



DANIDA

Département de Technologie
Alimentaire/IRSATCNRST, Burkina Faso

Department of Food Science, University of
Copenhagen Denmark

Microbiology Research Unit, HHS, London
Metropolitan University

Selection of *Bacillus* starter cultures for controlled production of **Soumbala**, a nutritious alkaline fermented food

* *Labia Irène Ivette OUOBA, Bréhima Diawara, Jane P. Sutherland, Mogens Jakobsen*

* *Senior research fellow, Microbiology Research Unit, London Metropolitan University*

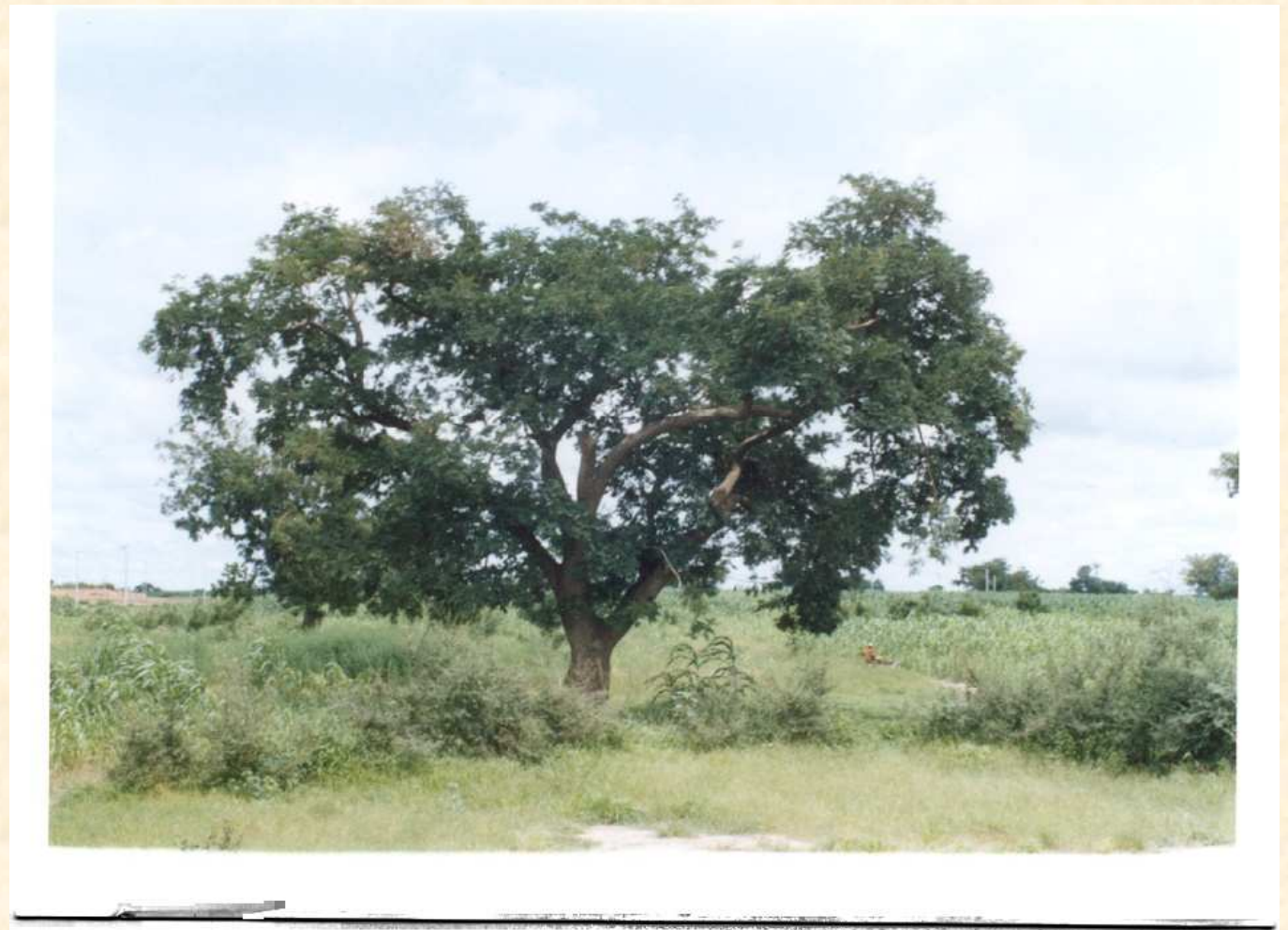
Introduction

- ❖ **Soumbala: product of a spontaneous and alkaline fermentation of African locust bean (*Parkia biglobosa*) seeds.**
- ❖ **Popular food condiment common in several countries of west and central Africa where it is used to flavour soups and sauces.**
- ❖ **Among other names, the product is known as Soumbala, Afitin, Dawadawa, iru, Nététou, Kinda.**
- ❖ **Soumbala is rich in proteins, lipids, carbohydrates, essential amino and fatty acids, vitamins and constitute a nutritional substitute of meat mainly for the poor and rural families.**
- ❖ ***Bacillus* spp. mainly *B. subtilis* as the main microorganisms responsible of the fermentation and proteolysis is the main enzymatic activity leading to a pronounced increase of FAA such as lysine.**

- ❖ **Production is still a traditional art with uncontrolled fermentation. This affect the quality and the stability of the product.**
- ❖ **In order to increase the nutritional and hygienic quality of Soumbala a modernization of the production techniques and an optimization of the processing conditions have been developed: GMP, HACCP, starter cultures, controlled fermentation, dehulling machine, training of producer, development of semi industrial productions sites.**
- ❖ **Besides its high nutritional value, Soumbala plays an economical, social and cultural role in Burkina Faso.**

African locust bean (*Parkia biglobosa*)

- ❖ *Parkia biglobosa* called *néré* in Burkina Faso is a tree of 10 to 25 m high that grows in open savanna woodlands.
- ❖ Other species of *Parkia* as *P. Clappertomiana*, *P. Filicoidea*, *P. Oliveri*, *P. Bicolor*, *P. Klanei* can also be found and have essentially the same use as *P. biglobosa*.
