Ministère de l'Enseignement Supérieur, de la Recherche Scientifique et de l'Innovation (MESRSI)
---
Centre National de la Recherche Scientifique et Technologique (CNRST)
---
Institut de Recherche en Sciences Appliquées et Technologies (IRSAT)
---
Département Technologie Alimentaire (DTA)
---

BURKINA FASO
---
UNITE - PROGRES - JUSTICE

# DANIDA GREEN GROWTH PROJECT REPORT

# Starter culture performances and consumer preferences in pilot plant and SME fermentation trials of soumbala

**Written by:**
**Dr Clarisse S. DAWENDE-COMPAORE**
**Miss Djeneba TAMBOURA**

**Approuved by:**
**Dr Hagrétou SAWADOGO-LINGANI**

**May 2018**

# Introduction

The overall objective of the DANIDA Green Growth project is to enable the West Africans to preserve and fully utilize their natural microbial resources, and to up-grade the food sector in an environmentally friendly way assuring the quality, safety and marketability of food products. The WP4 of the project consist of the implementation of starter cultures at SMEs with two main tasks: pilot plant trials and training and assistance of SMEs for implementation of starter cultures. Accomplishment of these tasks will allow evaluating starter cultures performance and consumer preference at pilot plant scale and SME fermentation trials.

The present report describes the methodology used to execute these tasks and the results obtained.

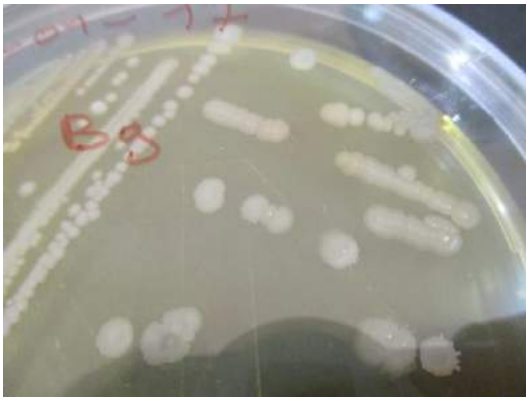
## I. Starter cultures used for the trials fermentation at DTA pilot plan and at SME

Four different *Bacillus* spp. strains selected in previous studies as starter cultures for the controlled fermentation of seeds condiments were used in this study. These starter cultures were maintained as stock cultures in the -80°C freezer of the Department of Food Technology (DTA) / IRSAT in Ouagadougou (BURKINA FASO). They origin and characteristics are presented in the following table.

**Table 1: Characteristics of starter cultures used**

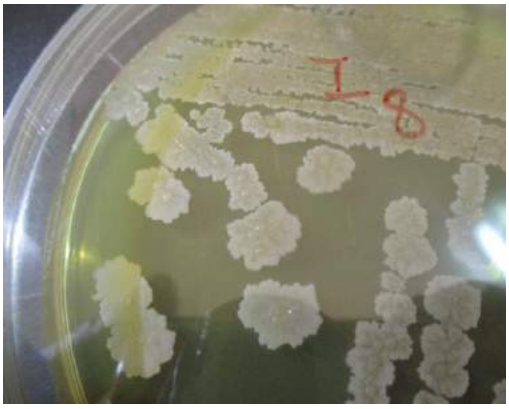
| Strain | Origin | Characteristics | References |
|---|---|---|---|
| *Bacillus subtilis* (B7 and B9) | Isolated from soumbala (fermented seeds of African locust beans | Antimicrobial activity against pathogens bacteria and fungi, proteolytic activity, lipolytic activity, degradation of polysaccharides and non-digestible oligosaccharides, safe based in the absence of enterotoxins genes | Ouoba *et al*. (2003, 2005, 2007, 2008) |
| *Bacillus amyloliquefaciens* (I8) | Isolated from bikalga (fermented roselle seeds) | High Antimicrobial activity against G+, G- bacteria and fungi, capacity to produce bacteriocins and lipopeptides, safe regarding presence of antimicrobial resistance genes | Compaoré (2013) |
| *Bacillus subtilis* (B3) | Isolated from maari (fermented seeds of baobab) | Antimicrobial activity, capacity to produce bacteriocins | Kaboré (2012) |


