Development of starter cultures carrier for the production of high quality soumbala, a food condiment based on Parkia biglobosa seeds

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Received 14 September, 2020; Accepted 17 November, 2020

In this study, three Bacillus subtilis (B7, B9 and B3) and one Bacillus amyloliquefaciens (I8) strains previously selected as potential starter cultures were cultivated on boiled dehulled African locust bean seeds to produce ready to use ferments. These ferments were then used to produce soumbala. The microbial load changes during ferments production were evaluated. Biochemical and microbiological characteristics of the obtained soumbala were also determined using standard methods. Variable growth ability on the carrier material was observed for the tested starters with Bacillus loads ranging between 8.21 and 10.37 Log CFU/g in the ferments. The highest microbial counts were observed for ferments prepared with the starters B9 and B7. These ferments also demonstrated the strongest fermentation capacity of soumbala. The ferment-based dried soumbala had a pH and moisture ranging from 7.17 to 7.37 and 5.67 to 8.46%, respectively. On dry matter (DM) basis, it contained 1.77 to 2.11% of ashes, 41 to 43% of proteins, 37 to 40% of fat and 13 to 15% of carbohydrates. Soumbala prepared with the starter B7 showed the highest content in valine, isoleucine, leucine, phenylalanine, tyrosine and proline.

Key words: African locust bean, starter culture, Bacillus, fermentation, soumbala, carrier.

INTRODUCTION

Fermented food condiments obtained by the fermentation of proteageneous seeds, are well appreciated in Africa for their high nutritional value and organoleptic properties. In Burkina Faso, the well-known of these fermented food condiments is soumbala, obtained by spontaneous alkaline fermentation of African locust bean (Parkia biglobosa) seeds (Parkouda et al., 2009). Soumbala is also well known and used in Côte d’Ivoire, Guinea, and Mali. It is known under different names such as dawadawa/iru in Nigeria and Ghana (Onzo et al., 2014; Ajavi et al., 2015), afitin/sorou/iru in Benin (Azokpota et al., 2006) and nététu in Senegal (N'Dir et al., 2000).
Soumbala is an affordable source of proteins (34-42%), fat (21-37%), carbohydrates (15-17%), minerals (calcium, phosphorus and iron), vitamins (B1 or thiamine, B2 or riboflavin and PP or niacin) and essential amino acids (Ouoba et al., 2003b) for low income inhabitants. In addition, the production of soumbala constitutes an income generator for producers who are generally illiterate women.

The production of soumbala includes successive cleaning of the seeds, a first cooking which often lasts more than 24 h, a dehulling of the cooked seeds, a second cooking which lasts between 1 and 2 h and then a spontaneous fermentation of 48-72 h (Sawadogo-Lingani et al., 2003). Bacillus subtilis group species were identified as the dominant Bacillus involved in the spontaneous fermentation of soumbala (Ouoba et al., 2004). Despite increasing consumption today in Burkina Faso, soumbala still faces competition from imported seasonings. This is partly due to the use of unsuitable fermentation methods in the production of traditional soumbala leading to a product with poor organoleptic and sanitary quality resulting sometimes in the presence of pathogenic bacteria and biogenic amines (Parkouda et al., 2010).

Several studies carried out on soumbala and other fermented condiments of Burkina Faso provided evidence on how to isolate and characterize some Bacillus species with potential uses as starter cultures in controlled fermentation to improve its hygienic, nutritional and organoleptic quality (Ouoba, 2003; Kaboré, 2012; Compaoré, 2013). However, the form in which these potential starter cultures can be easily transferred to soumbala production units has not been proposed yet. As a consequence, soumbala processing units are still producing soumbala in a traditional way with uncontrolled fermentation. The objectives of this study were, therefore on one hand to assess the possibility of using the dehulled seeds of African locust bean as local carrier material for the transfer of starter cultures of Bacillus spp. to soumbala production units, and on the other hand to compare the biochemical and microbiological characteristics of soumbala prepared with starter cultures used in single or in combination.

**MATERIALS AND METHODS**

**African locust bean seeds**

African locust bean seeds were purchased with a soumbala producer in Ouagadougou, Burkina Faso, stored in polypropylene bags and kept in the pilot plant of Département Technologie Alimentaire (CNRRST/IRSAT/DTA) at room temperature.

