Production methods and microbiological composition of soumbala (fermented african locust bean seeds) from different parts of Burkina Faso

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Abstract

Fermented African locust bean seeds, soumbala, is of socio-economic importance in Burkina Faso and there are differences in the appreciation of its organoleptic qualities by different socio-cultural groups. Two surveys were carried out to study differences in production methods and the composition of the microflora of soumbala from different production sites in four regions of Burkina Faso. Differences were found in the details of processing procedures and parameters at the different production sites. The wet cleaning method of preparing the seeds for dehulling was found to require less water and firewood, 18.6 - 34 l and 4.5 - 5.3 kg to produce one kg of soumbala than the dry method, 34.3 - 63.9 l and 4.9 - 11.9 kg respectively, hence considered more environmentally friendly. The duration of fermentation observed varied from 19 to 63 h and yield of soumbala from « néré » seeds ranged from 38.9 to 61.8%. The microflora of 13 regional samples of soumbala were fairly uniform and were dominated by gram-positive catalase-positive cocci and *Bacillus* spp.. *Bacillus* spp. were isolated at levels of at least 10^8 cfu/g in 8 of the samples and were predominantly *B. subtillis*. Pediococcus were isolated in 5 samples and other lactic acid bacteria also in 5 samples. In a sample purchased at a different period, 9 out of 35 isolates were found to be *Enteroccocus faecium*, 2 isolates were *Lactobacillus confusus* and the others isolates were *Bacillus* spp. and lactic acid bacteria which were cocci.

Keywords: African locust beans, soumbala, processing, fermentation, microflora.

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Résumé

Le soumbala, graines de néré fermentées, est d'une importance socio-économique au Burkina Faso, et on observe des différences dans l'appréciation des qualités organoleptiques par les différents groupes socio-culturels. Deux investigations ont été entreprises pour étudier les différences dans les méthodes de production et la composition de la microflore du soumbala dans quatre régions du Burkina Faso. Des différences ont été observées dans les détails des procédés de production et au niveau des paramètres de production. La méthode de nettoyage par voie humide des graines pour leur préparation au décorticage demande moins d'eau et de bois de chauffage, respectivement 18,6 - 34 l et 4,5 - 5,3 kg pour produire un kg de soumbala, que la méthode de nettoyage par voie sèche, 34,3 - 63,9 l et 4,9-11,9 kg respectivement et présente de ce fait des avantages environnementaux. Les durées de fermentation varient entre 19 heures et 63 heure et le rendement en soumbala par rapport aux graines se situe entre 38,9 et 61,8 %. La microflore de 13 échantillons régionaux est presque uniforme, dominée par des cocci Gram-positive catalase-positive et des *Bacillus* spp.. Les *Bacillus* spp. ont été dénombrés à un niveau d'au moins 10^8cfu/g dans 8 des échantillons avec une prédominance de *B. subtilis*. Des pédiococcus ont été isolés dans 5 échantillons et d'autres bactéries lactiques dans 5 échantillons également. Dans un échantillon acheté à une période différente, 9 isolats sur 35 ont été identifiés comme *Enteroccocus faecium*, 2 isolats comme *Lactobacillus confusus*, les autres isolats sont des *Bacillus* spp. et des bactéries lactiques en forme de cocci.

Mots-clés : Graines de néré, Soumbala, procédés de production, fermentation, microflore.

Introduction

Soumbala is the most important fermented food product in Burkina Faso. It is a food condiment with a pungent odour, produced by alkaline fermentation of the African locust bean seeds. It has a pH of 7.5 to 8.5, contains 10 % moisture, 30 to 40 % protein and 20 to 25 % fat. Because it contains high amounts of protein, essential amino acids, fatty acids, riboflavin, thiamin and niacin, it is consumed regularly in significant quantities where it plays an important role in the diet of many people in Burkina Faso (DIAWARA *et al.*, 1998)

Soumbala is widely consumed in West Africa and is variously known as dawadawa in Nigeria and Ghana, also as iru in Nigeria, nététu in Senegal, afiti in Benin and as soumbala in Mali, Côte d'Ivoire and Guinea Conakry. It is produced by the fermentation of the cooked dehulled seeds of the African locust beans and has been the subject of scientific studies mainly in Nigeria (ODUNFA 1981, 1983, 1985; ODUNFA and OYEWOLE 1986; ANTAI and IBRAHIM 1986; IKENEBOMEH and INGRAM 1986; ADERIBIGDE *et al.*, 1990; DIAWARA *et al.*, 1992).