***B. subtilis* B7**




***B. subtilis* B9**


***B. subtilis* B3**




***B. amyloliquefaciens* I8**

**Photo 1:** Aspect of the selected starter cultures on nutrient agar

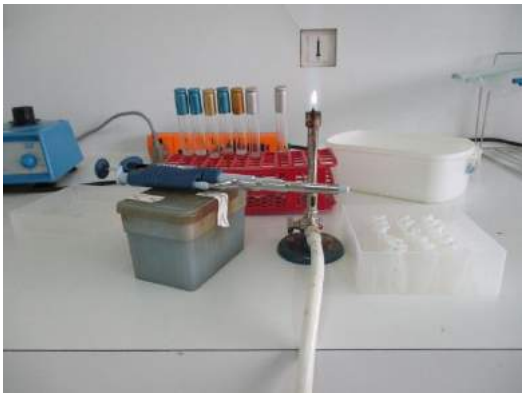
## II. Identification of carrier material for the production of starter cultures in the laboratory for their transfer to the SMEs

Due to the lack of a freeze dryer during the period of the study, we have chosen the dehulled seeds of *Parkia biglobosa* (photo 2) as a carrier material for the transfer of starter cultures to the SMEs. The production of the starter cultures on the carrier material in the laboratory is described below.

Dehulled seeds of African locust beans

## II.1. Preparation of the *Bacillus* inocula

Inocula of the *Bacillus* isolates were prepared as follows: the strains were first revivified by streaking on BHI agar. From BHI agar-plates incubated for 24 h at 37°C, one colony of each isolate was subcultured in 10 ml BHI broth for 24 h at 37°C. After the incubation, 1 ml of the culture was transferred in Eppendorf tube and centrifuged at 5000 g for 10 min. The supernatant was discarded and 1ml of sterile distilled water was added to the pellet for a second centrifugation. After that, the supernatant was discarded again, and the pellet containing the cells was resuspended in 20 ml of sterile distilled water. The number of cells was estimated by microscopy using a counting chamber and dilutions were made in sterile distilled water to obtain a rate of inoculation of $10^5$-$10^6$ cells/ml for the fermentation of the dehulled seeds of African locust beans. Four different inocula were prepared as follows: (i) inoculum of *B. subtilis* B7, (ii) inoculum of *B. subtilis* B9, (iii) inoculum of *B. amyloliquefaciens* I8 and (iv) inoculum of *B. subtilis* B3.



Preparation of *Bacillus* inocula

## II.2. Laboratory scale fermentation for the production of ferment

For the controlled fermentation in the laboratory, dehulled seeds were cleaned (by sorting and washing) before being cooked for 6 h from the boiling point. The cooked cotyledons were distributed (500 g) in baskets and autoclaved at 121°C for 30 min. After cooling at 45-50°C, inoculum was added (2% v/p) to each fermentation batch followed by incubation for 48 h at 37°C. Fermentation was conducted in monoculture with each selected *Bacillus* strain separately. After each batch fermentation, the obtained product was dried aseptically in incubator at 75°C during 24 h. The dried product was then aseptically grinded to obtain a powder of starter cultures. The power was packaged in sterile plastic bags (5 g and 10 g) and constitute the ready to be used as starter for pilot plan and SMEs trials fermentation Samples

were taken at 0 h (after inoculation), at the end of the fermentation (48 h) and after drying and grinding to determine pH and growth of starter culture. The assay was conducted in duplicate.

Four powders of starter cultures were then produced and constituted the ferments to be used for pilot plan and SMEs fermentation trials. They were designed as: Formulation B7 (FB7), Formulation B9 (FB9), Formulation I8 (FI8) and Formulation B3 (FB3).

Microbiological analysis showed that at 0h, the number of starter culture varied from 4, 33 to 4, 87 Log CFU/g. After the fermentation period, the number of each *Bacillus* strain increase with concentrations of 7, 11 to 9, 7 Log CFU/g. From the ferment (powder) , we noticed an increase again of the number of cells varying from 8, 21 to 10, 37 Log CFU/g, probably due to the continuous fermentation during the drying. It was observed that the best fermentations were obtained with the starter originating from soumbala ( FB7, FB9). The ferments produced were safe because no enterrobacteria and Yeasts and Molds were counted after 48 h of fermentation and in the ferments. This is due to the respect of the Good hygienic practices during the production and also the antimicrobial capacity of the selected starter.

