



## **Fate of proteic and lipidic compounds during production of a traditional legume condiment (Soumbala) made from African Locust Bean ( *Parkia biglobosa* ) seeds**

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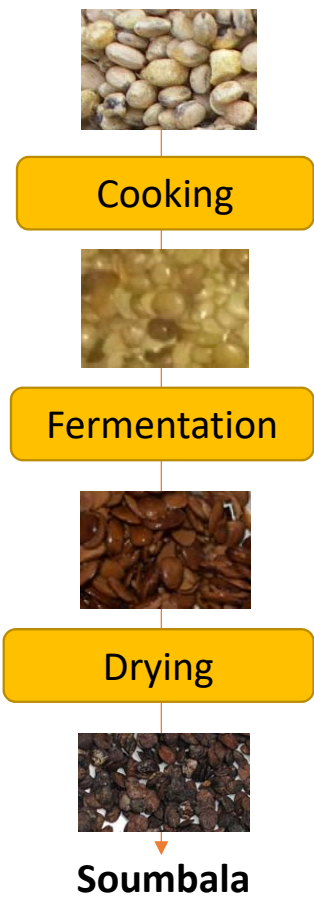


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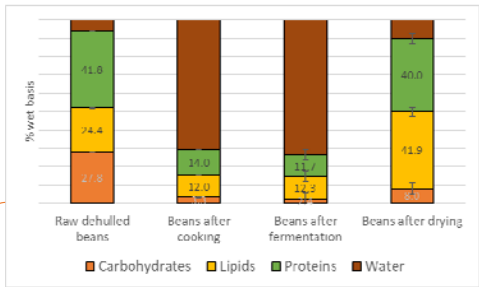
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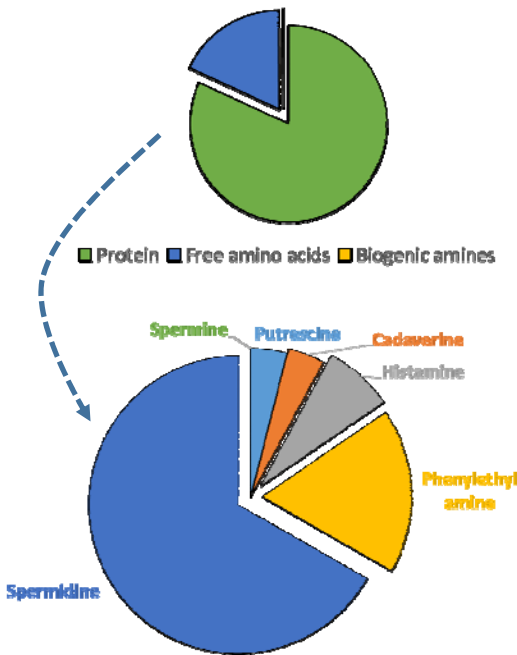
Parkia biglobosa seeds



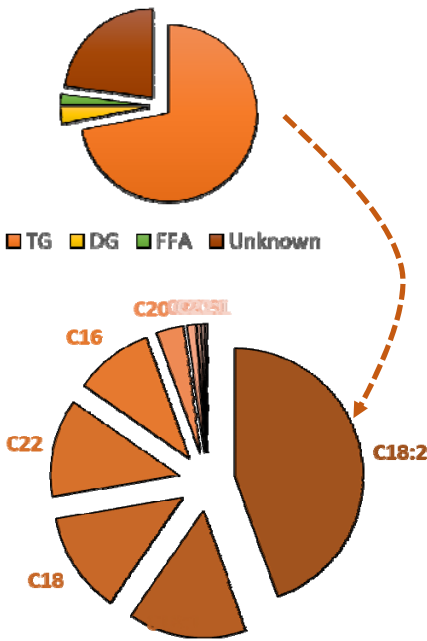
Macronutrients composition



Nitrogen fraction



Lipid extraction



# **Fate of proteic and lipidic compounds during production of a traditional legume condiment (Soumbala) made from African Locust Bean (*Parkia biglobosa*) seeds**

## **Running title: *Parkia biglobosa* seeds process & composition**

Michel Yavo Esse<sup>a</sup>, Tagro Simplicie Guehi<sup>a</sup>, Joël Grabulos<sup>b,c</sup>, Gilles Morel<sup>b,c</sup>, Robert Thierry Malomar<sup>b</sup>, Eric Tardan<sup>d,c</sup>, Christian Mestres<sup>b,c</sup>, Nawel Achir<sup>b\*</sup>

<sup>a</sup>Laboratoire de Biotechnologie et Microbiologie des Aliments, UFR de Sciences et Technologies des Aliments, Université Nangui Abrogoua

<sup>b</sup>QualiSud, Univ Montpellier, CIRAD, Institut Agro, Université d'Avignon, Université de la Réunion, Montpellier, France

<sup>c</sup>CIRAD, UMR QualiSud, F-34398 Montpellier, France

<sup>d</sup>BioWooEB, CIRAD, Montpellier, France

\*Corresponding author: Nawel Achir. 1101, avenue Agropolis, 34090 Montpellier Cedex 5, France.

E-mail: [nawel.achir@supagro.fr](mailto:nawel.achir@supagro.fr)

## **Abstract**

Soumbala is made from African locust bean seeds (*Parkia biglobosa*), a legume widely spread in west Africa. It is a lipid and protein-rich condiment obtained by three main steps: cooking, fermentation and drying. **Ten different analysis** were made on AFLBS samples at each step with a particular focus on protein and lipid fates. Results showed that cooking decreased markedly the carbohydrate content leaving mainly proteins and lipids as substrates for fermentation. During this step, 20% of the proteins were converted into free amino acids, mainly glutamic acid and tyrosine. Significant biogenic amines release occurred and reached 50 mg/100g of dried ALBS. Di-glycerides and fatty acids increased markedly and represented 11% and 7% of the total lipids of dried ALBS. These results give a complete view of the nutritional characteristics of dried ALBS, its benefit but also its defects that arise during fermentation and not suppressed by the subsequent processing steps.

**Keywords:** soumbala; nutritional composition; processing; biogenic amine; di-glyceride; free fatty acids

30

## 1. Introduction

Fermented products are the basis of food in many countries. They can be obtained from various raw materials such as cereals, fish, fruits but also legumes. Among them, fermentation of African locust bean seeds (*Parkia biglobosa*) gives an aromatic side dish or condiment consumed in African west coast countries. *Parkia biglobosa*, also known as néré in Francophone Africa, is a tree that has been used traditionally for medicine and food purposes (Abbas *et al.*, 2018). Fermented African locust bean seeds (ALBS) are traditionally called dawadawa (Nigeria, Ghana), netetu (Senegal), afitin (Benin) or soumbala (Mali, Burkina and Côte d'Ivoire) (Ndir *et al.*, 2000; Azokpota *et al.*, 2006a; Esenwah and Ikenebomeh, 2008). Fermentation of ALBS is made to develop the sensory properties of the final condiment and particularly its aroma. More precisely, final seeds are characterized by a strong flavour of ammonia also described as strong and pungent (Ouoba *et al.*, 2005; Amoa-Awua *et al.*, 2014). In appearance, they are brown because of polyphenol oxidation during processing. They can be dried and grounded to powder or moulded into balls of about 3 cm of diameter (Yusuf and Rahji, 2012). Because they are generally dried, they can be conveniently stored within a shelf life of one year till the next harvest. They are usually not consumed alone but added to stew or vegetables like bouillon cubes.

