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

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Manuscripts

Parkia biglobosa seeds



Cooking



Fermentation

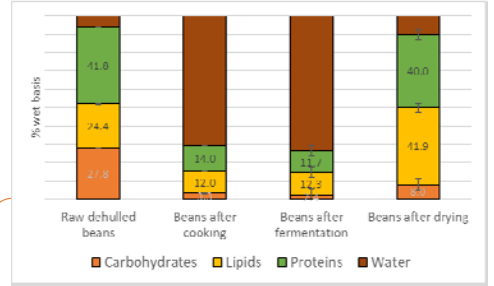


Drying

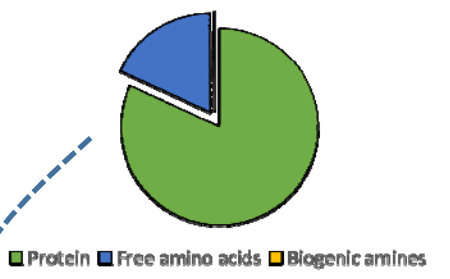


Soumbala

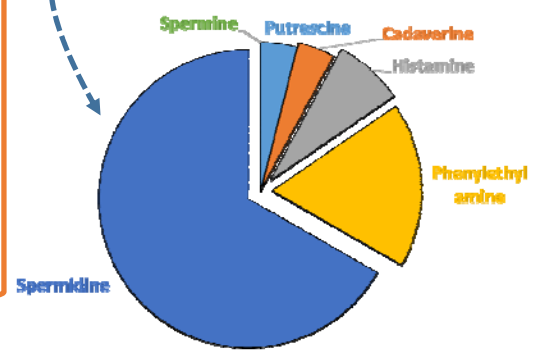
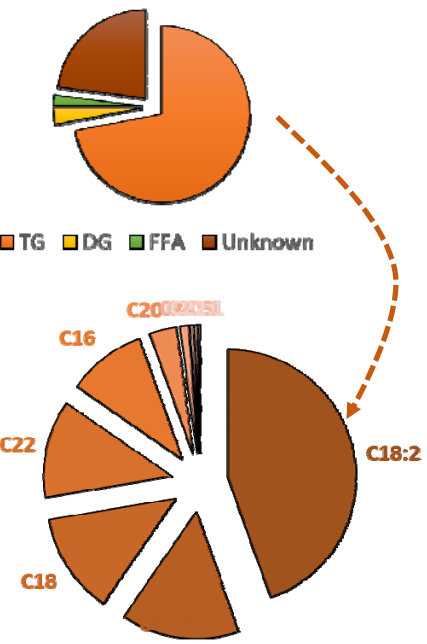
Macronutrients composition



Nitrogen fraction



Lipid extraction



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3 1 **Fate of proteic and lipidic compounds during production of a traditional legume condiment**
4 **(Soumbala) made from African Locust Bean (*Parkia biglobosa*) seeds**

5
6
7 3 **Running title: *Parkia biglobosa* seeds process & composition**

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33
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36
37 16 **Abstract**

38
39 17 Soumbala is made from African locust bean seeds (*Parkia biglobosa*), a legume widely spread
40 in west Africa. It is a lipid and protein-rich condiment obtained by three main steps: cooking,
41 18 in west Africa. It is a lipid and protein-rich condiment obtained by three main steps: cooking,
42 fermentation and drying. **Ten different analysis** were made on AFLBS samples at each step
43 with a particular focus on protein and lipid fates. Results showed that cooking decreased
44 markedly the carbohydrate content leaving mainly proteins and lipids as substrates for
45 20 with a particular focus on protein and lipid fates. Results showed that cooking decreased
46 markedly the carbohydrate content leaving mainly proteins and lipids as substrates for
47 21 markedly the carbohydrate content leaving mainly proteins and lipids as substrates for
48 fermentation. During this step, 20% of the proteins were converted into free amino acids,
49 22 mainly glutamic acid and tyrosine. Significant biogenic amines release occurred and reached
50 23 50 mg/100g of dried ALBS. Di-glycerides and fatty acids increased markedly and represented
51 24 11% and 7% of the total lipids of dried ALBS. These results give a complete view of the
52 25 nutritional characteristics of dried ALBS, its benefit but also its defects that arise during
53 26 fermentation and not suppressed by the subsequent processing steps.
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28 **Keywords:** soumbala; nutritional composition; processing; biogenic amine; di-glyceride; free
29 fatty acids

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31 **1. Introduction**

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

48 Consumption of such products, with a very distinctive odor and taste, has decreased over the
49 past decades for the benefit of industrial alternatives (Ham, 2017). However, current
50 nutritional and sustainability considerations encourage agronomist, nutritionist and food
51 scientists to pay attention again to this very interesting legume and its transformation (Bari *et al.*
52 *et al.*, 2016; Ham, 2017; Abbas *et al.*, 2018; Lado *et al.*, 2018; Ekperikpe *et al.*, 2019). ALBS are
53 nutritionally interesting since they present a balanced composition of protein (39 à 47%), lipids
54 (31 à 40%) and carbohydrates (12 à 16%) (Odunfa, 1985; Ndir *et al.*, 2000; Esenwah and
55 Ikenebomeh, 2008). Therefore, they can increase the daily intakes in proteins and lipids while
56 being more sustainable and affordable than meat products (Ham, 2017). As a legume, ALBS
57 contains anti-nutritional compounds (trypsin inhibitor, tannins, oligosaccharides and phytic

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3 58 acid) but once fermented, their level are fairly decreased (Esenwah and Ikenebomeh, 2008;
4
5 59 Yusuf and Rahji, 2012).