Dried ferment

Ferment powder

The flow diagram of the starter cultures production on the carrier material is presented below.

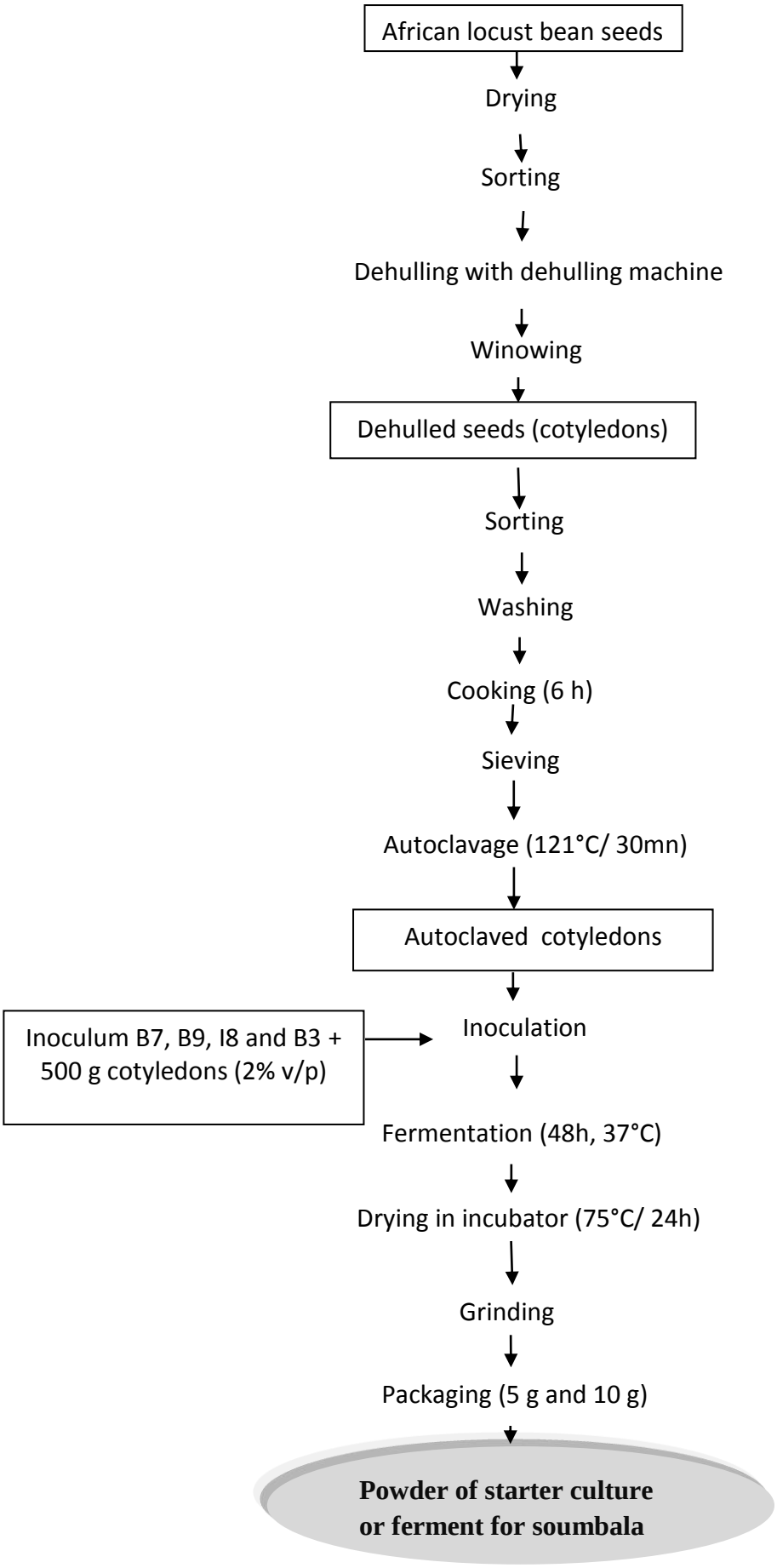



Figure 1: Diagram of starter culture production on carrier material

## III. Pilot plant scale fermentation with starter cultures and consumers preference

### III.1. Pilot plant scale fermentation trials

The ferments produced were used to produce soumbala at pilot plant scale in DTA with one producer of soumbala. The traditional process with non-dehulled seeds of *Parkia biglobosa* was used, except that the cooked cotyledons were inoculated with ferment in monoculture or mixed culture before the fermentation. Spontaneous fermentation of soumbala following the traditional process was also realized to serve as control. The diagram below shows the process of soumbala production with the ferments. The experiments were conducted in triplicate.

The different soumbala obtained were:

NS: Natural soumbala, soumbala obtained from monoculture fermentation (SB7, SB9, SI8 and SB3) and soumbala obtained from mix culture fermentation (SB7+B9, SB7+I8 and SB7+B3).