Consumption of such products, with a very distinctive odor and taste, has decreased over the past decades for the benefit of industrial alternatives (Ham, 2017). However, current nutritional and sustainability considerations encourage agronomist, nutritionist and food scientists to pay attention again to this very interesting legume and its transformation (Bari *et al.*, 2016; Ham, 2017; Abbas *et al.*, 2018; Lado *et al.*, 2018; Ekperikpe *et al.*, 2019). ALBS are nutritionally interesting since they present a balanced composition of protein (39 à 47%), lipids (31 à 40%) and carbohydrates (12 à 16%) (Odunfa, 1985; Ndir *et al.*, 2000; Esenwah and Ikenebomeh, 2008). Therefore, they can increase the daily intakes in proteins and lipids while being more sustainable and affordable than meat products (Ham, 2017). As a legume, ALBS contains anti-nutritional compounds (trypsin inhibitor, tannins, oligosaccharides and phytic

acid) but once fermented, their level are fairly decreased (Esenwah and Ikenebomeh, 2008; Yusuf and Rahji, 2012).

The traditional process of soumbala production is quite long (4 to 5 days). While it may vary slightly depending on the region of production, its process consists in different steps presented in Fig. 1. Four main unitary operations may affect the composition of ALBS: the first is cooking of seeds in boiling water during 12 to 24h to soften their shell before dehulling. The second step is the cooking of the resulting cotyledons in a small quantity of boiling water. This step softens the tissues before fermentation. The 3<sup>rd</sup> and major step is the fermentation of the cooked cotyledons. Finally, the post fermentation step is a stabilisation step, and is usually sun-drying (Odunfa, 1985; Dakwa *et al.*, 2005; Yusuf and Rahji, 2012). Literature about ALBS transformation is quite scarce. However, previous studies carried out on the transformation of ALBS into Soumbala have shown that there are significant composition modifications during processing (Odunfa, 1985; Azokpota *et al.*, 2006a). The operation unit most studied in literature is fermentation (Ouoba *et al.*, 2003b,a; Azokpota *et al.*, 2006a) while pre-fermentation steps are very poorly described and their consequence on the biochemistry of seeds, not studied. Indeed, fermentation is assumed to be the operation that gives the typical organoleptic properties of final product (Ouoba *et al.*, 2005; Azokpota *et al.*, 2006a; Amoa-Awua *et al.*, 2014). The microflora associated to the fermentation of ALBS is essentially composed of sporulated germs of the genus *Bacillus*. Their metabolic characteristic is to be able to use all macro-constituents as a substrate for their growth and particularly proteins, releasing free amino acids and basic amino molecules increasing pH (Ouoba *et al.*, 2003a; Azokpota *et al.*, 2006b). This alkaline fermentation is mainly responsible of the typical aroma of fermented ALBS (Omafuvbe *et al.*, 2004; Ouoba *et al.*, 2005). Pyrazines were also found in the fermented ALBS aroma compounds probably due to Maillard reactions that may occur during pre or post fermentation steps (Esenwah and Ikenebomeh, 2008; Amoa-Awua *et al.*, 2014). It was also shown that during fermentation, consumption of protein from *Bacillus* lead to free amino acids release at high quantity (Odunfa, 1985). It is known that free amino acids may lead to biogenic amines (Ruiz-Capillas and Herrero, 2019). A biogenic amine is a basic molecule with one or more amine groups generated by microbial decarboxylation of amino acids. When present in fermented products in high amount, biogenic amines may cause not only organoleptic defects but also adverse symptoms for human health. Indeed, biogenic

amines have biological effects and may among others cause some neurotransmission disorders, headache, gastrointestinal disorders, and edema by giving rise to blood vessel dilatations (Erdag *et al.*, 2018). Histamine is the most toxic of these amines and its action is enhanced by the presence of other amines such as putrescine, cadaverine, spermidine, tyramine and tryptamine. Histamine poisoning has been firstly diagnosed in fishery products (Ruiz-Capillas and Herrero, 2019). However, other products involving a fermentation step are likely to contain high amounts of biogenic amines such as wine, cheese or fermented soya products (Krause *et al.*, 1997; BIOHAZ, 2011; Byun and Mah, 2012).

The objective of this paper is to analyse the effect of the whole process of ALBS transformation in soumbala on the fate of the macromolecule of nutritive interest, i.e. proteins and lipids, but also of their resulting molecules that may be toxic and particularly biogenic amine that have never been studied in fermented ALBS products.

## 2. Material and methods

### 2.1. Plant material

African locus bean seeds and Soumbala samples were collected in the region of Khorogo, where most Soumbala production is located, in the north of Côte d'Ivoire.

### 2.2. Soumbala production from ALBS

The processing steps are described in Fig. 1. ALBS were firstly put into boiling water at a ratio seed/water of 1/3 during 24h. The seeds were dehulled in a laboratory mortar. Dehulled raw seeds were cooked in boiling water at a ratio seed/water of 1/1 during 1 hour. The cooked seeds were enclosed in jute bags and placed in small baskets in a chamber regulated at 40 ° C during 48h. Fermented seeds were dried in an oven at 50°C during 12h. The whole process was repeated 3 times. A total of 14 samples were collected throughout the production process and more precisely: 5 samples of raw seeds, 3 samples after cooking, 3 after 48 hours of fermentation and 3 after drying. All samples were dried and stored at -18 ° C.

### 2.3. Chemicals

1  
2  
3 119 All amino acids, biogenic amines (putrescine, cadaverine, histamine, phenethylamine,  
4 120 spermidine) and mono- di- and tri-olein standards and all solvents were of analytical grade  
5 121 and obtained from Merk (Germany).  
6  
7  
8  
9 122

## 11 123 **2.4. Analysis**

### 13 124 **2.4.1. Dry matter**

15  
16 125 The dry matter content was determined by oven drying at 105°C to a constant weight.  
17

### 18 126 **2.4.2. Protein**

19  
20  
21 127 The nitrogen content of all Samples was determined using Kjeldhal method and a conversion  
22 128 factor of 5.85 was used.  
23  
24

### 25 129 **2.4.3. Free and Total Amino Acid Analysis**

#### 27 130 **- Extraction procedure**

28  
29  
30 131 - Free amino acids were analysed following the method used by Moore *et al.*, 1958, with  
31 132 modifications. Briefly, 15 mg of sample was weighed and placed in a sealable test tube. To  
32 133 this, 50 µl of internal standard Norleucine (25 µM) and 4.95 ml of citrate buffer (pH 2.2) were  
33 134 added. The solution was mixed for 1 h on a rotational shaker.  
34  
35  
36  
37

38 135 - Total amino acids Samples (10 - 20 mg) of dried flour were weighed in a Schlenk tube and 50  
39 136 µl of 25 µM Norleucine and 450 µL of 4 M methanesulfonic acid were added. The tube was  
40 137 flushed with nitrogen, closed and heated at 150°C for 2 h. After cooling, 450 µL of 4 M NaOH  
41 138 was added to the hydrolysate, which was diluted to 5 ml with a loading buffer (citrate buffer  
42 139 at pH 2.2).  
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47

#### 48 140 **- Amino acid analysis**

49  
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51 141 Sample extracts for free and total amino acid analysis were filtered using a 0.45 µm membrane  
52 142 filter and injected into the amino acid analyzer (Biochrom 30+, Biochrom, France), using a  
53 143 lithium cation exchange resin column and ninhydrin as detection compound. All total and free  
54 144 amino acid contents were determined except total tryptophan, which is the most fragile  
55 145 amino acid and is destroyed by the extraction procedure. Amino acid standards were also run  
56 146 in a similar way as the samples.  
57  
58  
59  
60