The socio-economic importance of soumbala in Burkina Faso has been documented by SAWADOGO and OUÉDRAOGO (1996). The production of soumbala in Burkina Faso is carried out as a traditional commercial activity bothin rural areas. In fact, soumbala plays such an important socio-cultural role that in almost every rural Burkinabé family one female member specializes in the art of making soumbala (SAWADOGO and OUÉDRAOGO, 1996). Despite this reported importance, the microbiology of its fermentation has not been fully investigated. The studies are necessary as SAWADOGO and OUÉDRAOGO (1996) showed differences in the methods used to produce soumbala in different areas of Burkina Faso which effect the microbial flora of the product.

Traditional production of soumbala involves cleaning of the African locust bean seeds also called néré seeds followed by sorting, pounding, washing and sundrying. To dehull the cleaned seeds, they are boiled for up to 24 h and pounded with an abrasive material such as sand or rice hulls in a mortar with a pestle. The cotyledons are separated by washing, and sedimentation. The dehulled seeds are boiled for up to 4 h and fermented after the addition of a little wood ash or millet flour for 2-3 d in baskets or perforated earthernware pots or baskets lined with plastic or jute sacks. The fermented seeds are sundried and molded into balls.

Bacillus spp. dominated by *B. subtilis* have been reported by several workers to be responsible for the fermentation of the African locust beans in several West African countries. The fermentation has also been described as proteolytic and alkaline in nature (CAMPBELL-PLATT, 1980; ODUNFA and OYEWOLE, 1986; ANTAI and IBRAHIM, 1986; N'DIR *et al.*, 1994).The present study was carried out to characterize the microflora of soumbala fermentation from different parts of Burkina Faso, including the identification of the main microbial species involved.

Materials and methods

Survey

Surveys were conducted on two separate occasions covering selected towns in all four of the soumbala producing regions of Burkina Faso. In the first survey soumbala production methods were studied at production sites around Bobo Dioulasso, Péni, Banfora, Fada N'Gourma and Gaoua. Samples of soumbala were collected into sterile plastic bags, from surrounding villages and the local markets and taken to the laboratory of « Département Technologie Alimentaire » for analysis. In the second survey, detailed studies of the production procedures involving quantification of all ingredients used as well as intermediary and final products were carried out at two selected production sites each in Ouagadougou, Bobo Dioulasso, Fada N'Gourma and Diébougou near Gaoua. No samples were collected for laboratory analysis.

Microbiological analysis

10 g of each sample were added to 90 ml sterile diluent (containing 0.1% peptone, 0.8% NaCl, with pH adjusted to 7.2) and homogenized in a stomacher (Lab Blender, Model 4001, Seward Medical) for 4 min at 120 rev/s. From appropriate ten-fold dilutions, counts of aerobic mesophilic bacteria and lactic acid bacteria were carried out respectively on Plate Count Agar (PCA, Merck 5463, Darmstadt, Germany) incubated at 30°C for 3 d, on de Man Rogosa Sharp Agar (MRS CM 361, Oxoid, Hampshire, England) incubated anaerobically under anaerocult jar at 30°C for 5 d. (AMOA-AWUA, 1996).

All colonies loalling 30, from a segment of the highest dilution or suitable plate (> 15 % of the area) were subcultured in the corresponding broth medium and streaked on to the agar substrate until pure cultures were obtained. Bacterial colonies were examined by Gram reaction, catalase production, colony and cell morphology, aerobic and anaerobic growth.

Identification of Bacillus species

Gram positive catalase positive rods bearing phase bright spores which were isolated aerobically from PCA plates were classified in to the genus *Bacillus*. The species of these isolates were

identified by determining their pattern of fermentation of 49 carbohydrates in API 50 CHB galleries (bioMérieux sa, Marcy-l'Etoile, France) (CLAUS and BERKERLEY,1986).