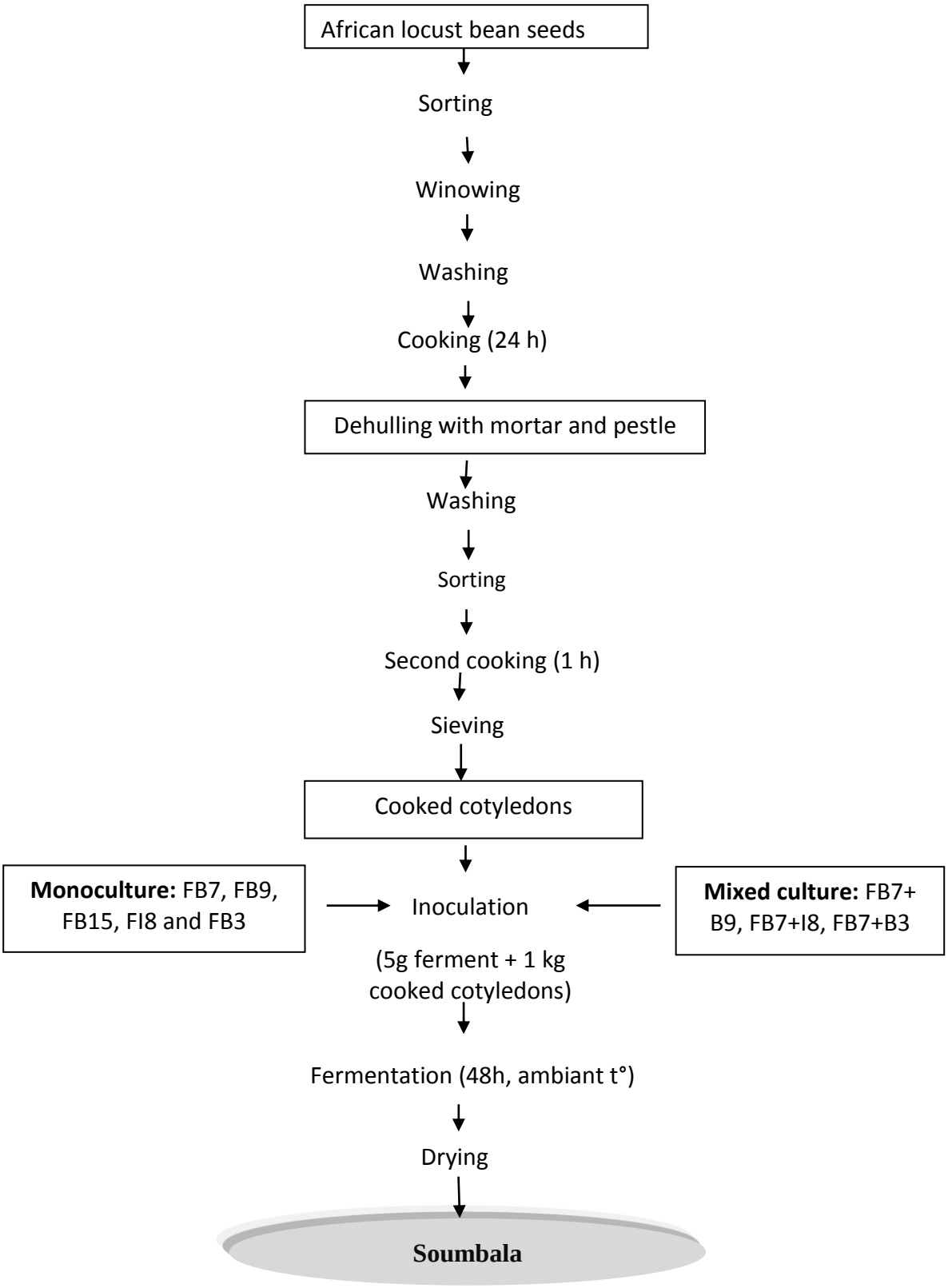



Figure 2: Diagram of soumbala production with the ferments

The microbiological quality (growth of *Bacillus* starter, and indicator of pathogenic microorganisms during the fermentation) and physico-chemical quality (pH, tritable acidity, moisture content, total ash, proteins, lipids, carbohydrates, amino acid profil) of the soumbala produced with the ferments was determined.

In addition, organoleptic analysis was conducted to identify the consumer preference.

### III.2. Sensory evaluation

The evaluation was carried out for each type of soumbala on two separate occasions by a trained panel of 12 producers and 12 regular consumers of soumbala. The analysis of samples of wet soumbala from the traditional and controlled fermentations was performed. The panelists were asked to score for overall acceptability according to appearance, colour, odour and taste.


Soumbala produced with the ferment in monoculture and mixed culture for the sensory evaluation

Preparation of samples for sensory evaluation

Sensory evaluation at DTA

The sensory evaluation revealed variable acceptability according to the type of soumbala and the evaluator.

Soumbala obtained from the monoculture fermentation was not appreciated by the panelists, in particular soumbala obtained with starter cultures of bikalga (I8) and maari (B3). They found that soumbala obtained with these starters is poorly hydrated, did not appear nice, did not smell good and did not taste good even if the seeds are well fermented. Comparing the soumbala from the monoculture fermentation, the one with starter originated from soumbala (SB7 and SB9) was better appreciated than the others because they presented organoleptic characteristics closed to those from the traditional soumbala. However, the panelist preferred the traditional soumbala than soumbala obtained with ferment in monoculture fermentation.