6
7 60 The traditional process of soumbala production is quite long (4 to 5 days). While it may vary
8
9 61 slightly depending on the region of production, its process consists in different steps
10
11 62 presented in Fig. 1. Four main unitary operations may affect the composition of ALBS: the first
12
13 63 is cooking of seeds in boiling water during 12 to 24h to soften their shell before dehulling. The
14
15 64 second step is the cooking of the resulting cotyledons in a small quantity of boiling water. This
16
17 65 step softens the tissues before fermentation. The 3rd and major step is the fermentation of
18
19 66 the cooked cotyledons. Finally, the post fermentation step is a stabilisation step, and is usually
20
21 67 sun-drying (Odunfa, 1985; Dakwa *et al.*, 2005; Yusuf and Rahji, 2012). Literature about ALBS
22
23 68 transformation is quite scarce. However, previous studies carried out on the transformation
24
25 69 of ALBS into Soumbala have shown that there are significant composition modifications during
26
27 70 processing (Odunfa, 1985; Azokpota *et al.*, 2006a). The operation unit most studied in
28
29 71 literature is fermentation (Ouoba *et al.*, 2003b,a; Azokpota *et al.*, 2006a) while pre-
30
31 72 fermentation steps are very poorly described and their consequence on the biochemistry of
32
33 73 seeds, not studied. Indeed, fermentation is assumed to be the operation that gives the typical
34
35 74 organoleptic properties of final product (Ouoba *et al.*, 2005; Azokpota *et al.*, 2006a; Amoa-
36
37 75 Awua *et al.*, 2014). The microflora associated to the fermentation of ALBS is essentially
38
39 76 composed of sporulated germs of the genus *Bacillus*. Their metabolic characteristic is to be
40
41 77 able to use all macro-constituents as a substrate for their growth and particularly proteins,
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43 78 releasing free amino acids and basic amino molecules increasing pH (Ouoba *et al.*, 2003a;
44
45 79 Azokpota *et al.*, 2006b). This alkaline fermentation is mainly responsible of the typical aroma
46
47 80 of fermented ALBS (Omafuvbe *et al.*, 2004; Ouoba *et al.*, 2005). Pyrazines were also found in
48
49 81 the fermented ALBS aroma compounds probably due to Maillard reactions that may occur
50
51 82 during pre or post fermentation steps (Esenwah and Ikenebomeh, 2008; Amoa-Awua *et al.*,
52
53 83 2014). It was also shown that during fermentation, consumption of protein from *Bacillus* lead
54
55 84 to free amino acids release at high quantity (Odunfa, 1985). It is known that free amino acids
56
57 85 may lead to biogenic amines (Ruiz-Capillas and Herrero, 2019). A biogenic amine is a basic
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59 86 molecule with one or more amine groups generated by microbial decarboxylation of amino
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61 87 acids. When present in fermented products in high amount, biogenic amines may cause not
62
63 88 only organoleptic defects but also adverse symptoms for human health. Indeed, biogenic

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3 89 amines have biological effects and may among others cause some neurotransmission
4 90 disorders, headache, gastrointestinal disorders, and edema by giving rise to blood vessel
5 91 dilatations (Erdag *et al.*, 2018). Histamine is the most toxic of these amines and its action is
6 92 enhanced by the presence of other amines such as putrescine, cadaverine, spermidine,
7 93 tyramine and tryptamine. Histamine poisoning has been firstly diagnosed in fishery products
8 94 (Ruiz-Capillas and Herrero, 2019). However, other products involving a fermentation step are
9 95 likely to contain high amounts of biogenic amines such as wine, cheese or fermented soya
10 96 products (Krause *et al.*, 1997; BIOHAZ, 2011; Byun and Mah, 2012).

11 97 The objective of this paper is to analyse the effect of the whole process of ALBS transformation
12 98 in soumbala on the fate of the macromolecule of nutritive interest, i.e. proteins and lipids, but
13 99 also of their resulting molecules that may be toxic and particularly biogenic amine that have
14 100 never been studied in fermented ALBS products.

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102 2. Material and methods

103 2.1. Plant material

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

107 2.2. Soumbala production from ALBS

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

118 2.3. Chemicals

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3 119 All amino acids, biogenic amines (putrescine, cadaverine, histamine, phenethylamine,
4 spermidine) and mono- di- and tri-olein standards and all solvents were of analytical grade
5 120 and obtained from Merk (Germany).
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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.
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18 126 **2.4.2. Protein**

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20
21 127 The nitrogen content of all Samples was determined using Kjeldhal method and a conversion
22 factor of 5.85 was used.
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24 129 **2.4.3. Free and Total Amino Acid Analysis**