Identification of lactic acid bacteria

Cultures were examined by anaerobic growth on MRS, colony morphology on ST agar, Gram reaction, catalase test, growth at different temperatures, production of CO_2 from glucose, production of NH_3 from arginin, fermentation of carbohydrates and determination of lactic acid isomers. For ovoid shaped cocci suspected of being enterococci, additional tests of growth at pH 9.6, in 6.5 % NaCl and 40 % bile were carried out (HUGH and LEIFSON, 1953).

For cell morphology, wet preparations were mounted and examined by phase contrast microscopy. Gram reaction was carried out by mixing a loopfull of culture with 3 % KOH. For Gram negative reaction, a stringy substance was produced. For growth at different temperatures, single colonies were inoculated into MRS broth and incubated at 15, 45 and 50°C for 3 d. Production of CO_2 from glucose was determined in MRS broth tubes containing Durham tubes. Production of ammonia from arginin was determined by inoculating Arginin media containing 0.3 % arginin with isolates and incubating for 48 h at 30°C. Presence of ammonia was detected by mixing 20 µl of broth culture with 20 µl of Nessler's reagent. In the presence of ammonia the mixture turned orange or brown. Fermentation of 49 carbohydrates was determined in API 50 CHL galleries (bioMérieux) (HUGH and LEIFSON, 1953).

Enzymatic profile of Bacillus isolates

Bacillus spp. were assayed for proteolytic activity on skim milk agar and for the production of 19 enzymes in API zym (bioMérieux) galleries.

Chemical analysis

Moisture content of samples was determined by the oven dry method and water activity in a water activity meter (Ebro Model AWX 3001, France). pH of samples was determined by blending 20 g of sample with 20 ml of distilled water and the pH measured with a pH meter (Hanna Instruments Model 8520, Singapore). Titratable acidity was determined by the titration of 80 ml of filtrate obtained from 10 g of sample dissolved in 200 ml distilled water against 0.1 N NaOH with 1 % phenolphthalein. one 1 ml of 0.1 N was taken as equivalent to 9.008×10^{-3}g lactic acid.

Calculation yields

The raw material, the intermediary products and the finished product are weighed and the yields are determined through the following ratios:

$$\text{Yield in fermented seeds (\% fresh soumbala)} = \frac{\text{Weight of fermented seeds} \times 100}{\text{Weight of raw material}}$$

$$\text{Yield in dried soumbala (\% final product)} = \frac{\text{Weight of final product} \times 100}{\text{Weight of raw material.}}$$

Results and discussion

Methods of soumbala production at different production sites in Burkina Faso

Results of two surveys carried out in the four soumbala producing regions of Burkina Faso showed differences in the methods of preparing soumbala (tables I and II). In general, the production of soumbala involves the following four basic operations: dehulling of African locust bean seeds, cooking of the cotyledons, their fermentation and preservation by sundrying. The survey was not extended to the Northern region because soumbala is neither produced nor an important article of diet in this part of the country. The northern region is sahelian and is unable to support *Parkia biglobosa* trees.

Table I: Methods for producing soumbala in different parts of Burkina Faso

Processing procedure	Bobo-Dioulasso	Péni	Banfora	Fada N'Gourma	Gaoua
African locust bean	+	+	+	+	+
Washing	+	+	+	-	-
Pounding (with added ash)	+	+	+	-	-
Sundrying (2-3 h)	+	+	+	-	-
Pounding (with added ash)	+	+	+	-	-
Winnowing	+	+	+	-	-
Boiling	15 h	18 h	15 h	25 h	25 h
Draining	+	+	+	+	+
Dehulling by pounding					
with added ash	-	+	-	-	-
with added sand	-	-	-	+	+
Washing	+	+	+	+	+
Decanting off of testa	-	-	+	+	+
Washing	-	-	+	+	+
Sorting	+	+	+	+	+
Boiling (2-4 h)	+	+	+	+	+
Draining	+	+	+	+	+
Addition of ash or millet flour	-	+	+	+	+
Fermentation (2 d)	+	+	+	+	+
Sundrying (1 d)	+	-	-	-	-
2nd fermentation	+	-	-	-	-
Sundrying (5-6 d)	+	+	+	+	+
Molding into balls	+	+	+	+	+
Sundrying	+	+	+	+	+

+:carried out; - : not carried out.