#### 147 **2.4.4. Biogenic amines**

##### 148 **- Extraction**

149 Analysis of biogenic amines was carried out based on the procedure described in Yoon *et al.*,  
150 (2015). Briefly, 20 mL of 0.4 M perchloric acid were added to 5 g of the samples, and the  
151 mixture was homogenized using a vortex mixer (Vortex-Genie, USA), reacted in a cold  
152 chamber at 4 °C for 2 hr, and centrifuged at 3000 ×g at 4 °C for 10 min. The supernatant was  
153 collected, and the residue was extracted again with an equal volume of 0.4 M perchloric acid.  
154 Both supernatants were combined, and the final volume was adjusted to 50 mL with 0.4 M  
155 perchloric acid. The extract was filtered through Whatman paper No. 1 (Whatman, UK). Then,  
156 derivatization of biogenic amines was carried out as follow: One milliliter of extract (or  
157 standard solution) was mixed with 200 µL of 2 M sodium hydroxide and 300 µL of saturated  
158 sodium bicarbonate solution. Two milliliters of a dansyl chloride solution (10 mg/mL) prepared  
159 in acetone were added to the mixture, which was then incubated at 40 °C for 45 min. Residual  
160 dansyl chloride was removed by adding 100 µL of 25% ammonium hydroxide. After incubation  
161 for 30 min at 25 °C, the volume of mixture was adjusted with acetonitrile to 5 mL. Finally, the  
162 mixture was centrifuged at 3000 ×g for 5 min, and the supernatant was filtered through 0.2  
163 µm-pore-size filters (Millipore Co., USA). The filtered supernatant was kept at -25 °C before  
164 HPLC injection.

##### 165 **- Chromatographic separations**

166 An HPLC unit equipped with a UV-Vis detector (Dionex Ultimate 3000, France) was employed.  
167 An ACE C18 column (250 mm × 4.6 mm, ACE, France) was used for the separation of biogenic  
168 amines, and the samples were eluted with a step gradient of ammonium acetate (0.1 M;  
169 solvent A) and acetonitrile (solvent B) as the mobile phases at the flow rate of 1 mL/min. The  
170 program was set for a linear gradient starting from 50% of solvent B to reach 90% of the  
171 solvent at 19 min. The sample volume injected was 10 µL and monitored at 254 nm. Standard  
172 stock solutions of biogenic amines, putrescin, cadaverin, histamine and β-phenylethylamin  
173 were separately prepared at 10 mg/L concentration in distilled water.

#### 174 **2.4.5. Total Volatile Basic Nitrogen**

175 Analysis of Total Volatile Basic Nitrogen (TVBN) was carried out based on the procedure  
176 described Malle (1989) (Malle and Poumeyrol, 1989). Briefly, 25 ml of a 16% aqueous

trichloroacetic acid solution was added to 0.5 or 1 g of raw seed or fermented soumbala respectively. After 30 min homogenization, the mixture was centrifuged at 400 g for 5 min and then filtered through a Whatman n°3 filter paper (Whatman, UK). Steam entrainment was performed using a Kjeldahl-type distillatory (Tecator Line 8400, Höganäs, Sweden). 5 ml of filtrate were loaded into the distillation tube followed by 6 ml of 10% NaOH. A beaker containing 10 ml of 4% boric acid and 0.04 ml of methyl red and bromocresol green indicator was placed under the condensor for the titration of ammonia (ref: Merck 6130). TVBN which was titrated with aqueous 0.025 N sulphuric acid solution and is expressed as mg or g nitrogen/100 g

#### **2.4.6. Lipids content**

About 3 g of sample were precisely weighed in a cellulose thimble and subjected to a Soxhlet extraction with 50 mL of solvent (petroleum ether) in aluminium capsule during 110 min at 130°C. After extraction and evaporation of solvent, the alumium were further dried at 60 °C for 30 min, after which the sample weight was constant.

#### **2.4.7. Lipid profile**

Lipids were separated and quantified by thin layer chromatography as described in Amenta (1964). Briefly, a solution of 10 mg/mL of extracted lipids in hexane was prepared. Injection was done automatically (CAMAG TLC, France) on a silica gel plate (20 cm x 10 cm). Migration of mobile phase (hexane/petroleum ether (50/50, v/v) was done in an automatic developing chamber (CAMAG TLC, France) during 20 minutes. Revelation of spots was done by immersing the silica gel plate in a mixture of saturated copper acetate and orthophosphoric acid 40% before drying on a hot plate. Revelation and integration of spot was done under UV light (CAMAG TLC, France). Lipid classes identification of the different spots was done by comparison of their retention factor (Rf) to that of standards of mono-, di- and tri-olein.

#### **2.4.8. Total Free fatty acids**

Three g of lipids were added to 100 mL ethanol preheated to 60° and 1 ml of phenolphthalein solution as titration indicator. Titration was immediately done with potassium hydroxide solution 0.1 N. Results were expressed in g of lineloic acid per 100 g of fat.

#### **2.4.9. Free fatty acid profile**

The free fatty acid profile was done only on the raw and final ALBS. Fatty acid methyl esters were obtained from about 50 mg of lipid fraction through esterification with 3 mL of HCl methanol solution prepared from 50mL of acetyl chloride dissolved in 625 mL of methanol (reflux time 15 min). 8 mL of hexane and 10 mL of water were used to extract the methyl esters. One  $\mu\text{L}$  of the hexane solution was injected in a capillary gas chromatography (GC) (Agilent model 7890, Santa Clara, CA, US) equipped with an injector (split-less mode), a capillary column (DB-Wax, J&W Scientific), a flame-ionization detector (FID). Flow rate of the carrier gas helium was 0.452 mL/min. The oven temperature was held at 130 °C-40 min, 1 °C/min to 160 °C kept 10min, 2 °C/min to 180 °C kept 20min, 2 °C/ min to 220°C kept 10 min. The temperature of injector and FID was 270 °C and 280 °C, respectively. Identification of the peaks was made by comparison with retention times of methyl esters of known oils.

#### 2.4.10. Conjugated dienes

Change in conjugated diene (CD) of lipids extracted were measured as an indication of oil primary oxidation products. 0.01 to 0.03 g oil or lipid extract sample were weighted into a 25-ml volumetric flask filled with isooctane as a solvent. Absorbance was read at 233 nm. CD concentration in  $\text{mol.L}^{-1}$  was obtained considering  $\epsilon$ , the molar absorptivity of linoleic acid hydroperoxide ( $2.5 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ ).

#### 2.5. Statistical analysis

All analyses previously described were performed in triplicate for each sample. One-way ANOVA was used to analyze the effect of the process step (effect considered significant when  $p < 0.05$ ) on the composition of seeds during soumbala production. If significantly different, means were further compared using Tukey's test. Statistical analyses were performed with Statistica® (StatSoft Inc., USA).

### 3. Results

#### 3.1. Major composition changes during soumbala production from ALBS

Figure 2 shows the composition of ALBS during soumbala production. Carbohydrates proportion was estimated by difference between the dry matter and the sum of proteins and

lipids. Raw ALBS were composed of carbohydrates 27.9 %, lipids 24.8 % and proteins 41.9 % (wb). This composition is typical of an oleoproteaginous seeds as soya and consistent with that already published on ALBS (Esenwah and Ikenebomeh, 2008; Elemo *et al.*, 2011). However, some previous results showed a higher content in carbohydrates sometimes superior to proteins that may due to variety variability. Anyway, our experiments and literature are in accordance with a dry matter superior to 90% (Omafuvbe *et al.*, 2004). The dry matter dropped markedly after cooking to 30% because of water transfer to the cotyledons leading a swelling ratio of 3. Water content was constant during fermentation. This is consistent with Omafuvbe *et al.* (2004) who found constant moisture content of 60% before and after fermentation. In a qualitative point of view, the main effect of cooking is a marked decreased in carbohydrate content divided by a 10-fold. This may be due to the very long cooking steps and a leak of the initial carbohydrates. Therefore, after cooking, protein and lipids constituted 46 and 40 % of the dry basis respectively. By comparison, Azokpota *et al.* (2006) found values of 43 and 31 % and Esenwah and Ikenebomeh (2008) found 33 and 35 % respectively (Azokpota *et al.*, 2006a; Esenwah and Ikenebomeh, 2008). After fermentation, only a significant decrease of protein was observed. After drying, the final water content is close to 10% enabling a stability of fermented ALBS at ambient temperature, and the major constituents are lipids and proteins at 41.3 and 39.0% and carbohydrates at 8.3%.