**Microorganisms**

The starter cultures used in this study included two strains of *B. subtilis* (B7 and B9) isolated from soumbala, one strain of *Bacillus amyloliquefaciens* (I8) isolated from bikalga (fermented seeds of Hibiscus sabdariffa) and one strain of *B. subtilis* (B3) originating from maari (fermented seeds of Adansonia digitata). These strains were identified based on molecular methods (Rep-PCR, ITS-PCR, M13-PCR, 16S rRNA and gyrB gene sequencing) and selected as starter cultures in previous studies based on their technological properties among other proteolytic, saccharolytic, lipolytic and antimicrobial activities (Ouoba et al., 2003a, 2003b, 2007; Kaboré et al., 2012; Compaoré et al., 2013b). All the strains were kindly provided by the laboratory of microbiology of CNRRST/IRSAT/DTA where they were stored in a -80°C freezer.

**METHODS**

**Preparation of the carrier material**

African locust bean seeds were first dried and cleaned by winnowing to remove light impurities. They were then dehulled using a mechanical dehuller (prototype CNRRST/IRSAT, Ouagadougou, Burkina Faso, 1997). Following dehulling, the cotyledons were separated from the hulls by winnowing and manual sorting. The cotyledons were then collected for use as carrier for the production of the fermentations.

**Preparation of the inocula**

The stock cultures were sub-cultured in Brain Heart Infusion (BHI) agar (Liofilchem, 610007, Italy) and incubated for 24 h at 37°C. From BHI agar plates, the Bacillus strains were sub-cultured for 18 h at 37°C in 10 ml of BHI broth (Liofilchem, 610008, Italy). After incubation, the cultures were centrifuged at 5 000 g for 10 min and the pellet resuspended in 5 ml of sterile diluent containing 8.5 g/l NaCl and 1.5 g/l peptone (Difco 218971, Becton Dickinson & Co, Sparks, MD, USA). The number of cells was then estimated by microscopy using a counting chamber (Neubauer, Werther, Germany) and dilutions were made in sterile diluent to obtain a rate of inoculation of 10^2 - 10^5 cells/ml.

Four different inocula were prepared: Inoculum of *B. subtilis* B7, inoculum of *B. subtilis* B9, inoculum of *B. subtilis* B3 and inoculum of *B. amyloliquefaciens* I8.

**Cultivation of the starter cultures on the carrier material**

The African locust bean cotyledons were weighed and washed before being boiled for 6 h. After cooking and draining, the cotyledones were distributed (500 g) in baskets and autoclaved at 121°C for 20 min. After cooling at 45 to 50°C, each basket was inoculated with each inoculum (2% v/w) in single and left to ferment for 48 h at room temperature (35 - 38°C). One non-inoculated basket served as control.

The fermented cotyledons from each basket were dried in an oven at 60 to 65°C for 24 h before being aseptically ground using a...
Production of soumbala with the ferments

The production of soumbala was carried out with non-dehulled African locust bean seeds following the traditional processing described by Sawadogo-Lingani et al. (2003) with slight modifications as follows: the seeds were cleaned, cooked for 18 h and dehulled manually with mortar and pestle; the dehulled seeds were cooked again for 1 h, drained, distributed in baskets, autoclaved at 121°C for 20 min and cooled to 45 to 50°C. Seven parallel fermentation batches were prepared as follows: the batches 1, 2, 3 and 4 containing 1 kg of sterilized cotyledons each, were inoculated with 5 g of each single ferment (FB7, FB9, FB3 and FI8); the batches 5, 6 and 7 containing 2 kg of sterilized cotyledons each were inoculated with 10 g of mixed ferments (FB7+FB3, FB7+FI8 and FB7+FB9). The batches were then left to ferment for 48 h at room temperature (35-38°C). Traditional spontaneously fermented soumbala was prepared according to Sawadogo-Lingani et al. (2003) to serve as a control (batch 8). After the fermentation, fermented cotyledons were sun-dried and kept in a dry place. Samples were collected at 0, 24 and 48 h after the fermentation (48 h) and after drying. The different types of soumbala produced were:

(1) Soumbala with single ferment: SB7, SB3, SI8, and SB9
(2) Soumbala with mixed ferments: SB7 + B3, SB7 + I8, SB7 + B9
(3) Spontaneously fermented soumbala: SN.