In all the soumbala production methods, some preliminary operations involving cleaning and boiling of the seeds are carried out to facilitate dehulling. Two types of preliminary procedures were observed and have been described as the wet and dry cleaning methods by SAWADOGO and OUÉDRAOGO (1996). The wet method as carried out at the production sites visited in Bobo-Dioulasso, Péni and Banfora (table I) involved labour intensive operations of washing or tempering of the seeds with water folowed by pounding, sundrying, further pounding and winnowing. The seeds were finally boiled for 15 to 18 h. In the dry method carried out at production sites in Fada N'Gourma and Gaoua, all these labour intensive operations were avoided, but the seeds had to be cooked for about 25 h.

Quantification of imputes during the second survey showed that less water and firewood were required for the more elaborated wet method (table II, Diébougou and Bobo-Dioulasso). This was because a shorter period was required for boiling the seeds till they were softened enough to be dehulled by pounding. The initial tempering and pounding removed adhering pulp to the seed coat facilitating transfer of heat during boiling.

Dehuling of the African locust bean at all the production sites visited involved pounding, washing, sedimentation of the cotyledons and sorting. At most of the production sites, an abrasive material such as wood ash or sand was added to the seeds during pounding to facilitate dehulling. After pounding, the broken seed coats were removed by washing. The broken seed coats floated on the water during washing and were removed by hand whilst most of the cotyledons sank to the bottom of the containers. At production sites in Banfora, Gaoua and Fada N'Gourma, clay was mixed into the wash water to improve separation of the seed coats from the cotyledons. Adding clay changed the specific gravity ot the wash water making it easier for the cotyledons to sink to the bottom of the container.

The dehulled seeds were re-cooked for periods varying from 2 to 8 h at the different production sites before they were fermented. Since the seeds were already cooked before they were re-boiled this operation was considered to have been carried out for microbiological reasons. Indeed, fermentation of African locust bean seeds was found to involve the hydrolysis of proteins by the proteolytic BACILLUS spp. (CAMPBELL-PLATT, 1980; ODUNFA and OYEWOLE, 1986; ANTAI and IBRAHIM, 1986; N'DIR *et al.*, 1994). The second boiling of the seeds might lead to the selection of the heat resistant spores of *Bacillus* at the expense of vegetative cells. Fermentation of soumbala is reported to be exothermic (ANTAI and IBRAHIM, 1986) and during the survey initial fermentation temperatures recorded varied from 38 to 58°C (table II). Temperatures recorded at the end of fermentation ranged from 28.5 to 50°C.

Table II. Processes, imputes and intermediary products recovery during the production of soumbala at different production sites of Burkina Faso.