25 130 **- Extraction procedure**

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27
28 131 - Free amino acids were analysed following the method used by Moore *et al.*, 1958, with
29 modifications. Briefly, 15 mg of sample was weighed and placed in a sealable test tube. To
30 this, 50 µl of internal standard Norleucine (25 µM) and 4.95 ml of citrate buffer (pH 2.2) were
31 added. The solution was mixed for 1 h on a rotational shaker.
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36 135 - Total amino acids Samples (10 - 20 mg) of dried flour were weighed in a Schlenk tube and 50
37 µl of 25 µM Norleucine and 450 µL of 4 M methanesulfonic acid were added. The tube was
38 flushed with nitrogen, closed and heated at 150°C for 2 h. After cooling, 450 µL of 4 M NaOH
39 was added to the hydrolysate, which was diluted to 5 ml with a loading buffer (citrate buffer
40 at pH 2.2).
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45 140 **- Amino acid analysis**

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48 141 Sample extracts for free and total amino acid analysis were filtered using a 0.45 µm membrane
49 filter and injected into the amino acid analyzer (Biochrom 30+, Biochrom, France), using a
50 lithium cation exchange resin column and ninhydrin as detection compound. All total and free
51 amino acid contents were determined except total tryptophan, which is the most fragile
52 amino acid and is destroyed by the extraction procedure. Amino acid standards were also run
53 in a similar way as the samples.
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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

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3 177 trichloroacetic acid solution was added to 0.5 or 1 g of raw seed or fermented soumbala
4
5 178 respectively. After 30 min homogenization, the mixture was centrifuged at 400 g for 5 min and
6
7 179 then filtered through a Whatman n°3 filter paper (Whatman, UK). Steam entrainment was
8
9 180 performed using a Kjeldahl-type distillatory (Tecator Line 8400, Höganäs, Sweden). 5 ml of filtrate
10
11 181 were loaded into the distillation tube followed by 6 ml of 10% NaOH. A beaker containing 10 ml
12
13 182 of 4% boric acid and 0.04 ml of methyl red and bromocresol green indicator was placed under the
14
15 183 condenser for the titration of ammonia (ref: Merck 6130). TVBN which was titrated with aqueous
16
17 184 0.025 N sulphuric acid solution and is expressed as mg or g nitrogen/100 g

18 185 **2.4.6. Lipids content**

19
20 186 **About 3 g of sample were precisely weighed** in a cellulose thimble and subjected to a Soxhlet
21
22 187 extraction with 50 mL of solvent (petroleum ether) in aluminium capsule during 110 min at 130°C.
23
24 188 After extraction and evaporation of solvent, the alumium were further dried at 60 °C for 30 min,
25
26 189 after which the sample weight was constant.

27 28 190 **2.4.7. Lipid profile**

29
30 191 Lipids were separated and quantified by thin layer chromatography as described in Amenta
31
32 192 (1964). Briefly, a solution of 10 mg/mL of extracted lipids in hexane was prepared. Injection
33
34 193 was done automatically (CAMAG TLC, France) on a silica gel plate (20 cm x 10 cm). Migration
35
36 194 of mobile phase (hexane/petroleum ether (50/50, v/v) was done in an automatic developing
37
38 195 chamber (CAMAG TLC, France) during 20 minutes. Revelation of spots was done by immersing
39
40 196 the silica gel plate in a mixture of saturated copper acetate and orthophosphoric acid 40%
41
42 197 before drying on a hot plate. Revelation and integration of spot was done under UV light
43
44 198 (CAMAG TLC, France). Lipid classes identification of the different spots was done by
45
46 199 comparison of their retention factor (Rf) to that of standards of mono-, di- and tri-olein.

47 48 200 **2.4.8. Total Free fatty acids**

49
50 201 Three g of lipids were added to 100 mL ethanol preheated to 60° and 1 ml of phenolphthalein
51
52 202 solution as titration indicator. Titration was immediately done with potassium hydroxide
53
54 203 solution 0.1 N. Results were expressed in g of lineloic acid per 100 g of fat.

55 56 204 **2.4.9. Free fatty acid profile**

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3 205 The free fatty acid profile was done only on the raw and final ALBS. Fatty acid methyl esters
4
5 206 were obtained from about 50 mg of lipid fraction through esterification with 3 mL of HCl
6
7 207 methanol solution prepared from 50mL of acetyl chloride dissolved in 625 mL of methanol
8
9 208 (reflux time 15 min). 8 mL of hexane and 10 mL of water were used to extract the methyl
10
11 209 esters. One μL of the hexane solution was injected in a capillary gas chromatography (GC)
12
13 210 (Agilent model 7890, Santa Clara, CA, US) equipped with an injector (split-less mode), a
14
15 211 capillary column (DB-Wax, J&W Scientific), a flame-ionization detector (FID). Flow rate of the
16
17 212 carrier gas helium was 0.452 mL/min. The oven temperature was held at 130 °C-40 min, 1
18
19 213 °C/min to 160 °C kept 10min, 2 °C/min to 180 °C kept 20min, 2 °C/ min to 220°C kept 10 min.
20
21 214 The temperature of injector and FID was 270 °C and 280 °C, respectively. Identification of the
22
23 215 peaks was made by comparison with retention times of methyl esters of known oils.

24 216 **2.4.10. Conjugated dienes**

25
26 217 Change in conjugated diene (CD) of lipids extracted were measured as an indication of oil
27
28 218 primary oxidation products. 0.01 to 0.03 g oil or lipid extract sample were weighted into a 25-
29
30 219 ml volumetric flask filled with isooctane as a solvent. Absorbance was read at 233 nm. CD
31
32 220 concentration in $\text{mol}\cdot\text{L}^{-1}$ was obtained considering ϵ , the molar absorptivity of linoleic acid
33
34 221 hydroperoxide ($2.5 \times 10^4 \text{ M}^{-1}\cdot\text{cm}^{-1}$).