The experiment was conducted in triplicate and 24 samples were taken at each assay. In total 72 samples were collected for microbiological analyses. Physicochemical analyses were performed only on the final dried products (24 samples).

Microbiological analyses

For all samples, 10 g were aseptically homogenized with 90 mL of sterile diluent by using a stomacher (Stomacher 400 lab blender, England) at normal speed for 2 min to obtain 10⁻¹ dilution (ISO 6887-1, 2017). Serial dilutions were made from the homogenate using 9 mL sterile diluent. From appropriate ten-fold dilutions, Bacillus strains were enumerated by pour plate technique using BHI Agar incubated aerobically at 37°C for 72 h. After incubation, plates with 15 to 300 colony forming units (CFU) were counted (ISO 4833, 2003) and results expressed as Log CFU/g.

Biochemical analyses

Ten grams of sample were homogenized with 20 mL of distilled water in a stomacher bag for 1 min at normal speed. The pH of the homogenate was determined using an electronic pH meter (Hanna, Romania) calibrated with standard buffer solutions pH 4.0 and 7.0. Moisture content was determined by drying the sample at 105 ± 2°C for 12 h according to ISO 712 (2009); total ash content was determined by incineration in a muffle furnace (Nabertherm, Germany) at 550°C for 4 h, according to ISO 2171 (2007); crude protein content (X×6.25) was determined by the Kjeldahl method after acid digestion (AFNOR NF V03-050, 1970); crude fat content was determined with Soxhlet apparatus using n-hexane according to ISO 659 (1998). Total carbohydrates content was determined by spectrophotometric method at 510 nm using sulfuric orcinol as reagent (Montreuil and Spik, 1969).

For amino acids profile determination samples were first defatted using Soxhlet method (ISO 659, 1998). The amino acid profile was carried out by high performance liquid chromatography (HPLC) using Waters PICO-TAG method (Kristofferson, 2011) which consists of three steps: hydrolysis of samples, sample derivatization pre-column and HPLC-reverse phase analysis. The identification and determination of the concentrations of the different amino acids were done from the Empower software by comparing retention times obtained with retention times of the standards.

Statistical analysis

All the data were submitted to Analysis of Variance (ANOVA) with the statistical software XLSTAT-Pro 7.5.2 and the means were compared using a Fischer test for post-hoc comparisons with a probability level p < 0.05. The curves and the standard deviation were obtained using Microsoft Excel 2007.

RESULTS

Microbial growth during the production of ferments

No microbial growth was observed after autoclaving of African locust bean cotyledons for all samples (results not shown). Figure 1 shows the growth ability of the different starter cultures inoculated in single in the cooked dehulled African locust bean seeds. At the onset of the fermentation (t = 0 h), Bacillus counts ranged between 4.33 and 4.87 Log CFU/g. At the end of the fermentation (t = 48 h), a significant increase of the Bacillus load was observed in all samples with values ranging between 7.11 and 9.70 Log CFU/g. However, the starter cultures showed variable ability to grow in the cooked dehulled seeds of P. biglobosa. Bacillus amyloliquefaciens I8 (originating from bikalga) counts were the lowest, followed by B. subtilis B3 (originating from maari). On the contrary, B. subtilis B7 and B. subtilis B9 (originating from soumbala) yielded the highest counts. In the final dried and ground products (ferments), the Bacillus counts had increased to between 8.21 and 10.37 Log CFU/g and the highest microbial counts were observed for ferments prepared with B. subtilis B9 and B7.

Microbial growth during controlled fermentation of soumbala using single and mixed ferments

The growth of Bacillus ferments inoculated in single during the production of soumbala is as shown in
Figure 1. Growth of Bacillus starter population during the production of ferments. FB7: Ferment produced with starter culture *B. subtilis* B7; FB3: Ferment produced with starter culture *B. subtilis* B3; FI8: Ferment produced with starter culture *B. amyloliquefaciens* I8; FB9: Ferment produced with starter culture *B. subtilis* B9.