	Ouagadougou		Fada N'Gourma		Diébougou		Bobo-Dioulasso	
Material or Process	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
Raw material								
Seeds per batch	18.5 kg	17 kg	12.1 kg	11.9 kg	13.8 kg	18.2 kg	17.3 kg	19.7 kg
Cleaning								
Water used	-	-	-	-	15.5l	19.7l	1.7l	5.25l
Duration of pounding	-	-	-	-	20mn	20mn	20mn	20mn
Duration of drying	-	-	-	-	2h	3h	3h	3h
Duration of pounding	-	-	-	-	30mn	30mn	45mn	30mn
Winnowed seeds	183kg	16.9kg	12.0kg	11.7kg	13.4kg	17.5kg	15.0kg	182kg
Water used	-	-	17.9l	-	-	-	-	-
Soaked seeds	-	-	13.9kg	-	-	-	-	-
First boiling								
Duration	21 h	21 h	22 h	21 h	18 h	20.5 h	21 h	18 h
Water used	124 l	106 l	107.5 l	99 l	77.7 l	87.8 l	85 l	91.8 l
Firewood consumed	44.6 kg	42.6 kg	35 kg	38 kg	22.4 kg	32.4 kg	38.9 kg	44.6 kg
Boiled seeds	47.4 kg	42.9 kg	30.3 kg	30.1 kg	30.5 kg	39.7 kg	ndt	41.3 kg
pH of water	4	5	5.5	5.5	ndt	ndt	ndt	ndt
Dehulling								
Ash used for pounding	2.2 kg	nil	6.4 kg	7.2 kg	-	-	-	-
Water used for washing	141.6 l	224.5 l	161 l	120 l	93.2 l	98.7 l	95 l	93.8 l
Dehulled seeds	23.3 kg	23.5 kg	14.7 kg	15.3 kg	18 kg	24.1 kg	22.6 kg	22.9 kg
Sorting								
Discarded seeds	0.36 kg	0.12 kg	0.47 kg	0.31 kg	0.5 kg	0.4 kg	0.1 kg	-
Second boiling								
Duration	2 h	2 h	7.7 h	8 h	5.5 h	6.3 h	3.3 h	2.5 h
Water used	35.4 l	17.2 l	71.6 l	70.4 l	14.5 l	16.5 l	24.4 l	17.6 l
Firewood consumed	9.6 kg	7.1 kg	18.4	25.3 kg	9 kg	16.2 kg	13.1 kg	8.7 kg
pH of water	5	5	6.5	6.5	5	5.5	5.5	5
Boiled seeds	29.0 kg	21.1 kg	13.3 kg	13.9 kg	16.9 kg	23.4 kg	21.6 kg	22.1 kg
Fermentation								
Sprinkled millet flour	95g	-	-	-	130g	615g	190g	340g
Sprinkled wood ash	-	-	+	+	6	6	6	6
Starting temperature	51 °C	92 °C	50 °C	45 °C	58 °C	38 °C	ndt	ndt
Duration	63 h	42 h	58 h	33 h	39 h	42.8 h	19 h	ndt
Final temperature	29.5 °C	50 °C	42 °C	42 °C	28.5 °C	35.6 °C	38 °C	40 °C
Final pH	9.3	9	8.5	8.5	8.5	9.5	7.5	9.5
Fermented seeds	16.8 kg	18.9 kg	11.1 kg	11.6 kg	12.7 kg	15.4 kg	16.3 kg	17.3 kg
Sundrying								
Duration	ndt	ndt	6 h	5.7 h	5.6 h	5.2 h	5 h	5h
Soumbala	7.2 kg	10.1 kg	5.6 kg	5.3 kg	5.9 kg	10.8 kg	10.7 kg	11.0 kg
Moisture	ndt	ndt	27%	32%	ndt	ndt	ndt	ndt

+: carried out; - : not carried out; ndt: not determined

Table II. (continued)

Material or Process	Ouagadougou		Fada N'Gourma		Diébougou		Bobo-Dioulasso	
	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2	Site 1	Site 2
Analysis								
Yield of fermented Seeds	90.8%	111.11%	91.7%	97.4%	92.0%	84.6%	94.2%	87.8%
Yield of soumbala	38.9%	59.4%	46.2%	44.5%	42.7%	59.3%	61.8%	55.8%
Total water used	301l	347.7l	358l	289.4l	200.9l	222.7l	206.1l	208.4l
Water used								
Per kg soumbala	41.8l	34.3l	63.9l	54.60l	34.0l	20.6l	19.3l	18.6
Total firewood used	54.2kg	49.7kg	53.4kg	63.3kg	31.4kg	48.6	52kg	53.3kg
Firewood used								
Per kg soumbala	7.5kg	4.9kg	9.5kg	11.9kg	5.3kg	4.5kg	4.8kg	4.8kg
Coast of materials								
Per kg soumbala	490F	ndt	420F	443F	ndt	ndt	ndt	ndt

For preservation soumbala is dried further to a moisture content of about 10%.
ndt: not determined; F: Franc CFA = 0,002 $

Various modes of handling the cotyledons during fermentation were used. The most common procedure observed was to pack the partially cooled cotyledons into perforated clay pots or baskets lined with plastic sacks, sprinkled with millet flour or wood ash and covered with a plastic sack or leaves. At a village near Fada N'Gourma, a completely different procedure was observed. The cotyledons were spread on a plastic sheet on top of the mud roof of the house, ash was sprinkled on the grains and covered with leaves and a jute sack.