- ❖ It can produce 25 to 100kg of fruit per year.





The fruits are elongated pods found in cluster. The mature pod is made up of a husk, which encloses a powdery yellow material in which dark brown seeds are embedded. These seeds are the most valuable part of the crops and constitute 30% of the fruit.

The principle of Soumbala production



Cleaned African locust bean seeds



First cooking(24-40h)



Manual dehulling



Second cooking(1-4h)



Fermentation (36-72h)

The principle of production



Moulding into balls



Dried balls of soubala



Soubala powder

Microorganisms associated with the fermentation of African locust bean

Odunfa and Oyewole(1986), Odunfa and adewuyi (1985b)	Antai and Ibrahim (1986)	N´Dir et al. (1994) N´Dir et al. (1997)	DTA*
<i>B. subtilis</i> , <i>B. pumilus</i> , <i>B. licheniformis</i> ,	<i>B. subtilis</i> <i>B.cereus</i>	<i>Bacillus spp.</i> <i>B. subtilis</i> <i>B. pumilus</i> ,	<i>B. subtilis</i> <i>B. pumilus</i> ,
<i>Staphylococcus Spp.</i>	<i>Staphylococcus spp.</i>	<i>B. licheniformis</i> <i>B. coagulans</i>	<i>B. licheniformis</i> <i>B. megaterium</i> <i>B. sphaericus</i> <i>B. cereus</i>
<i>Lactobacillus spp</i>	<i>Leuconostoc mesenteroides</i> , <i>Ln. dextranicus</i>	<i>Staphylococcus spp.</i>	<i>Staphylococcus spp.</i>
	<i>Micrococcus spp.</i>	<i>Streptococcus spp.</i>	<i>Micrococcus spp.</i>
			<i>Lactobacillus spp.</i>

Nutrition

Nutrients	Odunfa (1986)	Nordeide <i>et al</i> (1996)	Diawara <i>et al.</i> (1998) and DTA*	Laokole (1994)
%Proteins	40.0-47.4	37.2	30-40	40.30
%Lipids	31.4-42.9	35.5	20-25	32.50
%Carbohydrates	15.0	14.6	15-17	13.32
Energy Kcal/ 100g	480-546	526	464-543	506.98

Nutrition

Free amino acids	Free fatty acids	Minerals	Vitamins
Lysine, valine, isoleucine, leucine, methionine, histidine, phenylalanine, threonine, tryptophan, aspartic acid, glutamic acid, serine, proline, glycine, alanine, cysteine, tyrosine,	C14(myristic acid), C16(palmitic acid), C18 (stearic acid), C20(arachidic acid), C22 (behenic acid), C18:1(Oleic acid), C18:2(linoleic acid), C18:3 (linoleic acid), C20:4 (arachidonic acid)	Ca, Fe, Zn, Cu, Mg, P, Se, Na, Mn	Vitamine B: thiamine, riboflavine, niacine; Vitamin A; Vitamin C: ascorbic and folic acid