Figure 2. Growth of Bacillus starter population during the production of soumbala using single ferments. SN: spontaneous soumbala; SB7: soumbala produced with ferment B7; SB3: soumbala produced with ferment B3; SI8: soumbala produced with ferment I8; SB9: soumbala produced with ferment B9.

Figure 2. At the beginning of the fermentation, soumbala produced with single ferment (SB7, SI8, SB3 and SB9) had a *Bacillus* load between 6.74 and 7.66 Log CFU/g whereas spontaneous soumbala had a load of 2.86 Log CFU/g. At the end of the fermentation (48 h), the *Bacillus* loads increased in all types of soumbala and ranged...
between 7.92 and 9.54 Log CFU/g. The increase of *Bacillus* count was also observed in final dried products between 8.38 and 10.11 Log CFU/g. The highest *Bacillus* counts were obtained with ferments of *B. subtilis* B9 (10.11 Log CFU/g) and *B. subtilis* B7 (9.84 Log CFU/g). Regarding the *soumbala* obtained by spontaneous fermentation (SN), its *Bacillus* load after drying was 8.93 Log CFU/g.

Figure 3 shows the growth capacity of the *Bacillus* ferments used in mixture during the controlled fermentation of *soumbala*. Used in mixture, the starter cultures' loads increased from 7 Log CFU/g at the onset of the fermentation to 8 Log CFU/g at the end of the fermentation, while for spontaneous fermentation, microbial population increased from 2 to 8 Log CFU/g. After drying, the *Bacillus* counts were between 9.61 and 9.78 Log CFU/g for *soumbala* produced with ferments and 8.93 Log CFU/g for spontaneous *soumbala*.

### Proximate composition of *soumbala* produced with ferments of starter cultures

Results from Table 1 show the proximate composition of the different samples of *soumbala*. The pH of *soumbala* produced with ferments of starter cultures varied from 7.17 ± 0.01 to 7.25 ± 0.11 for single culture *soumbala* and from 7.31 ± 0.04 to 7.37 ± 0.02 for mixed cultures *soumbala*. However, *soumbala* from spontaneous fermentation had a pH of 7.21 ± 0.01. The lowest pH value (7.15 ± 0.01) was obtained with single starter culture *B. amyloliquefaciens* I8 (originating from *bikalga*) while the highest pH (7.37 ± 0.02) was recorded with the combination of *B. subtilis* B7 and B9 (originating from *soumbala*). There was no significant difference (p > 0.05) between the pH of the different *soumbala* produced using combined starter cultures. The moisture content of controlled fermented dried *soumbala* ranged between 5.67 ± 0.51 and 8.46 ± 0.67% whereas the spontaneous dried *soumbala* showed a moisture content of 6.19 ± 0.39%. Dried *soumbala* obtained with mixed starter culture SB7+I8 showed the highest moisture content (8.46 ± 0.67%). Single culture inoculated *soumbala* and mixed culture inoculated *soumbala* ash content ranged from 1.95 ± 0.16 to 2.11 ± 0.07%/DM and 1.77 ± 0.05 to 1.90 ± 0.04%/DM, respectively. Meanwhile, ash content of spontaneous *soumbala* was 2.11 ± 0.02%/DM. *Soumbala* obtained with a mixed culture of *B. subtilis* B7 and *B. amyloliquefaciens* I8 showed the lowest ash content (1.77 ± 0.05%/DM). Analyses showed that there was no significant difference (p > 0.05) between ash content of spontaneous *soumbala* and *soumbala* from starter B7 (SB7), I8 (SI8) and B9 (SB9). Protein content varied between 41 and 43%/DM with spontaneous *soumbala* giving the weakest rate (41.19 ± 0.89%/DM) compared to mixed culture *soumbala* (SB7+I8) which gave the highest rate (43.78 ± 0.13%/DM). There was significant difference (p < 0.05) between protein contents of all samples of *soumbala*. Regarding the crude fat content, it ranged from 37.46 ± 0.30%/DM (obtained with SB7+B3) to 40.67 ± 0.17%/DM (obtained with SB3) for *soumbala* produced with starter cultures and was

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**Figure 3.** Growth of *Bacillus* starter during the production of *soumbala* using mixed ferments. SN: spontaneous *soumbala*; SB7+B3: *soumbala* produced with ferment B7 in association with ferment B3; SB7+I8: *soumbala* produced with ferment B7 in association with ferment I8; SB7+B9: *soumbala* produced with ferment B7 in association with ferment B9.