### 3.2. Protein degradation products fate

Figure 3 present the degradation products from proteins during production of soumbala in a dry basis, expressed in the legal unit: Total Volatile Basic Nitrogen (TVBN, mg/100g), Biogenic Amines (BA, mg/kg), and Free amino acids (FAA) were expressed in g/100g. The 3 analytical procedures for TVBN, BA and FAA are more or less sophisticated. The simplest, oldest and cheapest is TVBN. This measurement is usual on fish and shrimps and is considered as a good spoilage index (Baixas-Nogueras *et al.*, 2003). Volatile bases are a mixture of ammonia, trimethylamine and dimethylamine, which each contain one basic nitrogen atom per molecule. Free amino acids and basic amines are more sophisticated measurements obtained with the sum of individual forms identified by HPLC. However, they are more precise since they give identification and quantification of free amino acids and biogenic amines that have different biological effects. On fish products, biogenic amines content was proven to be linked

to a high level of free amino acids and high TVBN (Ruiz-Capillas and Herrero, 2019). This link was also observed during soumbala production. Indeed, increase of biogenic amines contents was linked to that of TVBN and free amino acids content. TVBN increased from 270 to 881 mg/100 g, FFA from 0.6 to 8.7 g/100 g and BA from 105 to 512 mg/kg. While FAA were very low in the raw ALBS, content of TVBN and BA was already high certainly because of an endogenous enzymatic activity. After cooking, TVBN slightly decreased, FAA remained stable while BA increased significantly by a 5 fold. This may be due to a starting of fermentation of cotyledons during storage time. Fermentation has a major effect on the 3 indicators with a marked increase of 5, 9 and 2.5-fold for TVBN, FAA and BA respectively. This was caused by protease activity of microorganisms during fermentation. High proteinase activity has frequently been reported in the fermentation of similar protein-rich seeds like miso (Odunfa, 1985). After drying, no significant change of the indicators was observed and final TVBN content was close to 900 mg/100g which is much higher than the value of 30 to 35 mg/100g that European legislation considers to be unfit for human consumption in fish products (95/149/EC, 1995). However, soumbala is a dry condiment that is used in a small quantity. However, even used a 10% in a meal, it leads to 90 mg/100 g which is still high. Biogenic amine content was high as well with a total of 512 mg/kg. However, the content of individual amines and their free amino acids that may be precursors must be analysed.

### 3.2.1. Free amino acids release

Major amino acids which constitute proteins of raw ALBS are by order of importance glutamic and aspartic acids, leucine, lysine, arginine and valine (Fig. 4 A). This general profile remains after fermentation. These results are consistent with literature which also report low sulphur amino acids (cysteine and methionine), which is typical of legumes (Ndir *et al.*, 2000; Elemo *et al.*, 2011). The main FAA present in raw ALBS represent 2% (w/w) on a protein base. The main free forms were by importance glutamic and aspartic acids (60%) which seems logical. The remaining were valine (17%), cysteine (13%) and histidine (9%). While cysteine and histidine were in low quantities in ALBS proteins, they become majority in their free forms. However, the major release of FAA occurred during fermentation ( $p < 0.05$ ) and the content reached 8.7 g/100g making a FAA/protein ratio increase by a 10-fold till 20%. This FFA production was already observed in literature (Odunfa, 1985; Ouoba *et al.*, 2003b; Omafuvbe *et al.*, 2004). The

main FAA produced were glutamic acid, leucine, lysine, valine (total of 46%) which was consistent with the initial proteins composition. However, sum of tyrosine, phenylalanine, isoleucine, ornithine, cysteine and histidine represented also 47%. Therefore, it seemed that there was an oriented release or reaction/consumption of amino acids which may be the fact of micro-organisms. Besides, it was already noticed the release of cysteine, methionine, leucine, isoleucine, tyrosine and phenylalanine during fermentation of bacillus strains on a medium composed of 1% ALBS powder (Ouoba *et al.*, 2003b). In our case, no methionine was identified but instead we found ornithine and histidine among FAA. Ouoba *et al.* (2003) noticed differences in FAA release as a function of the bacillus strains involved during fermentation. In addition, some FAA may be subjected to a degradation during fermentation such as aspartic acids, arginine, alanine proline, and glycine. These reactions may be due to oxidative deamination and decarboxylation and furthest reaction that can lead to biogenic amines genesis.

### 3.2.1. Biogenic amines release

The biogenic amines identified during soumbala production were putrescine, cadaverine, histamine, phenethylamine and spermidine (Fig. 5). Like free amino acids, the main step increasing the most their content is fermentation ( $p < 0.05$ ), except for histamine that was present in the raw seeds. Histamine is considered as potentially toxic especially for sensitive population. Consumption of histamine in the range of 8–40 mg, 40–100 mg or higher than 100 mg in one meal may cause slight, intermediate and severe poisoning respectively (Biji *et al.*, 2016). Commission Regulation (EC) 2073/2005 lays down food safety limits for histamine between 100 and 200 mg/kg for certain fish and fishery products. Even though there are no legal limits for the concentration of biogenic amines in other products, some European countries only recommend maximum limits for histamine. Histamine final content after drying was 40 mg/kg product. Therefore, assuming a consumption of 50 g in a meal, the histamine intake would be 2 mg and may not be dangerous. Therefore, the high TVBN content observed after fermentation was due to other biogenic amines or other volatile compounds such as ammonia. By order of importance, the final content after drying was 325, 56, 37, 21 and 20 mg/kg for spermidine, phenylethylamine, histamine, cadaverine and putrescine respectively. Their origin is the decarboxylation of arginine, phenylalanine, histidine, lysine and ornithine



respectively. It is interesting to notice that phenylalanine, histidine, lysine and ornithine were the free amino acids found after fermentation while arginine was not found. As arginine is the 5<sup>th</sup> amino acid by order of importance in raw ALBS, the free amino acid may be formed and be decarboxylated very quickly to form spermidine. Other hypothesis is that spermidine can be also formed from putrescine (Biji *et al.*, 2016). Among free amino acids found after drying (Fig 4 B), it is also noteworthy that tyrosine could be decarboxylated in tyramine. However, this biogenic amine was not found in our case but could be potentially produced from ALBS as a function of the microorganism specie involved in the fermentation. Biogenic amines like spermidine, putrescine and cadaverine do not have any adverse health effect, but may react with nitrite to form carcinogenic nitrosoamines. In addition, they can potentiate the toxic effect of histamine by inhibiting intestinal histamine metabolizing enzymes like diamine oxidase. However, their toxicity level is 600, 2000 and >2000 ppm respectively, which is much higher than the amount that can be found in a dish with fermented ALBS (Biji *et al.*, 2016). However, when recommending fermented ALBS for nutritional purpose, the potential presence of biogenic amines should be taken in consideration especially for sensitive population.