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36 222

38 223 **2.5. Statistical analysis**

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40
41 224 All analyses previously described were performed in triplicate for each sample. One-way
42
43 225 ANOVA was used to analyze the effect of the process step (effect considered significant when
44
45 226 $p < 0.05$) on the composition of seeds during soubala production. If significantly different,
46
47 227 means were further compared using Tukey's test. Statistical analyses were performed with
48
49 228 Statistica® (StatSoft Inc., USA).

50
51 229

52 230 **3. Results**

54 55 231 **3.1. Major composition changes during soubala production from ALBS**

56
57 232 Figure 2 shows the composition of ALBS during soubala production. Carbohydrates
58
59 233 proportion was estimated by difference between the dry matter and the sum of proteins and
60

1
2
3 234 lipids. Raw ALBS were composed of carbohydrates 27.9 %, lipids 24.8 % and proteins 41.9 %
4
5 235 (wb). This composition is typical of an oleoproteaginous seeds as soya and consistent with that
6
7 236 already published on ALBS (Esenwah and Ikenebomeh, 2008; Elemo *et al.*, 2011). However,
8
9 237 some previous results showed a higher content in carbohydrates sometimes superior to
10
11 238 proteins that may due to variety variability. Anyway, our experiments and literature are in
12
13 239 accordance with a dry matter superior to 90% (Omafuvbe *et al.*, 2004). The dry matter
14
15 240 dropped markedly after cooking to 30% because of water transfer to the cotyledons leading a
16
17 241 swelling ratio of 3. Water content was constant during fermentation. This is consistent with
18
19 242 Omafuvbe *et al.* (2004) who found constant moisture content of 60% before and after
20
21 243 fermentation. In a qualitative point of view, the main effect of cooking is a marked decreased
22
23 244 in carbohydrate content divided by a 10-fold. This may be due to the very long cooking steps
24
25 245 and a leak of the initial carbohydrates. Therefore, after cooking, protein and lipids constituted
26
27 246 46 and 40 % of the dry basis respectively. By comparison, Azokpota *et al.* (2006) found values
28
29 247 of 43 and 31 % and Esenwah and Ikenebomeh (2008) found 33 and 35 % respectively
30
31 248 (Azokpota *et al.*, 2006a; Esenwah and Ikenebomeh, 2008). After fermentation, only a
32
33 249 significant decrease of protein was observed. After drying, the final water content is close to
34
35 250 10% enabling a stability of fermented ALBS at ambient temperature, and the major
36
37 251 constituents are lipids and proteins at 41.3 and 39.0% and carbohydrates at 8.3%.

252

253 3.2. Protein degradation products fate

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

1
2
3 265 to a high level of free amino acids and high TVBN (Ruiz-Capillas and Herrero, 2019). This link
4
5 266 was also observed during soumbala production. Indeed, increase of biogenic amines contents
6
7 267 was linked to that of TVBN and free amino acids content. TVBN increased from 270 to 881
8
9 268 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
10
11 269 low in the raw ALBS, content of TVBN and BA was already high certainly because of an endogen
12
13 270 enzymatic activity. After cooking, TVBN slightly decreased, FAA remained stable while BA
14
15 271 increased significantly by a 5 fold. This may be due to a starting of fermentation of cotyledons
16
17 272 during storage time. Fermentation has a major effect on the 3 indicators with a marked
18
19 273 increase of 5, 9 and 2.5-fold for TVBN, FAA and BA respectively. This was caused by protease
20
21 274 activity of microorganisms during fermentation. High proteinase activity has frequently been
22
23 275 reported in the fermentation of similar protein-rich seeds like miso (Odunfa, 1985). After
24
25 276 drying, no significant change of the indicators was observed and final TVBN content was close
26
27 277 to 900 mg/100g which is much higher than the value of 30 to 35 mg/100g that European
28
29 278 legislation considers to be unfit for human consumption in fish products (95/149/EC, 1995).
30
31 279 However, soumbala is a dry condiment that is used in a small quantity. However, even used a
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33 280 10% in a meal, it leads to 90 mg/100 g which is still high. Biogenic amine content was high as
34
35 281 well with a total of 512 mg/kg. However, the content of individual amines and their free amino
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37 282 acids that may be precursors must be analysed.
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284 3.2.1. Free amino acids release