![Growth of Bacillus starter during the production of soumbala using mixed ferments](image-url)
Amino acid profiles of *soumbala* produced with ferments of starter cultures

Amino acid profiles (in g/100 g DM) of *soumbala* produced with ferments of *Bacillus* starter as well as *soumbala* from spontaneous fermentation are presented in Table 2. The different *soumbala* presented variable content in essential amino acids. The highest contents in valine (1.038), leucine (1.138), isoleucine (0.772), phenylalanine (0.722), tyrosine (1.064) and proline (0.641) were obtained with *soumbala* fermented using the ferment produced with the starter B7. However, these amino acids were observed in low concentrations in *soumbala* produced with the combination of starter cultures B7 and B9. The *soumbala* SI8 presented the lowest content in histidine (0.102) while the highest content was observed for *soumbala* SB9 (0.208).

39.66 ± 0.05%/DM for spontaneously fermented *soumbala* (SN). Fat content of SB7+B9, SB7+I8 and SB7 was not significantly different (p > 0.05). Likewise, there was no significant difference (p > 0.05) between fat content of SN and SB9. The *soumbala* that was fermented using the ferment B9 had the highest total carbohydrates value (15.33 ± 0.57 %/DM), while the combination of ferments B7 and B3 gave the lowest content (13.81 ± 0.18%/DM). SN had a content of total carbohydrates of 14.57 ± 0.04%/DM.

**Table 1.** Proximate composition of spontaneous *soumbala* and *soumbala* produced using ferments in single or mixed culture.

| Type of formulation | Type of *soumbala* | pH            | Moisture (%) | Ashes (g/100 g DM) | Proteins (g/100 g DM) | Fat (g/100 g DM) | Carbohydrates (g/100 g DM) |
|---------------------|--------------------|---------------|--------------|--------------------|-----------------------|----------------|----------------------------|
| Without ferment     | SN                 | 7.21 ± 0.01<sup>cd</sup> | 6.19 ± 0.39<sup>bc</sup> | 2.11 ± 0.02<sup>a</sup> | 41.19 ± 0.89<sup>d</sup> | 39.66 ± 0.05<sup>bc</sup> | 14.57 ± 0.04<sup>abcd</sup> |
| Single culture      | S<sub>B7</sub>    | 7.17 ± 0.01<sup>cd</sup> | 5.67 ± 0.51<sup>c</sup> | 2.11 ± 0.07<sup>a</sup> | 42.51 ± 0.43<sup>bc</sup> | 39.13 ± 0.25<sup>c</sup> | 14.77 ± 0.26<sup>abcd</sup> |
|                     | S<sub>B3</sub>    | 7.25 ± 0.11<sup>bc</sup> | 6.23 ± 0.74<sup>bc</sup> | 1.95 ± 0.16<sup>bc</sup> | 41.58 ± 0.22<sup>cd</sup> | 40.67 ± 0.17<sup>a</sup> | 15.11 ± 0.47<sup>ab</sup> |
|                     | S<sub>B9</sub>    | 7.15 ± 0.01<sup>d</sup> | 6.06 ± 0.20<sup>bc</sup> | 2.05 ± 0.10<sup>ab</sup> | 41.87 ± 0.12<sup>cd</sup> | 40.28 ± 1.03<sup>ab</sup> | 14.22 ± 0.02<sup>cde</sup> |
|                     | S<sub>B7, B3</sub> | 7.21 ± 0.04<sup>cd</sup> | 6.30 ± 0.82<sup>bc</sup> | 2.09 ± 0.06<sup>ab</sup> | 42.75 ± 0.42<sup>cd</sup> | 39.68 ± 0.22<sup>bc</sup> | 15.33 ± 0.57<sup>a</sup> |
|                     | S<sub>B7, I8</sub> | 7.31 ± 0.04<sup>ab</sup> | 7.15 ± 0.90<sup>ab</sup> | 1.85 ± 0.01<sup>cd</sup> | 42.02 ± 0.23<sup>bcd</sup> | 37.46 ± 0.30<sup>d</sup> | 13.81 ± 0.18<sup>e</sup> |
|                     | S<sub>B7, I8</sub> | 7.35 ± 0.01<sup>a</sup> | 8.46 ± 0.67<sup>a</sup> | 1.77 ± 0.05<sup>d</sup> | 43.78 ± 0.13<sup>a</sup> | 38.76 ± 1.03<sup>c</sup> | 14.13 ± 0.51<sup>ab</sup> |
|                     | S<sub>B7, I8</sub> | 7.37 ± 0.02<sup>a</sup> | 6.74 ± 1.47<sup>bc</sup> | 1.90 ± 0.04<sup>cd</sup> | 41.47 ± 1.18<sup>d</sup> | 38.86 ± 0.38<sup>c</sup> | 14.16 ± 0.03<sup>ab</sup> |
| P value             | < 0.0001           | 0.0162        | 0.0003       | 0.0011             | 0.0001               | 0.0005         |
| F value             | 9.67               | 3.59          | 8.20         | 6.36               | 9.63                 | 7.31           |