### 3.3. Lipid fate

#### 3.3.1. Lipid profile of raw ALBS

The lipids of raw ALBS consisted mainly of triglycerides (>70%). The major fatty acids (FA) were by order of importance linoleic (C18:2), oleic (C18:1), stearic (C18), behenic (C22), palmitic (C16) and arachidic (C20), accounting for 45, 14, 13, 12, 9, and 3 % of total lipids respectively (Fig. 6 A). Therefore, ALBS oil is very rich in linoleic acid, an essential FA belonging to the omega 6 family. Once again, this composition was close to that of soya as observed in previous studies (Ndir *et al.*, 2000; Elemo *et al.*, 2011). The other major spot obtained but not identified by thin layer chromatography in raw ALBS accounted for 20% of lipid. It may gathered unsaponifiable compounds like vitamin E, phospholipids or phytosterols which were proven to be in high and interesting quantity in ALBS (Omafuvbe *et al.*, 2004; Olatunya *et al.*, 2019). It is noteworthy that these compounds were stable as their content did not change significantly during the

process (Fig. 6 B). Lastly, raw ALBS lipid fraction also contained 3% of di-glycerides and 2% of free fatty acids (FFA) probably due to a little intrinsic lipase activity

### 3.3.2. Di-glyceride and free fatty acid release during soumbala production

The fatty acid (FA) composition was not significantly different between raw and fermented or dried ALBS. However, lipid profile changed significantly as shown in Fig. 6 B. Triglycerides decreased by 10% as a consequence of cooking and fermentation ( $p<0.05$ ). During cooking, this degradation was concomitant with a significant increase by a 2-fold of di-glycerides ( $p<0.05$ ). Free fatty acids were mainly released during fermentation as their content increased markedly by a 4-fold ( $p<0.05$ ). These hydrolyses may be due to microorganism's lipases during fermentation but also in a lesser extend as soon as cotyledons were hydrated. Omafuvbe et al. (2004) also noticed a 2-fold increase of acid value (Omafuvbe *et al.*, 2004). Final composition of lipids did not change significantly after drying. This final composition may impact the organoleptic quality of soumbala because FFA and especially linoleic acid are very reactive and may cause rancidity of the product. Conjugated dienes were measured throughout soumbala production and did not increase significantly (results not shown). However, oxidation is a slow process that may occur during storage especially in hot conditions.

## Conclusion

The results presented in this paper showed that raw ALBS is an interesting food product with a balanced composition in protein, lipids and carbohydrates. Major composition changes were monitored during its transformation in soumbala. The first step of cooking affected the carbohydrates that dropped dramatically by leaking. As a consequence, proteins and lipids compounds became the major nutrients for microorganisms involved during fermentation. Scission reactions occurred on the proteins as a result of microorganism activity leading to free amino acids production. This conversion may increase the digestibility of fermented ALBS. However, it was also concomitant with conversion of free amino acids into biogenic amines production, caused by the decarboxylase activity of microorganisms, that may have adverse effect on human health. Concerning lipid matter, the fatty acid profile was not affected during



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soumbala production. However, lipid profile of ALBS was significantly affected. Indeed, because of lipase activity during cooking and fermentation, a significant production of di-glycerides and free fatty acids was remarkable. This production may have nutritional but also organoleptic quality of the product during storage because of the high reactivity of unsaturated free fatty acids. To synthetize the effect of processing, the initial and final composition of protein and lipid matter are presented in **supplementary material 1 and 2.**

In conclusion, fermented ALBS is an interesting product, but whose production should benefit a better comprehension and control to optimize its properties while decreasing its content in undesirable compounds such as peculiar biogenic amines or limit its free fatty acid content. Use of started or adequate fermentation parameters (temperature, pH..) must be deepen in future studies.

**Author statement**

Ethics approval was not required for this research. The authors have no conflict of interest to declare. The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Legends to Figures

**Figure 1.** Flowchart of ALBS transformation in Soumbala in Khorogo region

**Figure 2.** Composition in % **wet** basis of dehulled ALBS during soumbala production

**Figure 3.** Protein degradation products during soumbala production

**Figure 4.** A) Composition of initial total amino acids in raw ALBS and B) free amino acid (g/100 g dry basis) release during soumbala production

**Figure 5.** Content of biogenic amines in mg/kg during soumbala production

**Figure 6.** Composition A) in free fatty acids of raw ALBS B) and lipid fraction during soumbala production

**Supplementary material 1.** Nitrogen matter in raw and fermented ALBS and their recommended daily intake (RDI) or toxic value (mg)