Comparing soumbala from monoculture fermentation and soumbala from mixed culture fermentation, the sensory analysis showed that soumbala from mixed culture fermentation was preferred.

Regarding the comparison of soumbala obtained by traditional fermentation and soumbala obtained with mixed culture of *Bacillus*, the sample SB7+B9 (with starter from soumbala) was found to be very similar to the traditional soumbala in term of colour, odour, appearance and taste and was very well appreciated and preferred than soumbala obtained with the other mixed cultures. These results confirm first, that the starter cultures to be used for a specified product should be selected from the isolates of the same product; secondly, the starter cultures should be used in mixed culture to optimize their performance.

The conclusion here is that the consumers prefer soumbala obtained with mixed starter cultures of *Bacillus* isolated from the same product (soumbala).

**IV. Fermentation trials at SME by using starter cultures**

Based on the results of starter cultures performance and consumer preference from the pilot plant fermentation trials, ferments prepared with soumbala starter cultures were only used in this activity. The ferments were prepared in the laboratory at DTA using the methodology described in section II.2. The starter cultures used were *B. sutilis* B7, B9 and B15 and *B. safensis* B10 (also selected by Ouoba *et al* in 2007 as a potential starter culture for soumbala).

The SMEs of the women association called WENDEMI-PUGSONGO located in Ouagadougou was selected for the transfer of the technology of starter culture.

For the production of soumbala at the SME, the traditional process with non-dehulled seeds of *Parkia biglobosa* was used as described in section III.1. The difference here is that the inoculation was made with only mixed culture (2 different starter cultures and 3 different starter cultures). The diagram below shows the process of soumbala production with the ferments.