SN: Spontaneous *soumbala*; S<sub>B7</sub>: *soumbala* with ferment of *B. subtilis* B7; S<sub>B3</sub>: *soumbala* with ferment of *B. subtilis* B3; S<sub>B9</sub>: *soumbala* with ferment of *B. amyloliquefaciens* I8; S<sub>B7, B3</sub>: *soumbala* with ferment of *B. subtilis* B7 + *B. subtilis* B3; S<sub>B7, I8</sub>: *soumbala* with ferment of *B. subtilis* B7 + *B. amyloliquefaciens* I8; S<sub>B7, B9</sub>: *soumbala* with association of ferment of *B. subtilis* B7 + *B. subtilis* B9. For each column, the values with a common letter are not significantly different according to Fisher test at the 5% threshold.

DISCUSSION

The increase of *Bacillus* loads during the production of ferments indicates that the starter cultures used in the study are able to use African locust bean cooked cotyledons as substrate for their growth. However, the fermentation capacity varied among the strains. The highest loads observed with the starter B7 and B9 are probably due to the fact that these strains were previously isolated from the fermentation of the same substrate and are therefore more able to use this substrate for their growth. Indeed, the autochthonous character of these starters gives them a better implantation during the fermentation process (Fessard, 2017). The low concentrations of *Bacillus* in the ferments prepared with starter cultures B3 and I8 may be explained by their non-autochthonous character. Therefore, African locust bean seeds cotyledons may not be a favorite substrate for their growth.

The *Bacillus* loads (9.63 - 9.70 log CFU/g) found in the ferments prepared with starter cultures B7 and B9 were close to those of Agbobatinkpo et al. (2012) who also...
found *Bacillus* loads of 9.7 log CFU/g in *yanyanku* and *ikpiru*, two food additives (obtained by the fermentation of *H. sabdarif* seeds) used in Benin for the fermentation of African locust bean seeds into *sonru* and *iru*. In addition, during the controlled fermentation of *Aftitin* with *B. subtilis* starter cultures, the maximum load of *Bacillus* after fermentation was 9.5 log CFU/g (Ahonoukoun, 2014).

The increase in *Bacillus* load during the controlled fermentation of *soumbala* with the fermentates demonstrates the fermentation capacity of these fermentates. However, fermentates produced with starter cultures B7 and B9 demonstrated the strongest fermentation capacity, with the highest loads when used in monoculture (9.84-10.11 Log CFU/g) and in mixed culture (9.79 Log CFU/g). Similar range of bacterial counts in *soumbala* or similar products have been reported previously (Sawadogo/lingani et al., 2003; parkouda, 2009; Amoa-Awua et al., 2014; Ajavi et al., 2015; Guissou et al., 2020).

In this study, the pH of *soumbala* produced with ferments of starter cultures was alkaline, like that of traditional spontaneous *soumbala*. This result is in agreement with those recorded for similar African fermented condiments by other authors (Azokpota et al., 2006; Akabanda et al., 2018; Mohammadou et al., 2018; Ibrahim et al., 2018). This alkaline pH is due to the proteolytic activity of the fermenting microorganisms, which degrade proteins and release ammonia in the medium (Mohammadou et al., 2018). The results reported here corroborate those of Agbobatin et al. (2012) in Benin, during the study of the fermentation ability of *yanyanku* and *ikpiru*, who found an average pH ranging from between 7.1 and 7.3 for African locust bean cotyledons fermented with or without additives. However, Sawadogo et al. (2003) and Guissou et al. (2020) during spontaneous fermentation of *P. biglobosa* seeds to produce *soumbala* found lower pH values in the dried products. The low water content observed in the various *soumbala* would promote their conservation (Ajavi et al., 2015).