**Supplementary material 2.** Lipidic matter in raw and fermented ALBS and their recommended daily intake (RDI)

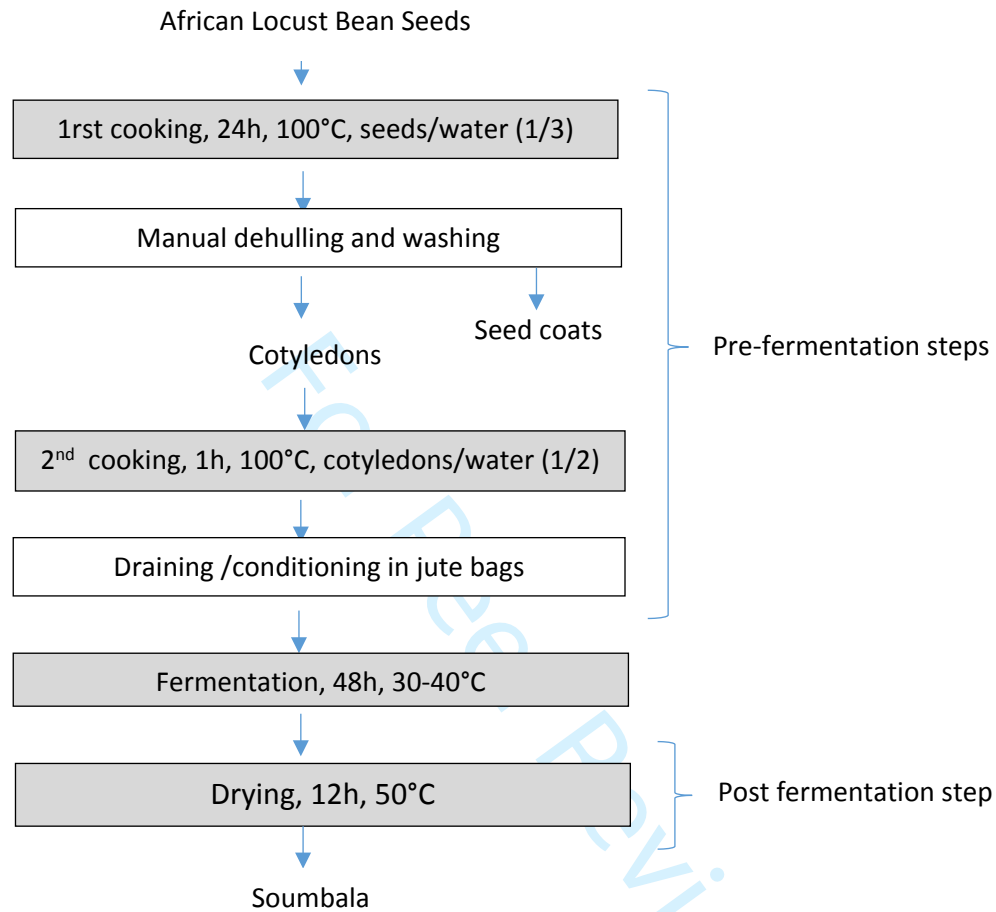
## 418 5 Annotated References in yellow

## 419 References

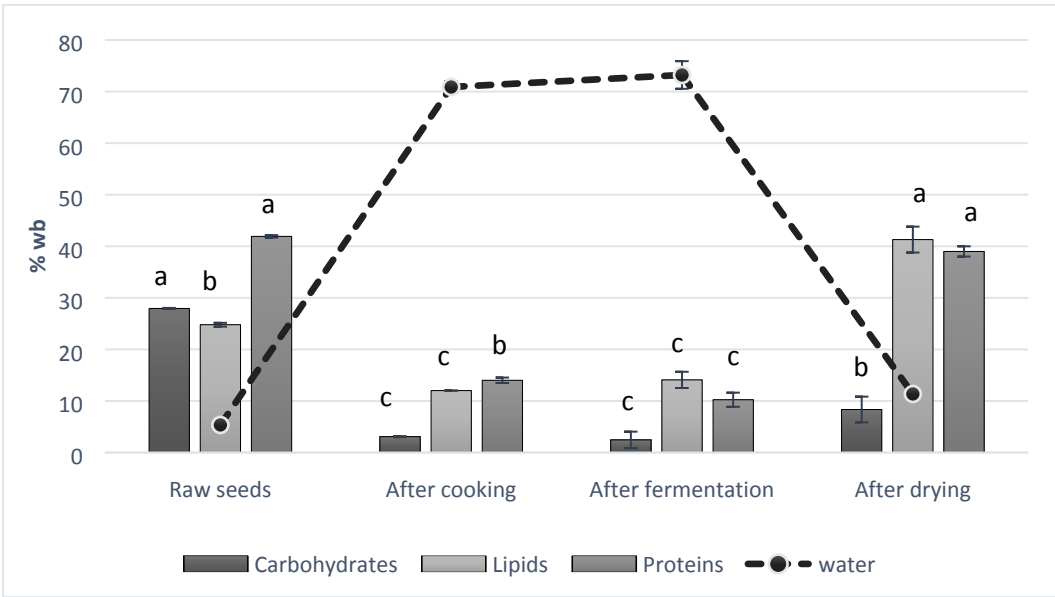
- 420 95/149/EC. (1995). fixing the total volatile basic nitrogen (TVB-N) limit values for certain categories of  
421 fishery products and specifying the analysis methods to be used. *Official Journal of the*  
422 *European Communities*, 15 p.
- 423 Abbas, M.S., El-Shabrawi, H.M., Soliman, A.Sh. & Selim, M.A. (2018). Optimization of germination,  
424 callus induction, and cell suspension culture of African locust beans *Parkia biglobosa* (Jacq.)  
425 Benth. *Journal of Genetic Engineering and Biotechnology*, **16**, 191–201.
- 426 Amenta, J.S. (1964). A rapid chemical method for quantification of lipids separated by thin-layer  
427 chromatography. *The Journal of Lipid Research*, **5**, 270–272.
- 428 Amoa-Awua, W.K., Awusi, B., Owusu, M., Appiah, V., Ofori, H., Thorsen, L. & Jespersen, L. (2014).  
429 Reducing the atypical odour of dawadawa: Effect of modification of fermentation conditions  
430 and post-fermentation treatment on the development of the atypical odour of dawadawa.  
431 *Food Control*, **42**, 335–342.
- 432 Azokpota, P., Hounhouigan, D.J. & Nago, M.C. (2006a). Microbiological and chemical changes during  
433 the fermentation of African locust bean (*Parkia biglobosa*) to produce afitin, iru and sonru,  
434 three traditional condiments produced in Benin. *International Journal of Food Microbiology*,  
435 **107**, 304–309.
- 436 Azokpota, P., Hounhouigan, D.J., Nago, M.C. & Jakobsen, M. (2006b). Esterase and protease activities  
437 of *Bacillus* spp. from afitin, iru and sonru; three African locust bean (*Parkia biglobosa*)  
438 condiments from Benin. *African Journal of Biotechnology*, **5**, 265–272.
- 439 Papers of Azokpota et al. are reference in microbiology of African traditional fermented food and  
440 particularly alkaline-fermented indigenous seeds.
- 441 Baixas-Nogueras, S., Bover-Cid, S., Veciana-Nogués, M.T. & Vidal-Carou, M.C. (2003). Suitability of  
442 Volatile Amines as Freshness Indexes for Iced Mediterranean Hake. *Journal of Food Science*,  
443 **68**, 1607–1610.
- 444 Bari, A.U., Santiago, M.Q., Osterne, V.J.S., Pinto-Junior, V.R., Pereira, L.P., Silva-Filho, J.C., Debray, H.,  
445 Rocha, B.A.M., Delatorre, P., Teixeira, C.S., Neto, C.C., Assreuy, A.M.S., Nascimento, K.S. &  
446 Cavada, B.S. (2016). Lectins from *Parkia biglobosa* and *Parkia platycephala*: A comparative  
447 study of structure and biological effects. *International Journal of Biological Macromolecules*,  
448 **92**, 194–201.
- 449 Biji, K.B., Ravishankar, C.N., Venkateswarlu, R., Mohan, C.O. & Gopal, T.K.S. (2016). Biogenic amines in  
450 seafood: a review. *Journal of food science and technology*, **53**, 2210–2218.
- 451 BIOHAZ, E.P. on B.H. (2011). Scientific Opinion on risk based control of biogenic amine formation in  
452 fermented foods. *EFSA Journal*.
- 453 Byun, B.Y. & Mah, J.-H. (2012). Occurrence of Biogenic Amines in Miso, Japanese Traditional Fermented  
454 Soybean Paste. *Journal of Food Science*, **77**, T216–T223.

- 455 Dakwa, S., Sakyi-Dawson, E., Diako, C., Annan, N.T. & Amoa-Awua, W.K. (2005). Effect of boiling and  
456 roasting on the fermentation of soybeans into dawadawa (soy-dawadawa). *International*  
457 *Journal of Food Microbiology*, **104**, 69–82.
- 458 Ekperikpe, U.S., Owolabi, O.J. & Olapeju, B.I. (2019). Effects of *Parkia biglobosa* aqueous seed extract  
459 on some biochemical, haematological and histopathological parameters in streptozotocin  
460 induced diabetic rats. *Journal of Ethnopharmacology*, **228**, 1–10.
- 461 Elemo, G.N., Elemo, B.O., Oladunmoye, O.O. & Erukainure, O.L. (2011). Comprehensive investigation  
462 into the nutritional composition of dehulled and defatted African locustbean seed (*Parkia*  
463 *biglobosa*). *African Journal of Plant Science*, **5**, 291–195.
- 464 Erdag, D., Merhan, O. & Yildiz, B. (2018). Biochemical and Pharmacological Properties of Biogenic  
465 Amines. In: *Biogenic Amines*.
- 466 Esenwah, C. & Ikenebomeh, M. (2008). Processing effects on the nutritional and anti-nutritional  
467 contents of African locust bean (*Parkia biglobosa* Benth.) seed. *Pakistan Journal of Nutrition*,  
468 **7**, 214–17.
- 469 This paper gives the composition of macronutrients during ALBS processing. It makes a focus on anti-  
470 nutritional molecules like tannins, phytic acid, and anti-trypsin factors which is complementary to our  
471 results.
- 472
- 473 Ham, J.R. (2017). Cooking to be Modern but Eating to be Healthy: The Role of Dawa-Dawa in  
474 Contemporary Ghanaian Foodways. *Food, Culture & Society*, **20**, 237–256.
- 475 Krause, I., Bockhardt, A. & Klostermeyer, H. (1997). Characterization of cheese ripening by free amino  
476 acids and biogenic amines and influence of bactofugation and heat-treatment of milk, 101–  
477 108.
- 478 Lado, A., Umar Sani, F., Usman Yahaya, S. & Kwalle Karaye, A. (2018). Efficacy of *Parkia biglobosa* fruit  
479 powder on the control of *Striga* in cowpea cropping systems in the Sudan-Savanna, Nigeria.  
480 *Heliyon*, **4**, e00733.
- 481 Malle, P. & Poumeyrol, M. (1989). A New Chemical Criterion for the Quality Control of Fish:  
482 Trimethylamine/Total Volatile Basic Nitrogen (%). *Journal of Food Protection*, **52**, 419–423.
- 483 Moore, S., Spackman, D.H. & Stein, W.H. (1958). Chromatography of Amino Acids on Sulfonated  
484 Polystyrene Resins. An Improved System. *Analytical Chemistry*, **30**, 1185–1190.
- 485 Ndir, B., Lognay, G., Wathelet, B., Cornelius, C., Marlier, M. & Thonart, P. (2000). Composition chimique  
486 du nétéu, condiment alimentaire produit par fermentation des graines du caroubier  
487 africain *Parkia biglobosa* (Jacq.) Benth. *Biotechnol. Agron. Soc. Environ.*, **4**, 101–105.
- 488 Odunfa, S.A. (1985). Biochemical changes in fermenting African locust bean (*Parkia biglobosa*) during  
489 'iru' fermentation. *International Journal of Food Science & Technology*, **20**, 295–303.
- 490
- 491 This paper deals with protease and lipase activities during soumbala production: the release of amino  
492 acids during fermentation was particularly compared to our results.