Various dishes prepared with soubala



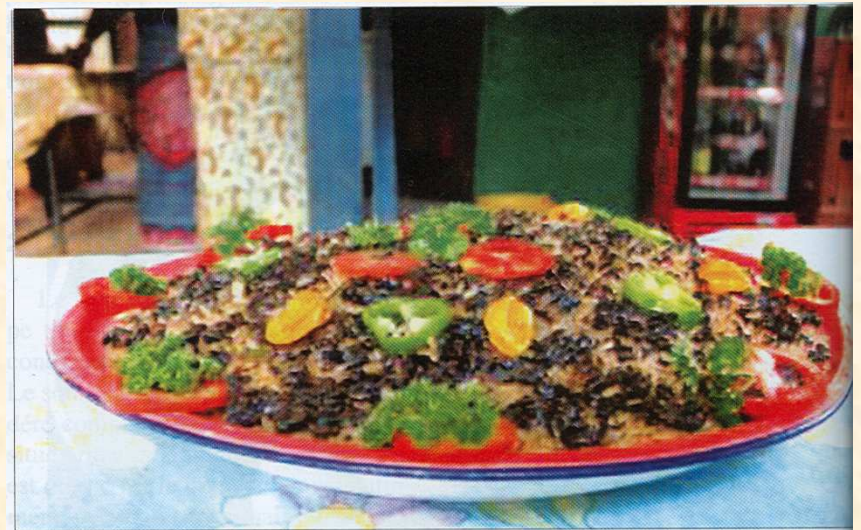
Soubala sauce with `to` (thick maize porridge)



Guinea fowl with Soubala also called `Guinea fowl dish of mother in law`



Pasta with Soubala



Fried rice with Soubala

Medical properties

- ❖ In Burkina Faso, traditional physicians prescribe Soumbala against high blood pressure.
- ❖ Soumbala is also used sometimes in the preparation of potions to cure sexual deficiency(Men)
- ❖ To prevent aging,
- ❖ *Parkia biglobosa* is efficient against some pathologies. Leaves can be use against diarrhoea, dysentery, rubella, pneumonia; the pulp against fever and malaria; the bark against intestinal trouble, Candida, malaria and fever,

Socio- cultural and economical role of soumbala

- ❖ Soumbala is used by many tribes to strengthen social relationships; it can be given as a gift or a symbol of respect or attachment to someone.
- ❖ Soumbala has a cultural importance mainly during weddings and other traditional events. Among some tribes as Mossi, Gourounsi, Sénoufo, Gourmantché and Lobi, a part of the duty to the bride during the weeding is constituted by four big balls of Soumbala.
- ❖ Among the Bobo tribe it is use during the traditional youth initiation practices.
- ❖ Among the Gourmantché tribe, it is use in potions to gain girls affection and to enhance sexual strength.
- ❖ Soumbala is prepared traditionally by women in rural and urban families or by women associations. A few part of the production is consumed by those families while a large part is sold at the market and constitutes then an economical source for the producers

Optimization of the processing conditions during the production of Soumbala

- **GMP and HACCP:**
- **Dehulling machine(CNRST): decrease of the processing time and energy,**
- **Development of starter cultures (Ouoba *et al*, 2003 a, Ouoba *et al*, 2003b, Ouoba *et al*, 2004, Ouoba *et al*, 2005) : controlled fermentation with enhanced nutritional and hygienic quality as well as stability,**
- **Soumbala cube machine(CNRST),**
- **Protected dryer,**
- **Improved packages,**
- **Semi- industrial production sites.**

Selection of *Bacillus* starter cultures

- ❖ Isolation and identification of *Bacillus* spp from soumbala: phenotyping and genotyping (ITS-PCR, PFGE, DNA Sequencing)
- In total 17 isolates were identified :
 - 8 *B.subtilis* (B3, B4, B11, B15, B16, B7, B9):3 different subspecies
 - 2 *B. pumilus* (B6, B10): 2 different subspecies
 - 1 *Brevibacillus Bortelensis* (B14)
 - 6 *B. cereus* (B1, B2, B8, B18 B13, B17)
- Publication:

Ouoba, L. I.I., Diawara, B., Amoa-Awua, W.K., Traoré , A.S. and Lange Møller, P. (2004) Genotyping of starter cultures of *Bacillus subtilis* and *Bacillus pumilus* for fermentation of African locust bean (*Parkia biglobosa*) to produce Soumbala. *International Journal of Food microbiology* 90, 197-205.