The content of ashes obtained for the different *soumbala* (1.77 - 2.11%) was lower than those found by Agbobatin et al. (2012) which were 2.6 to 3.2%. This difference could be explained by the addition of ash solution during the preparation of the additives *yanyanku* and *ikpiru* used for the fermentation of *P. biglobosa* seed-based condiments in Benin, or by the difference in ash content of the seeds used in each country. The spontaneous *soumbala* as well as *soumbala* produced with ferments of starter cultures also presented lower concentrations of ashes compared to the results recently

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### Table 2. Amino acid profiles of spontaneous *soumbala* and *soumbala* produced using ferments in single or mixed culture (g/100 g DM).

| Amino acid   | SB7   | SB3   | SB8   | SB9   | SB7+B3 | SB7+I8 | SB7+B9 | SN | P value | F value |
|--------------|-------|-------|-------|-------|--------|--------|--------|----|---------|---------|
| Aspartic acid| 0.128 | 0.351 | 0.140 | 0.214 | 0.248  | 0.211  | 0.143  | 0.141 | 0.0056  | 4.57    |
| Glutamic acid| 0.077 | 0.089 | 0.062 | 0.112 | 0.073  | 0.076  | 0.086  | 0.110 | 0.0011  | 6.38    |
| Serine       | 0.069 | 0.054 | 0.035 | 0.093 | 0.062  | 0.095  | 0.052  | 0.060 | 0.1475  | 1.84    |
| Glycine      | 0.276 | 0.260 | 0.165 | 0.227 | 0.231  | 0.238  | 0.177  | 0.204 | 0.0673  | 2.43    |
| Histidine    | 0.161 | 0.192 | 0.102 | 0.208 | 0.181  | 0.176  | 0.146  | 0.169 | 0.0025  | 5.42    |
| Arginine     | 0.075 | 0.075 | 0.071 | 0.075 | 0.096  | 0.137  | 0.079  | 0.102 | 0.1615  | 1.76    |
| Threonine    | 0.083 | 0.057 | 0.049 | 0.053 | 0.074  | 0.109  | 0.054  | 0.062 | 0.0022  | 5.57    |
| Alanine      | 0.399 | 0.425 | 0.275 | 0.465 | 0.453  | 0.506  | 0.376  | 0.482 | 0.0631  | 2.48    |
| Proline      | 0.641 | 0.355 | 0.484 | 0.560 | 0.344  | 0.488  | 0.428  | 0.528 | 0.0058  | 4.55    |
| Tyrosine     | 1.064 | 0.992 | 0.786 | 0.876 | 0.815  | 0.919  | 0.655  | 0.794 | 0.0252  | 3.21    |
| Valine       | 1.038 | 0.682 | 0.603 | 0.677 | 0.728  | 0.877  | 0.493  | 0.708 | 0.0042  | 4.87    |
| Methionine   | 0.076 | 0.066 | 0.065 | 0.063 | 0.069  | 0.077  | 0.032  | 0.062 | 0.0326  | 1.27    |
| Cysteine     | 0.012 | 0.030 | 0.015 | 0.044 | 0.020  | 0.040  | 0.036  | 0.0012| 6.21    |
| Isoleucine   | 0.772 | 0.392 | 0.374 | 0.370 | 0.439  | 0.566  | 0.330  | 0.436 | <0.0001 | 12.03   |
| Leucine      | 1.138 | 0.685 | 0.617 | 0.711 | 0.794  | 0.950  | 0.594  | 0.790 | 0.0003  | 8.09    |
| Phenylalanine| 0.723 | 0.595 | 0.432 | 0.526 | 0.556  | 0.651  | 0.427  | 0.555 | 0.0052  | 4.66    |
| Lysine       | 0.707 | 0.791 | 0.520 | 0.751 | 0.670  | 0.715  | 0.565  | 0.673 | 0.0300  | 3.07    |