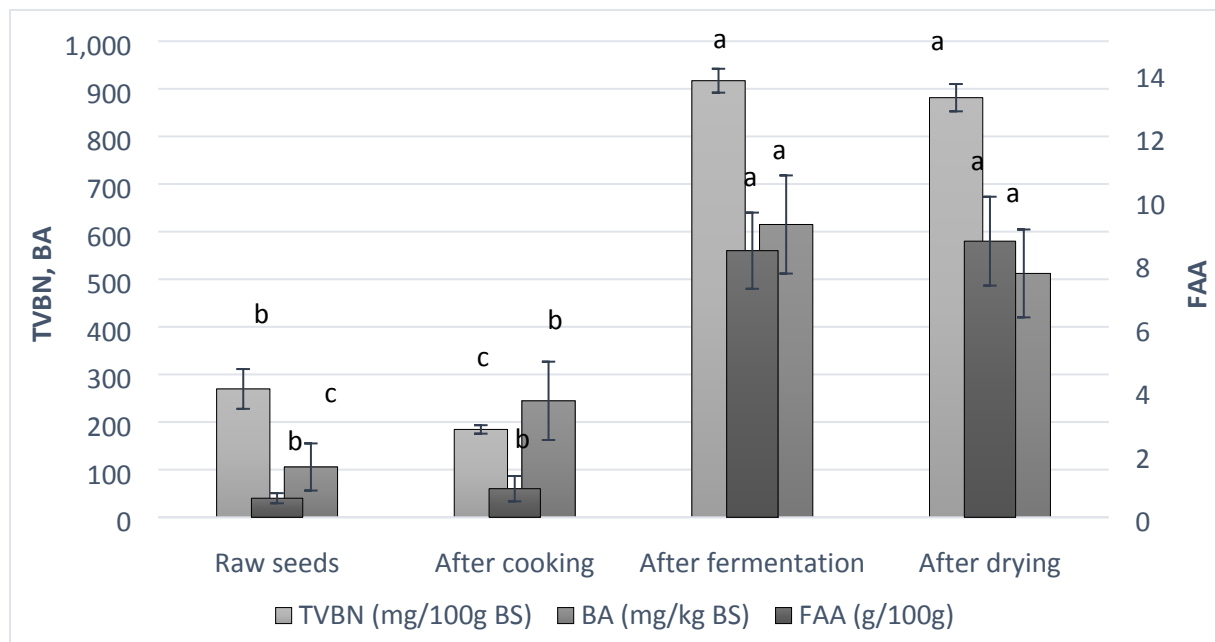
- 493
- 494 Olatunya, A.M., Omojola, A., Akinpelu, K. & Akintayo, E.T. (2019). Vitamin E, Phospholipid, and  
 495 Phytosterol Contents of *Parkia biglobosa* and *Citrullus colocynthis* Seeds and Their Potential  
 496 Applications to Human Health. *Preventive nutrition and food science*, **24**, 338–343.
- 497 Omafuvbe, B., Falade, O., Osuntogun, B. & Adewusi, S. (2004). Chemical and biochemical changes in  
 498 African locust bean (*Parkia biglobosa*) and melon (*Citrullus vulgaris*) seeds during fermentation  
 499 to condiments. *Pakistan Journal of Nutrition*, **3**, 140–45.
- 500 Omafuvbe et al. followed protein and lipid fate during fermentation of various seeds. Particularly, they  
 501 monitored lipase and protease activity and measured acid value and glycine release as indicators of  
 502 their activity.
- 503
- 504 Ouoba, L.I.I., Cantor, M.D., Diawara, B., Traoré, A.S. & Jakobsen, M. (2003a). Degradation of African  
 505 locust bean oil by *Bacillus subtilis* and *Bacillus pumilus* isolated from soumbala, a fermented  
 506 African locust bean condiment. *Journal of Applied Microbiology*, **95**, 868–873.
- 507 Ouoba, L.I.I., Diawara, B., Annan, N.T., Poll, L. & Jakobsen, M. (2005). Volatile compounds of Soumbala,  
 508 a fermented African locust bean (*Parkia biglobosa*) food condiment. *Journal of Applied  
 509 Microbiology*, **99**, 1413–1421.
- 510 Ouoba, L.I.I., Rechinger, K.B., Barkholt, V., Diawara, B., Traore, A.S. & Jakobsen, M. (2003b).  
 511 Degradation of proteins during the fermentation of African locust bean (*Parkia biglobosa*) by  
 512 strains of *Bacillus subtilis* and *Bacillus pumilus* for production of Soumbala. *Journal of Applied  
 513 Microbiology*, **94**, 396–402.
- 514 Ouoba et al. did a lot of work on ALBS and particularly on the microbiology of its fermentation. They  
 515 isolated *Bacillus* strains and made experimental works to understand their metabolism and particularly  
 516 on the resulting aromatic properties of fermented ALBS.
- 517
- 518 Ruiz-Capillas, C. & Herrero, A.M.I. (2019). Impact of Biogenic Amines on Food Quality and Safety. *Foods*,  
 519 **8**.
- 520 Yoon, H., Park, J.H., Choi, A., Hwang, H.-J. & Mah, J.-H. (2015). Validation of an HPLC Analytical Method  
 521 for Determination of Biogenic Amines in Agricultural Products and Monitoring of Biogenic  
 522 Amines in Korean Fermented Agricultural Products. *Toxicological research*, **31**, 299–305.
- 523 Yusuf, O.I.S. & Rahji, M.A.Y. (2012). The Processing and Preference for Locust Bean Products (*Parkia  
 524 Biglobosa*) in Lagos, Nigeria. *Journal of Biology, Agriculture and Healthcare*, **2**.

**Figure 1.** Flowchart of ALBS transformation in Soumbala

**Figure 2.** Composition in % wet basis of ALBS during soumbala production



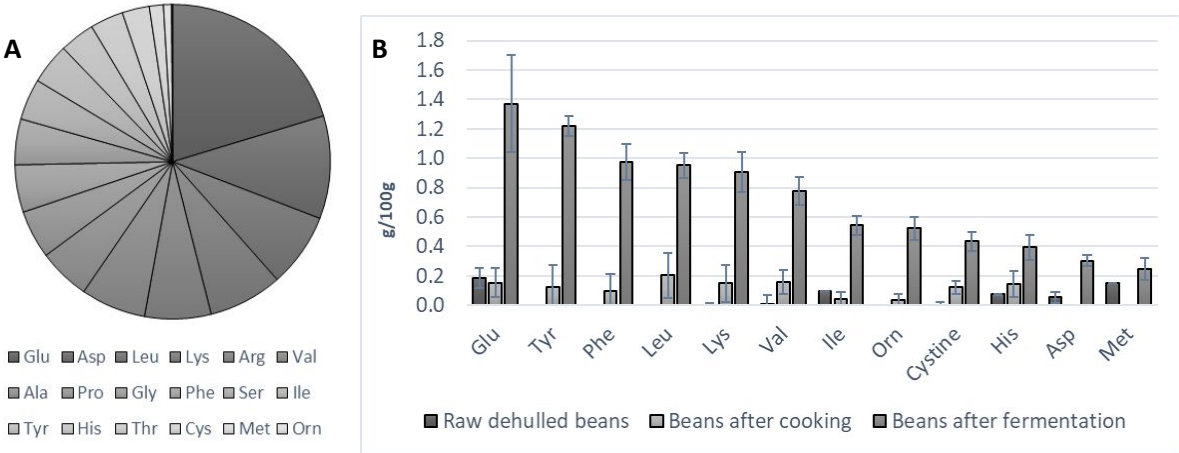
Values bearing different letters for the same parameter are significantly different ( $p<0.05$ ).