Fermentation was carried out for periods varying from 19 to 63 h at the different undying production sites before the product was preserved by partial sundrying. At a production site in Bobo Dioulasso, a stepwise fermentation was observed in which the seeds were fermented in a perforated clay pot for 2 d, sundried for 1 d and fermented further for 1 d. The processor claimed that the final product had a superior organoleptic quality.

The fermented products were only partially sundried before they were pounded and molded into balls for marketing as an intermediate moisture product. For prolonged storage, products were sundried for about 3 d, often after the addition of salt.

Imputes and intermediary products yield

Ingredients used at every stage of processing and the weight of the intermediary products at the different production sites are shown in Table II. The volume of water used to produce one kg of soumbala by the wet method (18.6 to 34l in Diédougou and Bobo-Dioulasso) was less than the volume required for the dry method (34.3 to 63.9l in Ouagadougou and Fada N'Gourma). The amount of firewood used during boiling could not be related strictly to the duration of cooking. This was because different types of woods were used and efficiency of the combustion systems could also have been different. However, generally less firewood (4.5 to 5.3 kg), was required for the production of 1 kg of soumbala by the wet method than by the dry method (4.9 to

11,9 kg). The wet cleaning method was therefore considered to be more economical and environmentally friendly than the dry cleaning method, water and firewood based on requirements. This is very significant due to the prevailing semi-arid conditions in the soumbala producing areas. The yield of soumbala from néré seeds at the different production sites ranged from 38.9 to 61.8 %.

The effects of both cleaning methods were however considered rather severe and alternative procedures have been sought to dehull néré seeds. Consequently at a semi-industrial plan in Ouagadougou, néré seeds are currently dehulled mechanically using a machine made by the «Département Mécanisation » of the « Centre National de la Recherche Scientifique et Technologique, 03 BP 7047 Ouagadougou, Burkina Faso ». But this mechanical dehulling procedure does not require neither water nor firewood. It is envisaged to cook the dehulled seeds using fuel thus completely eliminating the use of firewood processing.

Variations in the details of soumbala production methods observed during the surveys to have possibly evolved as a result of constraints for water and firewood availability for processing but now manifest as socio-cultural differences. These differences may therefore also account for differences in the appreciation of the organoleptic qualities of soumbala by different socio-cultural groups in Burkina Faso.

Microbiology of soumbala

pH and microbial counts of thirteen samples of soumbala collected from the various towns, villages and markets are shown in table III. The pH of all the samples were near neutral even though the fermentation process has been described as alkaline by several workers (ODUNFA, 1981; ANTAI and IBRAHIM, 1986). This is possibly because, after the alkaline fermentation of the seeds, lactic acid fermentation takes place during sundrying, pounding and molding into balls, slightly decreasing pH of the product and contributing to the organoleptic quality of soumbala from Burkina Faso.

Table III. The acidity and microbial compositiopn of soumbala produced in different area of Burkina Faso.