❖ Proteolytic activity

The studied bacteria comprised:

- 8 isolates of *B. subtilis* (B3, B4, B11, B15, B16, B7, B9)
- 2 isolates of *B. pumilus* (B6, B10)

➤ **Method:** agar diffusion tests, fermentation, SDS-Page electrophoresis (proteins profiles), cation exchange chromatography (quantity and profile of amino acids)

➤ **Results:** *B. subtilis* B9 and B15 were the best in the degradation of African locust bean proteins leading to the liberation of high quality and quantity of essential amino acids

➤ Publication

Ouoba, L. I.I., Rechinger, K. B., Diawara, B., Traoré, A.S. and 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. *Journal of Applied Microbiology* 94, 396-402.

❖ Lipolytic activity

The studied bacteria comprised:

- 8 isolates of *B. subtilis* (B3, B4, B11, B15, B16, B7, B9)
- 2 isolates of *B. pumilus* (B6, B10)
- **Method:** agar diffusion tests, fermentation, gas chromatography (quantity and profile of free fatty acids)
- **Results:** *B. pumilus* B6, B10 and *B. subtilis* B7 were the best in the degradation of African locust bean oil leading to the liberation of high quality and quantity of essential fatty acids
- **Publication**
Ouoba, L. I.I., Cantor, M. D., Diawara, B., Traoré, A.S. and Jakobsen, M. (2003) Degradation of African locust bean oil by *Bacillus subtilis* and *Bacillus pumilus* isolated from Soubala, a fermented African locust bean condiment. *Journal of Applied Microbiology* 95, 868-873.

❖ Degradation of polysaccharides and non-digestible oligosaccharides

The studied bacteria comprised:

- 3 isolates of *B. subtilis* (B7, B9, B15)
- 2 isolates of *B. pumilus* (B6, B10)

- **Method:** degradation of polysaccharide gels, fermentation, pH measurement, High Performance Anion Exchange Chromatography, Pulsed amperometric detection (HPAEC-PAD) for the profile and quantity of raffinose, stachyose, arabinogalactan and residual simple sugars.

- **Results:** *B. subtilis* B15 and B7 were the best in the degradation of polysaccharides and non digestible oligosaccharides as stachyose and raffinose present in high amount in the beans

- **Publication**
Ouoba , L. I.I., Diawara, B., Christensen, T., Mikkelsen, J.D. and Jakobsen, M. (2005) Degradation of polysaccharides and oligosaccharides by *Bacillus subtilis* and *Bacillus pumilus* isolated from Soumbala, a fermented African locust bean food condiment. *European Food Research and Technology*, 224, 689-694.

❖ **Antimicrobial activity**

The studied bacteria comprised:

- 3 isolates of *B. subtilis* (B7, B9, B15)
- 2 isolates of *B. pumilus* (B6, B10)

➤ **Method:** inhibition tests in agar, inactivation tests in broth and during the fermentation of African locust beans against pathogens as *Micrococcus luteus*, *Staphylococcus aureus*, *Bacillus cereus*, *Enterococcus faecium*, *Listeria monocytogenes*, *Escherichia coli*, *Salmonella typhimurium*, *Shigella dysenteria*, *Yersinia enterocolitica*, *Aspergillus ochraceus* and *Penicillium roqueforti*.

➤ **Results:** *B. subtilis* B7 and B15 showed the best antimicrobial activity and were able to inactivate Gram positive and Gram negative indicators of pathogenic bacteria and moulds during the fermentation of African locust beans

➤ **Publication**

Ouoba , L. I.I., Diawara, B., Jespersen, L and Jakobsen, M. (2007) Antimicrobial activity of *Bacillus subtilis* and *Bacillus pumilus* during the fermentation of African locust bean (*Parkia biglobosa*) for Soumbala production. *Journal of Applied Microbiology*, 102, 963-970.