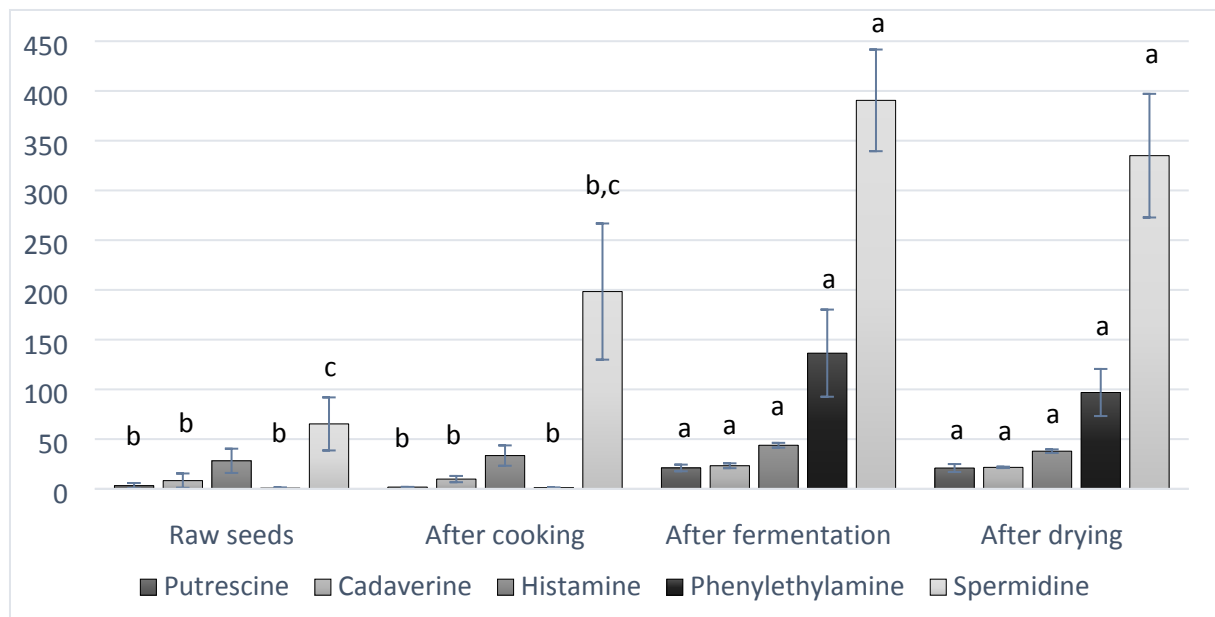
**Figure 3.** Protein degradation products during soumbala production

Values bearing different letters for the same parameter are significantly different ( $p < 0.05$ ).



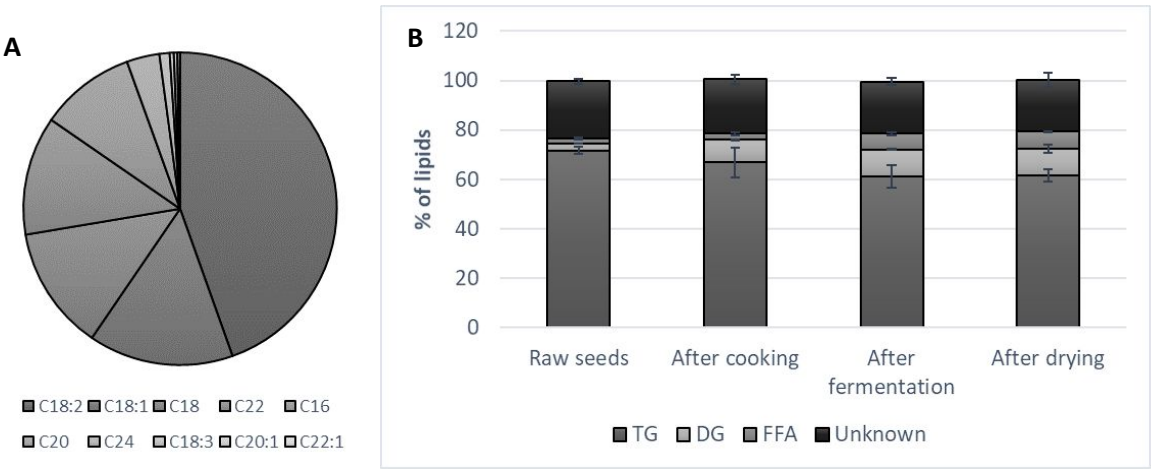
**Figure 4.** A) Composition of initial total amino acids in raw ALBS and B) free amino acid (g/100 g db) release during soumbala production



**Figure 5.** Content of biogenic amines in mg/kg during soumbala production

Values bearing different letters for the same parameter are significantly different ( $p < 0.05$ ).

**Figure 6.** Composition A) in free fatty acids of raw ALBS B) and lipid fraction during soumbala production



**Supplementary material 1.** Nitrogen matter in raw and fermented ALBS and their recommended daily intake (RDI) or toxic value (mg)

	Raw ALBS	Dried fermented ALBS (g/100g)		RDI (g)			
		Total AA	Free AA		Biogenic amines (mg/100g)	TV (mg)	
<b>Proteins</b>	41.9	39.0		50			
<b>Glutamic acid</b>	5.00	7.76	1.32				
<b>Aspartic acid</b>	2.50	3.96	0.29				
<b>Leucine</b>	1.75	3.38	0.92	2.73			
<b>Lysine</b>	1.75	3.26	0.87	2.10	Cadaverine	2.1	>2000
<b>Phenylalanine</b>	1.25	2.53	0.94	0.85	Phenylethylamine	9.7	
<b>Serine</b>	1.00	1.60					
<b>Valine</b>	1.75	2.23	0.75	1.82			
<b>Arginine</b>	1.75	2.03			Spermidine	33.5	600
<b>Alanine</b>	1.25	1.89					
<b>Proline</b>	1.25	1.92					
<b>Isoleucine</b>	1.25	1.97	0.52	1.40			
<b>Glycine</b>	1.25	1.79					
<b>Tyrosine</b>	1.00	2.29	1.17	0.85			
<b>Histidine</b>	0.75	1.30	0.38	0.70	Histamine	3.8	100
<b>Cystine</b>	0.25	0.40	0.42	0.50			
<b>Methionine</b>	0.25	0.40	0.24	0.50			
<b>Ornithine</b>	0.25	0.40	0.50		Putrescine	2.1	2000

**Supplementary material 2.** Lipidic matter in raw and fermented ALBS and their recommended daily intake (RDI)

	Raw ALBS (g/100g)	Dried fermented ALBS (g/100g)	RDI (g)
Lipids	24.4	41.9	100
C18:2	10.92	19.11	4.00
C18:1	3.58	6.27	17.00
C18:3	0.10	0.17	1
C20:1	0.08	0.14	
C22:1	0.15	0.27	
C18	3.18	5.56	<12
C22	2.90	5.07	
C16	2.26	3.95	
C20	0.83	1.45	
C24	0.31	0.53	
Acid value	2	7	<5