SN: Spontaneous *soumbala*; SB7: *soumbala* with ferment of *B. subtilis* B7; SB8: *soumbala* with ferment of *B. subtilis* B8; SB9: *soumbala* with ferment of *B. subtilis* B9; SB7+B3: *soumbala* with association of ferment of *B. subtilis* B7 + *B. subtilis* B3; SB7+I8: *soumbala* with association of ferment of *B. subtilis* B7 + *B. amyloliquefaciens* I8; SB7+B9: *soumbala* with association of ferment of *B. subtilis* B7 + *B. subtilis* B9. For each line, the values with a common letter are not significantly different according to Fisher test at the 5% threshold.
presented by Guissou et al. (2020).

The protein levels obtained in this study were higher than those obtained for *sonru* and *iru* fermented with *yanyanku* and *irkiru* additives, which average was 35% (Agbobatinkpo et al., 2012). The variation of the protein contents may be due to the proteolytic activity of the fermenting strains (Mohammadou et al., 2018) and also to the difference in the physicochemical composition of African locust bean seeds according to the localities. Results demonstrated that controlled fermented *soumbala* as well as spontaneous fermented *soumbala* were rich in proteins (> 40%). Therefore, *soumbala* could be a source of protein that could help poor population to meet their requirement for this nutrient, particularly in developing countries. High amount of protein was also noted for other alkaline fermented products and was related to *Bacillus* counts (Terlabie et al., 2006; Mohammadou et al., 2018).

The different *soumbala* prepared with the ferments of *Bacillus* spp. presented interesting fat contents (37 - 40%). The fat content of *soumbala* prepared using single ferment is comparable to that reported by Guissou et al. (2020), which was 40.47%. The carbohydrate contents found are also in agreement with that reported by Guissou et al. (2020).

Results showed that *soumbala* produced with ferments of *Bacillus* spp. contained more essential amino acids than the traditional spontaneous *soumbala*. *Soumbala* produced using the starter culture B7 had the highest levels of valine, leucine, isoleucine and phenylalanine. Similar results were previously obtained by Ouoba et al. (2003b) in *soumbala* produced by controlled fermentation using the same *B. subtilis* as starter culture. As reported by the same authors (Ouoba et al., 2003b), it was also found that *soumbala* produced with starter culture B9 contained high content of histidine compared to *soumbala* produced with starter culture B7.

The presence of high amounts of lysine is particularly interesting because lysine is a limiting amino acid in cereals and seeds that constitute the staple diet of the majority of African populations (Diawara et al., 2004). The *soumbala* produced with this starter culture could then be used to fortify foods. The presence of non-essential amino acids such as tyrosine, proline and glycine at significant content in certain samples is also of interest since these amino acids could be essential in some human physiological circumstances (Ouoba et al., 2003b). Variable concentrations of amino acids in African fermented condiments have been reported in other studies (Parkouda et al., 2015; Akabanda et al., 2018; Ibrahim et al., 2018).

**Conclusion**

In the present study, four *Bacillus* strains (B. *subtilis* B7, B. *subtilis* B9, B. *subtilis* B3 and B. *amyloliquefaciens* I8) previously isolated from spontaneous fermentation of three different condiments and selected as starter cultures were successfully developed on dehulled African locust bean seeds used as carrier material to produce ferments. These ferments have been used separately or in combination to control the fermentation of African locust bean seeds into *soumbala*, which present interesting microbiological and nutritional characteristics. The obtained results indicate that dehulled African locust bean seeds are a promising carrier material for the transfer of *Bacillus* starter cultures to *soumbala* production units. These results may help to standardize the *soumbala* production process as well as its quality. However, further investigations need to be performed to evaluate the performance of these ferments in real environment and assess their stability during storage.

**CONFLICT OF INTERESTS**

The authors have not declared any conflict of interests.

**ACKNOWLEDGEMENTS**

The authors are grateful to the Ministry of Foreign Affairs of Denmark (Danida) for funding through the project “Preserving African food microorganisms for Green Growth” (project number DFC No. 13-04KU). The authors would like to appreciate all the soumbala producers involved in the study for their collaboration.

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