❖ Enterotoxins production

The studied bacteria comprised:

- 3 isolates of *B. subtilis* (B7, B9, B15)
- 2 isolates of *B. pumilus* (B6, B10)
- 6 isolates of *B. cereus* (B1, B2, B8, B13, B17, B18)

Method:

❖ Haemolytic activity on blood agar, detection of genes encoding cytotoxin K, non-haemolytic (NheA, NheB, NheC) and haemolytic enterotoxins (Hbl A, Hbl C, Hbl D),, emetic toxin : PCR

Production of enterotoxins in broth and during the fermentation of African locust beans for soumbala production:

B. cereus Enterotoxin Reverse Passive Latex Agglutination test kit (BCET-RPLA, Oxoid): detection of haemolytic toxins

Bacillus Diarrhoeal Enterotoxin Visual Immunoassay (BDEVIA): detection of non- haemolytic toxins

Results:

➤ Genes encoding enterotoxins and cytotoxin K were detected in all isolate of *B. cereus* isolates .

- All isolate of *B. cereus* were able to produce enterotoxins in broth and during the fermentation of African locust bean
- No toxin gene was detected in the isolates of *B. subtilis* and *B. pumilus* from Soumbala
- No enterotoxin production was observed nor in broth neither in African locust bean inoculated with *B. subtilis* and *B. pumilus* isolates

Publication

Ouoba L.I.I., Thorsen, L., Varnam, A.H. (2008) Enterotoxins and emetic toxins production by *Bacillus cereus* and other species of *Bacillus* isolated from Soumbala and Bikalga, African alkaline fermented food condiments. International Journal of Food Microbiology 124,224-230. (Poster session)

❖ Antibiotic resistance: detection of transferable genes

The studied bacteria comprised:

3 isolates of *B. subtilis* (B7, B9, B15)

2 isolates of *B. pumilus* (B6, B10)

6 isolates of *B.cereus* (B1, B2, B8, B13, B17, B18)

Method:

❖ Determination of Minimal Inhibitory concentration(MIC) for 25 antimicrobials by micro-broth and agar dilution according to standards set by CLSI(Clinical and Laboratory Standards Institute).

❖ Detection of resistance of resistance gene by PCR Detection of resistance genes for Erythromycin, Gentamycin, Kanamycin, Penicillin, Spectinomycin, Streptomycin ,Tetracycline and Trimethoprim was performed by PCR reactions using specific primers and positive control bacteria.

Minimal Inhibitory concentration(MIC, (µg/ml)

Antimicrobials	Bacillus isolates											Resistance
	B7	B9	B15	B6	B10	B1	B2	B8	B13	B17	B18	
Ampicilin	<2s	<2s	<2s	<2s	<2s	8r	8r	32r	>64r	>64r	<2s	6/11 Bc
Avilamycin	<2s	<2s	<2s	<2s	<2s	<2s	<2s	<2s	<2s	<2s	<2s	0
Bacitracin	>64r	>64r	>64r	>64r	>64r	>64r	>64r	>64 r	>64r	>64 r	>64r	11/11
Chloramphenicol	4 s	<2 s	<2s	16 i	8 S s	4 s	4 s	4 s	4s	4 s	4 s	0
Ceftiofur	0.5s	0.25s	<0.12s	8r	16r	16 r	16r	16r	>16r	>16r	>16r	8/11,Bc, Bp
Ciprofloxacin	0.25s	<0.12s	<0.12s	0.5s	0.5s	0.25s	0.25s	1s	0.25s	0.25s	0.25s	0
Daptomycin	4s	2s	4s	4s	4s	1s	1s	16r	8r	8r	1s	3/11
Erythromycin	<0.12s	<0.12s	<0.12s	0.25s	<0.12s	2i	4i	2i	0.25s	0.25s	2i	0
Flavomycin	>32r	>32r	>32r	>32r	>32r	<4s	<4s	<4s	<4s	<4s	<4s	5/11, Bs, Bp
Florfenicol	8s	<1s	<1s	2s	2s	<1s	<1s	<1s	<1s	<1s	<1s	0
Gentamycin	≤2s	≤2s	≤2s	≤2s	4i	≤2s	≤2s	≤2s	≤2s	≤2s	≤2s	0
Kanamycin	4s	4s	4s	≤2s	32r	4s	8s	8s	4s	4s	8s	1/11, Bp
linezolid	2s	<1s	<1s	<1s	2s	<1s	<1s	<1s	<1s	<1s	<1s	0
Penicillin	<0.06s	<0.06s	<0.06s	0.12s	<0.06s	>16r	8r	>16r	>16r	>16r	8r	6/11, Bc
Salynomycin	4s	<2s	<2s	<2s	<2s	<2s	<2s	<2s	<2s	<2s	<2s	0
spectinomycin	64s	32s	64s	128r	256r	64s	64s	64s	128r	128r	64s	4/11, Bp, Bc
Streptomycin	16i	4s	4s	16i	8s	4s	8s	8s	16i	16i	4s	0
Sulphamethoxazol	512r	64s	512r	512r	512r	>512r	>512r	>512r	>512r	>512r	>512r	10/11,all
Synercid	4r	<0.5s	4r	2s	2s	2s	2s	16r	2s	2s	2s	3/11, Bs, Bc
Tetracycline	16r	<0.5s	8i	<0.5s	<0.5s	1s	<0.5s	<0.5s	4s	4s	1s	1/11, Bs
Tiamulin	>32r	<0.25s	>32r	>32r	>32r	>32r	>32r	>32r	>32r	>32r	>32r	10/11, all
Tigecycline	0.06s	0.25s	0.03s	0.03s	0.03s	0.03s	0.03s	<0.015s	0.06s	0.06s	0.03s	0
Trimethoprim	<1s	<1s	<1s	<1s	4s	>32r	>32r	>32r	>32r	>32r	>32r	6/11, Bc
TMP+SMX	<0.25s	<0.25s	<0.25s	<0.25s	<0.25s	>16r	>16r	>16r	>16r	>16r	>16r	6/11,Bc
Vancomycin	<2s	<2s	<2s	<2s	<2s	<2s	<2s	4s	<2s	<2s	<2s	0

