is a process where proteolysis, lipolysis as well as degradation of carbohydrates seem to be equally important, each metabolism being predominant at a specific time. For most *Bacillus* alkaline fermentations, proteolysis is the most important metabolism throughout the fermentation (Odunfa, 1985; Wang and Fung, 1996; Diawara et al., 1998). There is need to undertake other studies in order to standardize first the production process of Bikalga, then determine hazards and critical control points during the process, and propose adequate corrections. Good Manufacturing Practices (GMP) as well as a quality system as HACCP should be establish for Bikalga as it has already be done for other African products as Soumbala (Diawara et al., 1998) and Kenkey (Amoa-Awa et al., 2007). Studies should also address a selection of starter cultures for controlled fermentation of *H. sabdariffa* leading to a final product with increased nutritional and hygienic qualities as well as increased stability. Due to the fact that Bikalga has been often reported locally (in Burkina Faso) as having a benefic impact in curing Diarrhea and eye infection, studies on the probiotic properties of the bacteria involved in fermentation should be of high consideration.

ACKNOWLEDGMENTS

The financial support of the ENRECA program under DANIDA and London Metropolitan University as well as the scientific and technical support of Département de Technologie Alimentaire (DTA/IRSAT/CNRST) in Ouagadougou, Burkina Faso, Michel Combari, Roland Sanou, Donatien Kaboré in Ouagadougou, Burkina Faso and all consulted women involved in the production of Bikalga in Burkina Faso are gratefully acknowledged.

REFERENCES

Abu-Tarboush HM, Ahmed AA, Kahtani HA (1997). Some nutritional and functional properties of Karkade (*Hibiscus sabdariffa*) seed products. Cereal. Chem. 74: 352-355.

Aderibigbe EY, Odunfa SA (1990). Growth and extracellular enzyme production by strains of *Bacillus* species isolated from fermenting African locust bean, iru. J. Appl. Bacteriol. 69: 662-671.

Al Wandawi H, Al-Shaikhly K, Abdul-Rahman M (1983). Roselle seeds: a new proteins source. J. Agric. Food. Chem. 32: 510-512.

Allangheny NO, Banu, ZA, Campbell-Platt G, Owens JD (1996). Control of ammonia formation during *Bacillus* fermentation of legumes. Int. J. food. Microbiol. 20: 321-333.

AOAC (1995). Official Methods of Analysis. Association of Official Analytical Chemists Washington DC, USA

Amoa-Awua KW, Ngunjiri P, Anlobe J, Kpodo K, Halm M, Ewurafua HA, Jakobsen M (2007). The effect of applying GMP and HACCP to traditional food processing at semi-commercial kenkey production plant in Ghana. Food Contr. 18: 1449 -1457.

Bengaly M, Bere A, Traore AS (2006). The chemical composition of bikalga, a traditional fermented roselle (*Hibiscus sabdariffa* L) seeds condiment Part I: proximate analysis and protein quality evaluation. Electron. J. Food. Plants Chem. 1: 1-6.

Diawara B, Sawadogo L, Amoa Amwa WKA, Jakobsen M (1998). HACCP system for traditional African fermented foods: Soumbala. ISBN 87-90737-00-8 WAITRO Danish Technological Institute, Taastrup.

El Adawy TA, Khalil AH (1994). Characteristic of roselle seeds as a new source of protein and lipid. J. Agric. Food. Chem. 42: 1896-1900.

Harper DB, Colins MA (1992). Leaf and seed fermentations of Western Sudan. In: Gaden EL, Bokanga M, Harlander S, Hesseltine CW, Steinkraus KH (eds). Applications of Biotechnology in traditional Fermented Foods. National Academic Press Washington DC, pp. 105-113.

Ibrahim MH, Antai SP (1986). Chemical changes during the fermentation of African locust bean (*Parkia filicoidea* Welw) seeds for production of \`Daddawa´. *Qualitas plantarum*. Plant Foods Hum. Nutr. 36: 179-184.

Ikenebomeh MJ, Kok R, Ingram JM (1986). Processing and fermentation of the African locust bean (*Parkia filicoidea* Welw) to produce Dawadawa. J. Sci. Food Agric. 37: 273-282.

Lakshimi MB, Naidu MV, Reddy DS, Reddy CV (1995). Effect of time of sowing and topping on seed yield of roselle (*Hibiscus sabdariffa*). Indian. J. Agric. 40: 682-685.

Mohamadou BA, Mofung CMF, Thouvenot D (2007). Functional potential of a product from traditional biotechnology: antioxidant and probiotic potential of Mbuja, produced by fermentation of *Hibiscus sabdariffa* seeds in Cameroon. J. Food. Technol. 5: 164-168.
Odunfa SA (1985). Biochemical changes during “iru” fermentation. J. Food. Technol. 20: 295-303.
Omobuwajo TO, Sanni LA, Balami YA (2000). Physical properties of sorrel (*Hibiscus sabdariffa*) Seeds. J. Food. Eng. 45: 37-41.
Ouoba LII, Diawara B, Annan NT, Poll L, Jakobsen M (2005). Volatiles compounds of Soumbala, a fermented African locust bean (*Parkia biglobosa*) food condiment. J. Appl. Microbiol. 99: 1413-1421.
Ouoba LII, Parkouda C, Diawara B, Scotti C, Varnam AH (2007). Identification of *Bacillus* spp from Bikalga, fermented seeds of *Hibiscus sabdariffa*: phenotypic and genotypic characterization. J. Appl. Microbiol. 104: 122-131.
Ouoba LII, Rechinger KB, Diawara B, Traoré AS, Jakobsen M (2003). Degradation of proteins during the fermentation of African locust bean (Parkia biglobosa) by strains of *Bacillus subtilis* and *Bacillus pumilus* for production of Soumbala. J. Appl. Microbiol. 94: 396-402.
Pu R (1996). Nutrient composition and biological evaluation of Mestor (*Hibiscus sabdariffa*) seeds. Plants Foods Hum Nutr 49: 27-34.
Sanni AI (1993). The needs of optimisation of African fermented foods and beverages. Int. J. Food. Microbiol. 18: 85-95.
Walinga I, van Vark W, Houba VJG, Van der Lee JJ (1989). Plant Analysis Procedures, Part 7. Department of Soil Science and Plant Nutrition, Wageningen Agricultural University, pp. 197-200.
Wang J, Fung DYC (1996). Alkaline fermented foods: a review with emphasis on pidan fermentation. Crit. Rev. Microbiol. 22: 101-138.
Yagoub AEGA, Mohamed BE, Ahmed AH, Tinay AHE (2004). Study on Furundu, a traditional Sudanese fermented roselle (*Hibiscus sabdariffa*) seed: effect on in vitro protein digestibility, chemical composition and functional properties of the total proteins. J. Agric. Food. Chem. 52: 6143-6150