Source of sample	pH	Acidity	Microbial composition in cfu/g: Aerobic mesophilic bacteria	*Bacillus* subtillis	*Bacillus* cereus	*Bacillus* spp.	*Pediococus* spp.	Gm +ve Cat +ve cocci	Gm +ve Cat-ve cocci	Gm-ve Cat+ve cocci
Central Province										
Ouagadougou	7.1	ndt	$4.6x10^{9}$	nd	nd	nd	nd	1.8×10^{9}	nd	1.5×10^{8}
South -West Province										
Banfora	6.1	0.12	$1.1x10^{9}$	6.6×10^{8}	nd	nd	nd	4.4×10^{8}	nd	nd
Village 1 near Gaoua	7.8	0.13	$5.9x10^{9}$	nd	nd	nd	nd	5.9×10^{9}	nd	nd
Village 2 near Gaoua	6.7	0.14	$2.3x10^{9}$	9.2×10^{8a}	nd	4.6×10^{8}	nd	9.2×10^{8}	nd	nd
Village 3 near Gaoua	6.9	0.16	$2.7x10^{10}$	nd	2.2×10^{9}	nd	nd	2.2×10^{10}	2.2×10^{9}	nd
Gaoua market 1	7.2	0.13	$2.4x10^{9}$	1.8×10^{8}	1.8×10^{8}	nd	1.8×10^{8}	1.3×10^{9}	3.7×10^{8}	1.8×10^{8}
Gaoua market 2	6.7	0.10	$4.2x10^{9}$	1.8×10^{8}	nd	nd	6.9×10^{8}	2.3×10^{9}	nd	nd
Gaoua market 3	7.1	0.15	$1.0x10^{11}$	nd	nd	nd	1.4×10^{10}	7.1×10^{10}	1.4×10^{10}	nd
Western Province										
Péni	7.3	0.11	$1.7x10^{10}$	nd	3.6×10^{9}	nd	nd	2.3×10^{12}	nd	nd
Bobo-Dioulasso	7.4	0.16	$1.5x10^{10}$	7.5×10^{9}	nd	nd	nd	7.5 x 109	nd	nd
Eastern Province										
Fada N'Gourma	7.4	0.12	$1.6x10^{11}$	5.4×10^{10}	nd	nd	2.7×10^{10}	8.1×10^{10}	nd	nd
Fada N'Gourma marke1	7.4	0.10	$3.8x10^{12}$	1.5×10^{12}	nd	nd	nd	2.3×10^{12}	nd	nd
Fada N'Gourma marke2	7.0	0.10	$4.2x10^{10}$	nd	nd	nd	4.7×10^{9}	2.8×10^{10}	4.7×10^{9}	4.7×10^{9}

ndt: not determined; nd: not detected on the dilution plate examined, a: an unidentified Bacillus spp. was isolated at 4.6 x 108 in this sample
Gm: Gram; Cat: Catalase

Wide variations were observed in the population of aerobic mesophiles in the different samples collected, ranging from 10^9 to 10^{12} cfu/g. The bacterial populations grown anaerobically on MRS not dominated by lactic acid bacteria since they were mostly Gram-positive and catalase-positive. A major factor which may account for the wide variations in the population of aerobic mesophiles were differences in the duration of fermentation carried out at the different production sites. The recorded fermentation periods ranged from 19 to 63 h over such a wide range of fermentation period it is not expected that the microbial population could be stable.

The most frequently occurring types of microorganisms found in all the soumbala samples were a variety of Gram-positive cocci. Most of these cocci which were dominant catalase on both PCA and MRS plates were catalase-positive and only a few were catalase-negative. In seven of the thirteen samples, Gram-positive catalase-positive rods bearing spores occurred in fairly equal proportions with the Gram-positive catalase-positive cocci in the population of aerobic mesophiles. The Gram-positive catalase-positive rods bearing phase bright spores were Bacillus spp. which have been reported by several works as responsible for the fermentation of the African locust beans in other West African countries (CAMPBELL-PLATT, 1980; ODUNFA, 1981). Gram-positive catalase-negative cocci as well as some pediococcus were observed.

The frequent isolation of Gram-positive catalase-positive cocci from soumbala samples suggests that after fermentation by *Bacillus* i e during sundrying, a microbial succession occurs to an extent. One disturbing observation made during the survey was the extent to which the very strong smelling unprotected soumbala attracted houseflies during sundrying. Houseflies are prevalent in Burkina Faso and the flora of traditionally produced soumbala is certain to be influenced by microbial contamination resulting from the activities of houseflies.