285 Major amino acids which constitute proteins of raw ALBS are by order of importance glutamic
286
287 after fermentation. These results are consistent with literature which also report low sulphur
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289 amino acids (cysteine and methionine), which is typical of legumes (Ndir *et al.*, 2000; Elemo *et*
30
31 *al.*, 2011). The main FAA present in raw ALBS represent 2% (w/w) on a protein base. The main
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33 free forms were by importance glutamic and aspartic acids (60%) which seems logical. The
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35 remaining were valine (17%), cysteine (13%) and histidine (9%). While cysteine and histidine
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37 were in low quantities in ALBS proteins, they become majority in their free forms. However,
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39 the major release of FAA occurred during fermentation ($p < 0.05$) and the content reached 8.7
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41 g/100g making a FAA/protein ratio increase by a 10-fold till 20%. This FFA production was
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43 already observed in literature (Odunfa, 1985; Ouoba *et al.*, 2003b; Omafuvbe *et al.*, 2004). The
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3 296 main FAA produced were glutamic acid, leucine, lysine, valine (total of 46%) which was
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5 297 consistent with the initial proteins composition. However, sum of tyrosine, phenylalanine,
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7 298 isoleucine, ornithine, cysteine and histidine represented also 47%. Therefore, it seemed that
8
9 299 there was an oriented release or reaction/consumption of amino acids which may be the fact
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11 300 of micro-organisms. Besides, it was already noticed the release of cysteine, methionine,
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13 301 leucine, isoleucine, tyrosine and phenylalanine during fermentation of bacillus strains on a
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15 302 medium composed of 1% ALBS powder (Ouoba *et al.*, 2003b). In our case, no methionine was
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17 303 identified but instead we found ornithine and histidine among FAA. Ouoba *et al.* (2003)
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19 304 noticed differences in FAA release as a function of the bacillus strains involved during
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21 305 fermentation. In addition, some FAA may be subjected to a degradation during fermentation
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23 306 such as aspartic acids, arginine, alanine proline, and glycine. These reactions may be due to
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25 307 oxidative deamination and decarboxylation and furthest reaction that can lead to biogenic
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27 308 amines genesis.

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310 **3.2.1. Biogenic amines release**

311 The biogenic amines identified during soubala production were putrescine, cadaverine,
312 histamine, phenethylamine and spermidine (Fig. 5). Like free amino acids, the main step
313 **increasing the most their content is fermentation** ($p < 0.05$), except for histamine that was
314 present in the raw seeds. Histamine is considered as potentially toxic especially for sensitive
315 population. Consumption of histamine in the range of 8–40 mg, 40–100 mg or higher than 100
316 mg in one meal may cause slight, intermediate and severe poisoning respectively (Biji *et al.*,
317 2016). Commission Regulation (EC) 2073/2005 lays down food safety limits for histamine
318 between 100 and 200 mg/kg for certain fish and fishery products. Even though there are no
319 legal limits for the concentration of biogenic amines in other products, some European
320 countries only recommend maximum limits for histamine. Histamine final content **after drying**
321 was 40 mg/kg product. Therefore, assuming a consumption of 50 g in a meal, the histamine
322 intake would be 2 mg and may not be dangerous. Therefore, the high TVBN content observed
323 **after fermentation** was due to other biogenic amines or other volatile compounds such as
324 ammonia. By order of importance, the final content **after drying** was 325, 56, 37, 21 and 20
325 mg/kg for spermidine, phenylethylamine, histamine, cadaverine and putrescine respectively.
326 Their origin is the decarboxylation of arginine, phenylalanine, histidine, lysine and ornithine

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3 327 respectively. It is interesting to notice that phenylalanine, histidine, lysine and ornithine were
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5 328 the free amino acids found after fermentation while arginine was not found. As arginine is the
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7 329 5th amino acid by order of importance in raw ALBS, the free amino acid may be formed and be
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9 330 decarboxylated very quickly to form spermidine. Other hypothesis is that spermidine can be
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11 331 also formed from putrescine (Biji *et al.*, 2016). Among free amino acids found after drying (Fig
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13 332 4 B), it is also noteworthy that tyrosine could be decarboxylated in tyramine. However, this
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15 333 biogenic amine was not found in our case but could be potentially produced from ALBS as a
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17 334 function of the microorganism specie involved in the fermentation. Biogenic amines like
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19 335 spermidine, putrescine and cadaverine do not have any adverse health effect, but may react
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21 336 with nitrite to form carcinogenic nitrosoamines. In addition, they can potentiate the toxic
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23 337 effect of histamine by inhibiting intestinal histamine metabolizing enzymes like diamine
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25 338 oxidase. However, their toxicity level is 600, 2000 and >2000 ppm respectively, which is much
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27 339 higher than the amount that can be found in a dish with fermented ALBS (Biji *et al.*, 2016).
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29 340 However, when recommending fermented ALBS for nutritional purpose, the potential
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31 341 presence of biogenic amines should be taken is consideration especially for sensitive
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33 342 population.

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344 3.3. Lipid fate

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346 3.3.1. Lipid profile of raw ALBS

347 The lipids of raw ALBS consisted mainly of triglycerides (>70%). The major fatty acids (FA) were
348 by order of importance linoleic (C18:2), oleic (C18:1), stearic (C18), behenic (C22), palmitic
349 (C16) and arachidic (C20), accounting for 45, 14, 13, 12, 9, and 3 % of total lipids respectively
350 (Fig. 6 A). Therefore, ALBS oil is very rich in linoleic acid, an essential FA belonging to the omega
351 6 family. Once again, this composition was close to that of soya as observed in previous studies
352 (Ndir *et al.*, 2000; Elemo *et al.*, 2011). The other major spot obtained but not identified by thin
353 layer chromatography in raw ALBS accounted for 20% of lipid. It may gathered unsaponifiable
354 compounds like vitamin E, phospholipids or phytosterols which were proven to be in high and
355 interesting quantity in ALBS (Omafuvbe *et al.*, 2004; Olatunya *et al.*, 2019). It is noteworthy
356 that these compounds were stable as their content did not change significantly during the

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3 357 process (Fig. 6 B). Lastly, raw ALBS lipid fraction also contained 3% of di-glycerides and 2% of
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5 358 free fatty acids (FFA) probably due to a little intrinsic lipase activity
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360 3.3.2. Di-glyceride and free fatty acid release during soumbala production