Detection of Resistance genes

Using PCR with specific primers, no positive amplicon was obtained for the resistance gene screened: (erm(A), erm(B), erm(C), aac(6')aph(2''), aph(3'')-I, aph(3'')-III, aac(3'')IV, ant(2'')-I1, blaZ, aadA, aadE, StrA, StrB, ant(6)-Ia, tet(S), tet(O,) tet(K), tet(M) tet(L), tet(Q), dfr(A)).

The resistance observed for some antimicrobials seem to be intrinsic. The isolates are not likely to introduce antimicrobial resistance and are safe regarding their ability to host and transfer resistance genes

.

❖ Controlled fermentation, Sensory evaluation, Aroma compounds analysis

- 3 isolates of *B. subtilis* (B7, B9, B15)
- 2 isolates of *B. pumilus* (B6, B10)

Ouoba , L. I.I., Diawara, B., Annan, N.T., Poll, L. and Jakobsen, M. (2005a) Volatiles compounds of Soumbala, a fermented African locust bean (*Parkia biglobosa*) food condiment. *Journal of Applied Microbiology* 99, 1413-1421

Table 1 Sensory evaluation of various samples of Soumbala

Samples of Soumbala	Characteristics of 48h Soumbala (appearance, colour, odour, taste)	Overall acceptability (scores)*	
		Soumbala	Rice [‡]
B7	Very slimy, red-brown, mild odour and taste	4.40 ^{at}	3.85 ^{ba}
B9	Individual, dark brown, very strong odour and taste	3.75 ^a	4.05 ^a
B15	Slimy, yellow-brown, slightly strong odour and taste	4.25 ^a	4.20 ^a
BT:7,9,15	Middling slimy, brown, strong odour and taste	4.15 ^a	4.20 ^a
BP: 7.9, 15+6	Individual, brown, very strong odour and taste	3.25 ^{ba}	2.85 ^b
Sa	Individual, light brown, strong odour and taste	2.20 ^b	3.70 ^{ba}

* Scores: from six to one, 6=like extremely, 1=dislike extremely)

† Letters are used in the order of alphabet to indicate decreasing score. Means with the same letter are not significantly different at 95% confidence level at the time considered.

‡ Rice: rice flavored with Soumbala (180g of dry Soumbala per 1500g of rice).

Aroma volatiles of Soumbala

116 compounds in total were identified as aroma volatiles of soumbala

- ❖ 20 aldehydes,
- ❖ 16 pyrazines,
- ❖ 14 alcohols,
- ❖ 12 Ketones,
- ❖ 10 esters,
- ❖ 7 alkanes
- ❖ 7 acids,
- ❖ 5 benzenes,
- ❖ 3 alkenes,
- ❖ 3 amines,
- ❖ 4 pyridines,
- ❖ 4 sulphur compounds,
- ❖ 1 furan
- ❖ and 6 other compounds

Conclusions and perspectives

- ❖ *B. subtilis* isolates especially B15 and B7 showed the best ability to ferment African locust bean leading to a final product with improved nutritional and hygienic quality
- ❖ Soumbala produced with starter cultures preferred
- ❖ Transfer of the use of starter cultures to producers
- ❖ Standard for Soumbala