Identification of bacteria was first directed at the Bacillus species since they were expected to be the primary fermenting organisms in the fermentation of the African locust bean seeds. Even though *Bacillus* spp, were not isolated from all the PCA plates examined, they were present in all samples. The most frequently isolated Bacillus species were identified as Bacillus subtilis (table III) at the levels of at least 10^8 cfu/g in seven out of the thirteen samples. The highest population of *B. subtilis* (1.5×10^{12} cfu/g) was found in one sample purchased from the market in Fada N'Gourma. *Bacillus cereus* was identified at the levels of 10^8 and 10^9cfu/g in three out of the thirteen samples. In two of these samples, no *B. subtilis* was isolated at the dilution level. A third type of Bacillus was isolated in one of the samples but attempts at identification of the species based on the results of the API test were inconclusive. CAMPBELL-PLATT (1980) as reported by ODUNFA (1985) indicated that *B. subtilis* represented the dominant microorganisms isolated from numerous soumbala samples collected from different countries. In some samples, this percentage reached 61-69 % of all the total isolates whilst all *Bacillus* spp constituted 83-93 % of all isolates. *B. subtilis* has also been confirmed as the predominant species in dawadawa fermentation in Nigeria by ODUNFA (1981), and ODUNFA and OYEWOLE (1986). The results of the present findings are therefore in agreement with the reports of previous workers and confirm the role of *Bacillus* spp dominated by *B. Subtilis* in the fermentation of African locust beans into soumbala in Burkina Faso. The two species of Bacillus identified in the present work, *B. subtilis* and *B. cereus*, were also isolated by ANTAI and IBRAHIM (1986) from market samples of dawadawa in Nigeria.

The Gram-positive catalase-positive cocci were not identified. They may be dominated by staphylococci as suggested by ODUNFA (1981) who considers staphylococci as part of the natural flora of soumbala and who identified two varieties of *Stapylococcus saprophyticus* in some Nigerian samples. ANTAI and IBRAHIM (1986) isolated spathyloccocci at a rather low level of 4.4×10^4 cfu/g in a market sample of dawadawa in Nigeria.

In five out of the thirteen samples analysed during the present work, pediococci constituted a substantial part of the microfora. Gram-positive catalase-negative cocci also formed a substantial part of the microflora in five of the samples. Efforts were made to identify the species of the Gram-positive catalase-negative cocci. In preliminary identifications carried out before the survey on a sample of soumbala purchased from the central market in Ouagadougou, enterococci were found to account for 26 % of the isolates. In this preliminary work before the surveys were carried out, 35 cultures were isolated from the soumbala sample. Nine isolates were ovoid shaped cocci and grew at 45°C, in 6.5 % NaCI, at pH 9.6 and produced NH_3, from arginin but failed to grow in 40 % bile or produce CO_2 from glucose. Most of the isolates fermented arabinose, cellobiose, galactose, maltose, melibiose, rabinose, ribose, trehalose and amygdalin. Only half of these isolates fermented xylose and non fermented raffinose. All nine ovoid cocci were identified as *Enterococcus faecium*. Two other isolates identified were found to be lactic acid bacteria. They were heterofermentative lactobacilli which grew at both 15 and 45°C and produced NH_3 from arginin and fermented arabinose, xylose, maltose, salicin, amydalin. One fermented melibiose and the other dextrin and were both identified as *Lactobacillus confusus*. Twelve other cocci representing 34 % of the isolates were found to be lactic bacteria but no further tests were carried out for identification purposes. Two of the rest of the isolates were found to be *Bacillus* spp. The rest representing 29 % of the population could not be cultured anaerobically on MRS and were considered as non lactic acid bacteria but tests for further identification were not carried out.

The presence of *Bacillus cereus* detected in the samples of two towns (Gaoua and Péni) is related to the environment and could constitute health risk for the consumers. Further investigations should be done on the sanitary quality of the soumbala from these towns for more precisions about the conditions and the frequency of *Bacillus cereus* appearance.

Acknowledgements

This work was funded by the Danish International Development Assistance (DANIDA) project and involved collaboration between the « Département Technologie Alimentaire, IRSAT/ CNRST, Burkina Faso », the Food Research Institute of Ghana and the Royal Veterinary and Agricultural University, of Copenhagen, Denmark. The authors are grateful to Mme Fatimata Drabo from the « Département Technologie Alimentaire » for her assistance in the laboratory.

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