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

375 376 Conclusion

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

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3 387 soumbala production. However, lipid profile of ALBS was significantly affected. Indeed,
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5 388 because of lipase activity during cooking and fermentation, a significant production of di-
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7 389 glycerides and free fatty acids was remarkable. This production may have nutritional but also
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9 390 organoleptic quality of the product during storage because of the high reactivity of
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11 391 unsaturated free fatty acids. To synthesize the effect of processing, the initial and final
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13 392 composition of protein and lipid matter are presented in **supplementary material 1 and 2.**

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15 393 In conclusion, fermented ALBS is an interesting product, but whose production should benefit
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17 394 a better comprehension and control to optimize its properties while decreasing its content in
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19 395 undesirable compounds such as peculiar biogenic amines or limit its free fatty acid content.
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21 396 Use of started or adequate fermentation parameters (temperature, pH..) must be deepen in
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23 397 future studies.

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27 399 **Author statement**

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29 400 Ethics approval was not required for this research. The authors have no conflict of interest to
30
31 401 declare. The data that support the findings of this study are available from the corresponding
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33 402 author upon reasonable request.
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3 403 **Legends to Figures**
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8 405 **Figure 1.** Flowchart of ALBS transformation in Soumbala in Khorogo region
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10 406 **Figure 2.** Composition in % **wet** basis of dehulled ALBS during soumbala production
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13 407 **Figure 3.** Protein degradation products during soumbala production
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15 408 **Figure 4.** A) Composition of initial total amino acids in raw ALBS and B) free amino acid (g/100
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17 409 g dry basis) release during soumbala production
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20 410 **Figure 5.** Content of biogenic amines in mg/kg during soumbala production
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22 411 **Figure 6.** Composition A) in free fatty acids of raw ALBS B) and lipid fraction during soumbala
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24 412 production
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27 413 **Supplementary material 1.** Nitrogen matter in raw and fermented ALBS and their
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29 414 recommended daily intake (RDI) or toxic value (mg)
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31 415 **Supplementary material 2.** Lipidic matter in raw and fermented ALBS and their recommended
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418 **5 Annotated References in yellow**419 **References**

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Figure 1. Flowchart of ALBS transformation in Soubala