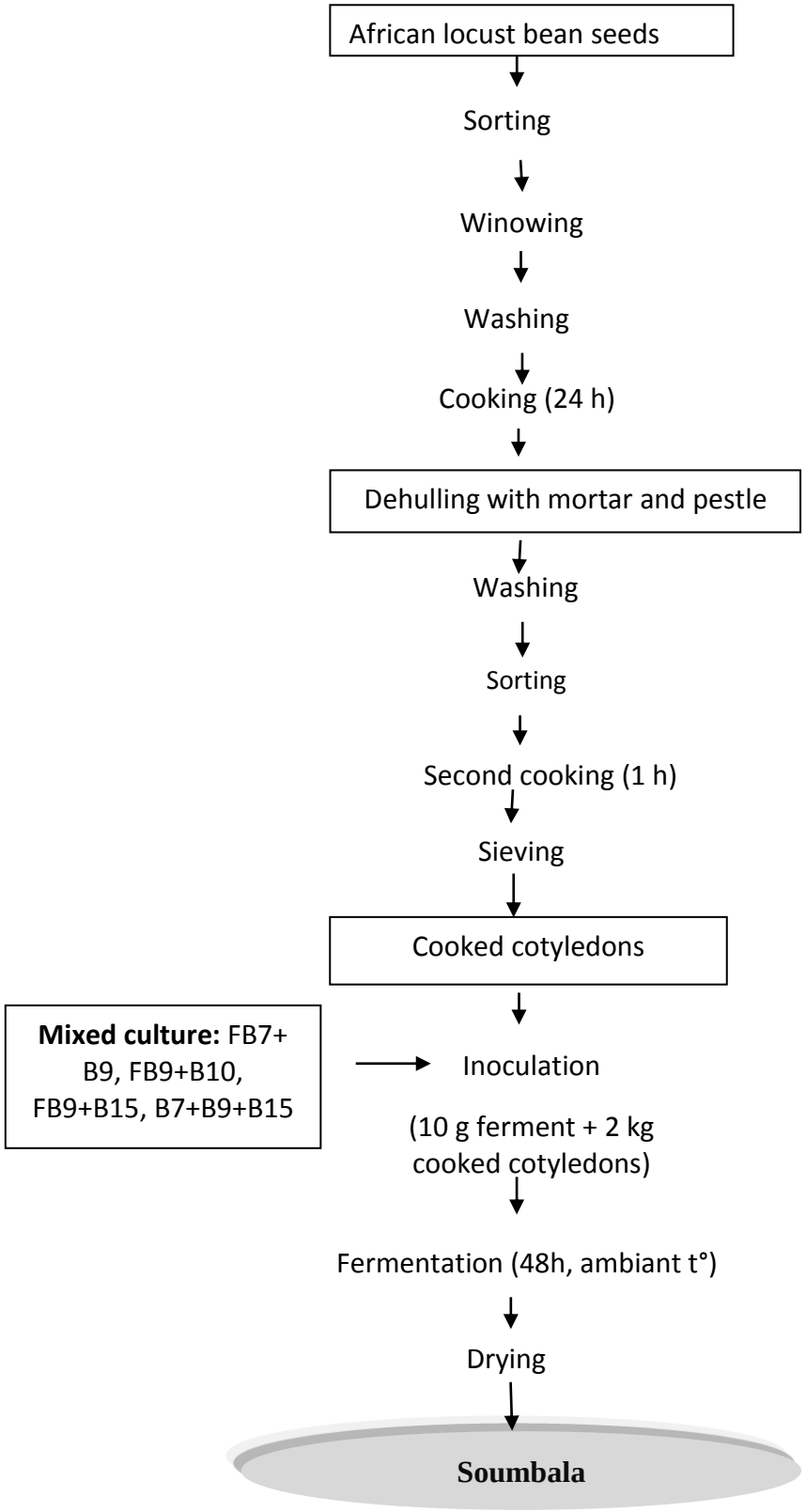





Figure 3: Diagram of soumbala production with the ferments at the SME

Four different fermentation batches were prepared and inoculated as follow:

FB7+B9, FB9+B10 and FB9+B15: 2 kg of cooked cotyledons + 5g of each ferment; FB7+B9+B15: 3 kg of cooked cotyledons + 5 g of each ferment.