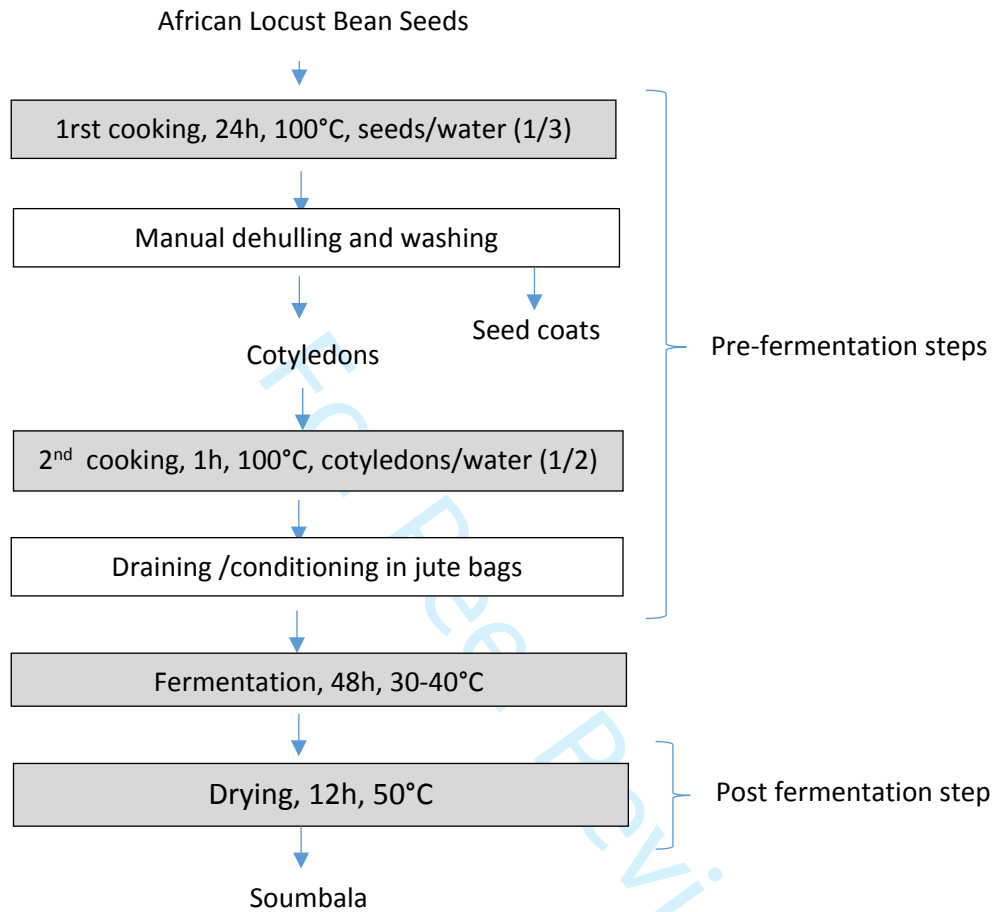
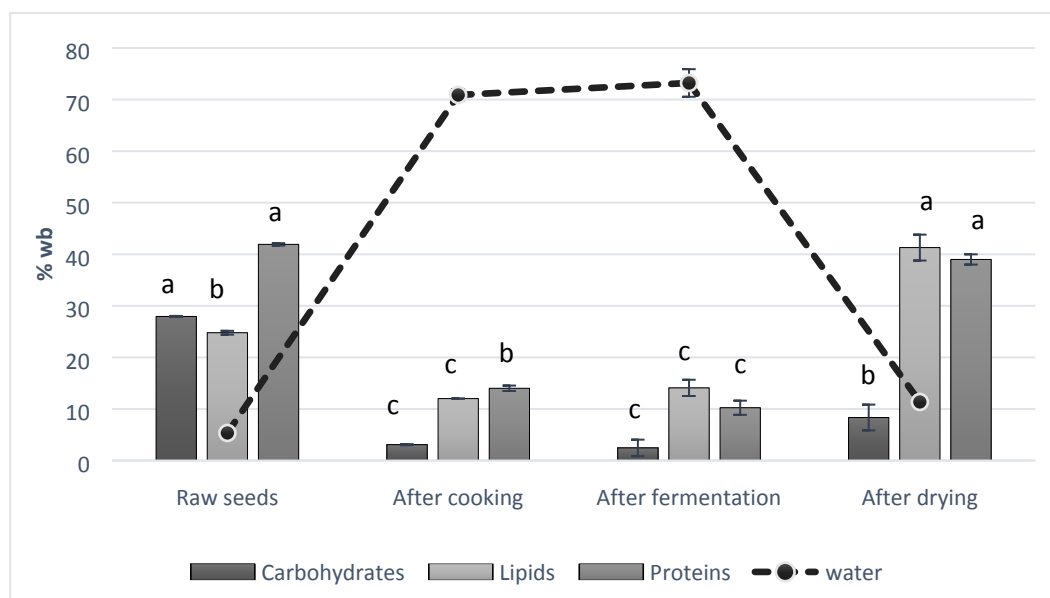
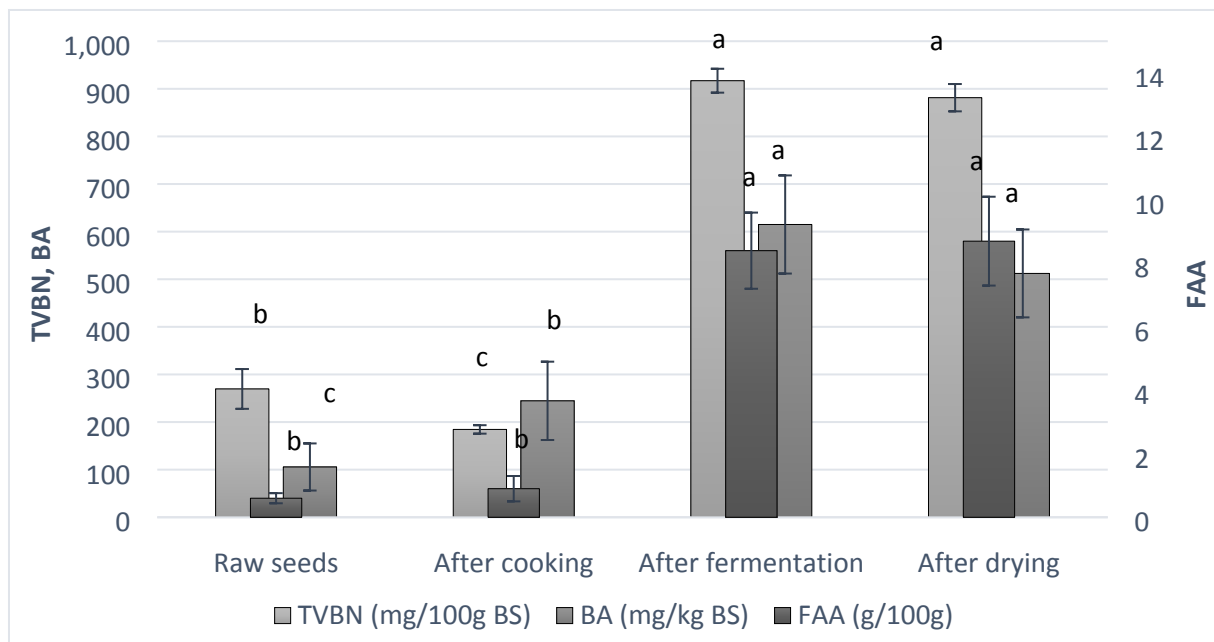