Spontaneous fermentation of soumbala following the traditional process was also realized to serve as control.

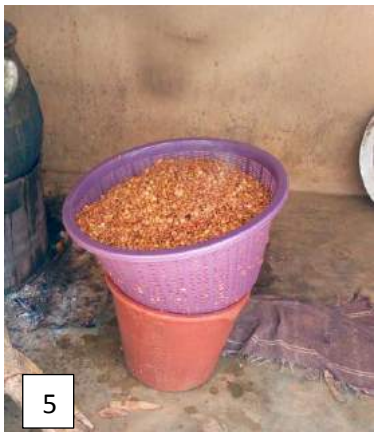
At the end of the fermentation, sampling was done in each fermentation batch for plate counting on nutrient agar in the aim to know the concentration of each starter in the fermented product.





Steps of the soumbala processing at SMEs with the starter cultures:

1. Cooking of Parkia seeds. 2: Traditional dehulling (with motar and pestle) and washing. 3: Shorting of non - dehulled seeds. 4. Rinsing of cooked dehulled cotyledons


5


6


7


8


9


10

Steps of the soumbala processing at SMEs with the starter cultures (continued):

5: Draining of washed cotyledons. 6: Second cooking of the cotyledons. 7, 8, 9 and 10: Weighing, setting of the batches of fermentation and inoculation of each batch of fermentation with the starter cultures

Inoculation of fermentation batch

Inoculation of fermentation batch

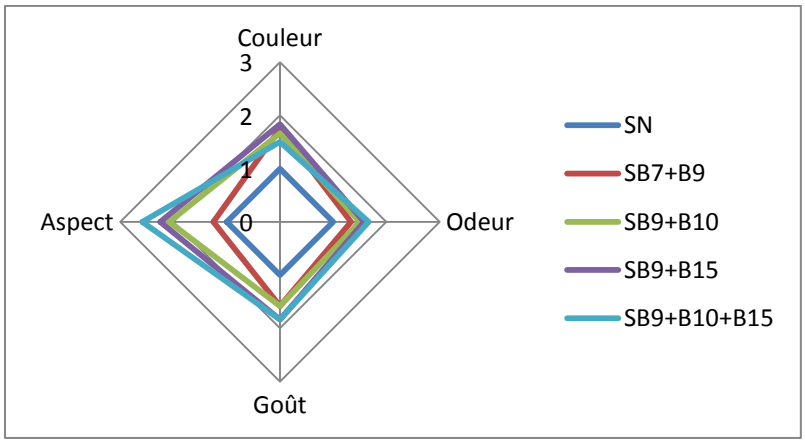
Fermentation batches during the fermentation


**Soumbala produced with starter cultures at the SME**

The fresh fermented soumbala was also submitted to sensory evaluation by a total of 12 panelists including producers of the SME and some consumers.

Sensory evaluation of soumbala produced with starter cultures at the SME

**Sensory evaluation results:**




Sensory evaluation of soumbala produced with starter cultures showed that formulation SB9+B7 (soumbala produced with a mixed culture of two *B. subtilis*) was the best formulation in term of odour, taste, and appearance. This result confirms the results of the pilot plant scale fermentation. The formulation with 3 starter cultures (2 *B. subtilis* + 1 *B. safensis*) was not appreciated by the producers because this formulation was too much fermented and did not have a good taste and good odour.

When we compared the texture of traditional fermented soumbala with soumbala fermented with starter cultures, it was clear that the producer preferred the traditional one. They found that soumbala fermented with starter culture did not have a nice texture compare to traditional soumbala.

**Conclusion**

This study has proven the performance of soumbala starter cultures selected in previous studies for the fermentation of African locust bean. To obtain a desirable product, the starter cultures should not be used in monoculture but in mixed culture. Starter cultures selected from other products similar to soumbala could ferment African locust bean, however, the obtained product was not appreciated by the consumer.

DTA was provided with a fermenter now, therefore it is necessary to repeat this experiment in order to optimize all the parameters of the controlled fermentation for the production of ferments. Better results could also be obtained if the starter cultures are freeze dried before being transferred to the SMEs.