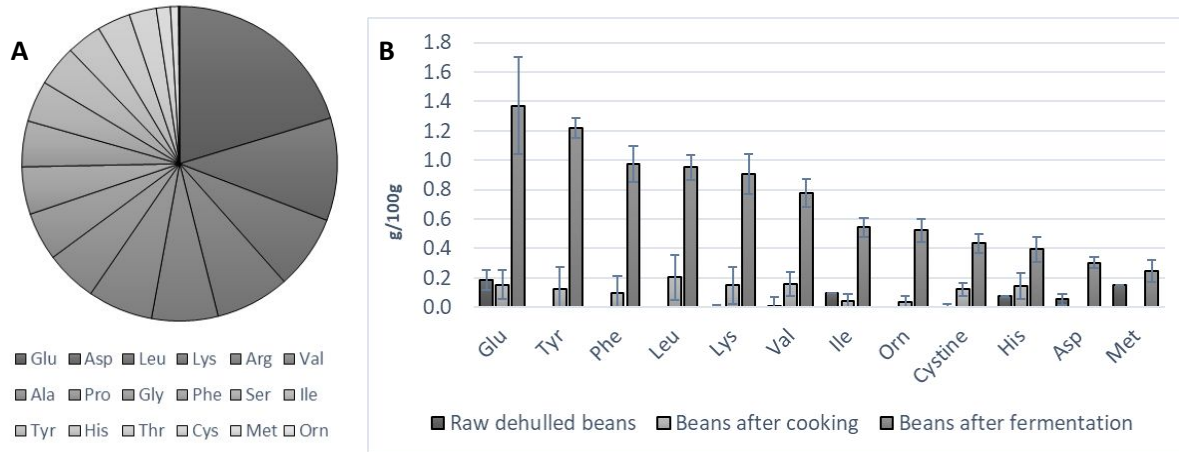
Figure 2. Composition in % wet basis of ALBS during soumbala production

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

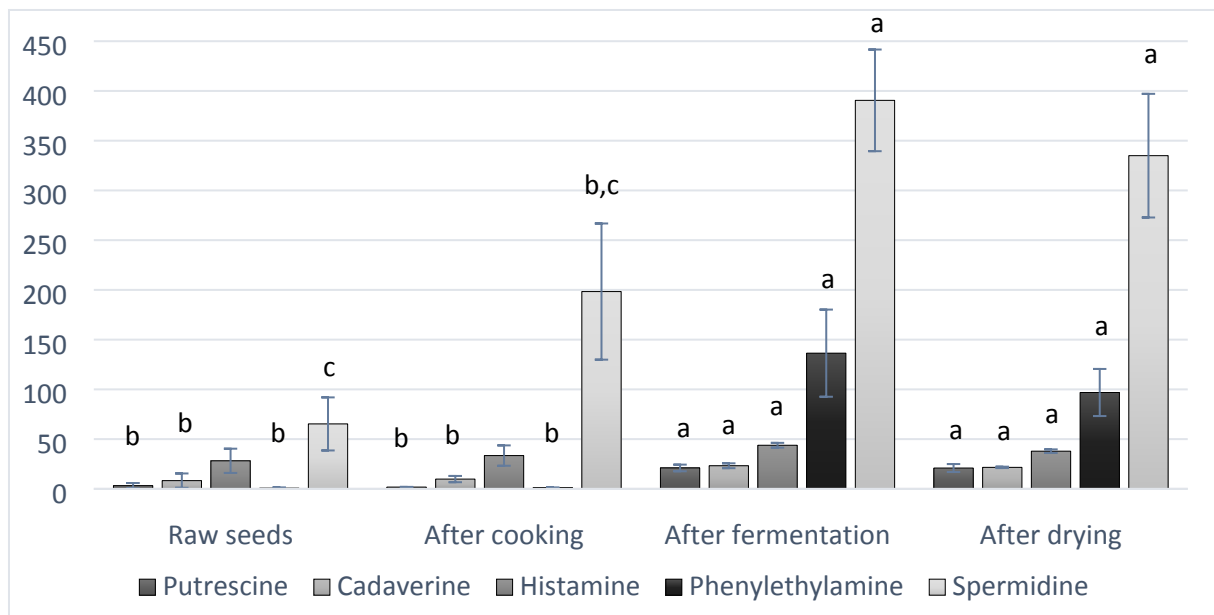
Figure 3. Protein degradation products during soubbala production

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

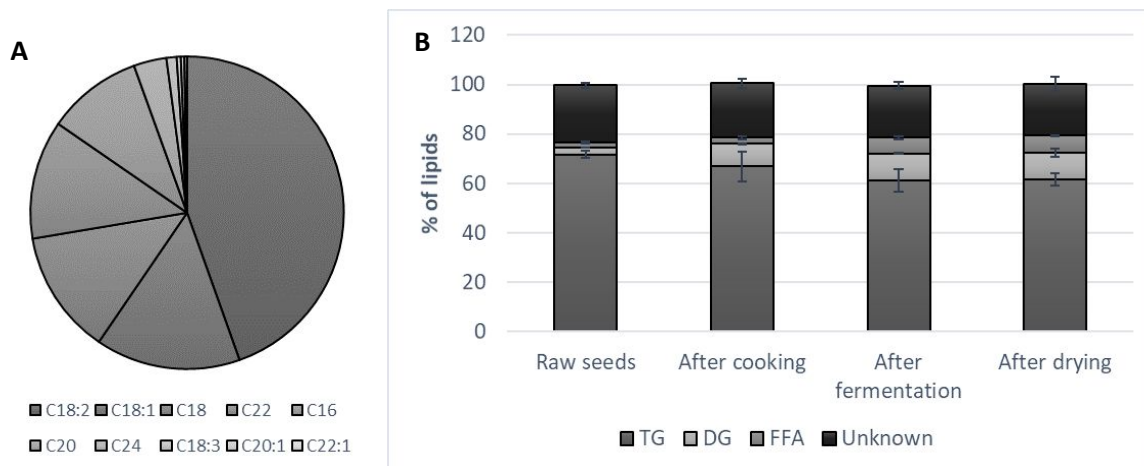


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Figure 5. Content of biogenic amines in mg/kg during soubbala production

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