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Kinetic study of some flavor and bioactive compounds during fermentation of *Parkia biglobosa*

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3 1 **Kinetic study of some flavor and bioactive compounds during fermentation of *Parkia***
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5 2 ***biglobosa***

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3 17 **Abstract**
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5 18 Seeds of *Parkia biglobosa* also called African locust bean (ALBS) are used to produce a
6
7 19 condiment by subjecting them to cooking, alkaline fermentation and drying. The objective of
8
9 20 this work was to monitor the evolution of the composition of ALBS during spontaneous
10
11 21 fermentation. Results showed that the seeds at initial time contained mainly proteins (45%)
12
13 22 and lipids (36%). During fermentation, conversion of this initial composition took place to
14
15 23 produce ammonium, free amino acids and short chain fatty acids (SCFA) that accounted for 2,
16
17 24 10 and 1.5% db respectively which explains the typical flavor of fermented ALBS. This release
18
19 25 was also concomitant with biogenic amine production. Along with ammoniac and SCFA
20
21 26 release, pyrazines were the major volatile organic compounds. Composition modulation was
22
23 27 well represented by a Verhulst equation of which the dynamic could be modified by the initial
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25 28 composition of the seed, the pre-cooking step, and the fermentation conditions (temperature,
26
27 29 aeration).
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30 31 **Key words: soumbala, aroma compounds, short chain fatty acids, biogenic amines, kinetics,**

31 32 **Verhulst equation**
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3 34 **Practical applications**
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8 36 For nutritional and sustainability reasons, re-integration of vegetable in general and legumes
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10 37 in particular in our diet is a current trend. However, only dish that are appealing to consumers
11 38 are concretely adopted. Fermentation of legumes is interesting in more than one way. It does
12
13 39 not involve heat, so thermo-labile molecules can be preserved and the financial and energetic
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15 40 costs are low. Biological interesting molecules are produced from the metabolism of
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17 41 microorganisms and among them, volatile organic compounds that contribute to enhance the
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19 42 flavour of legume dishes. Soumbala is a meal and/or condiment obtained from the
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21 43 fermentation of African locust bean (*Parkia biglobosa*) seeds (ALB), a legume widely spread in
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23 44 west Africa. The final typical sensory properties are known to be due to the alkaline
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25 45 fermentation step by *Bacillus* species. This paper presents the kinetics of release of various
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27 46 compounds during spontaneous fermentation that may impact the sensory and nutritional
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29 47 properties of the final product. More precisely, the dynamic of the production of free amino
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31 48 acids, free fatty acids, volatile compounds and biogenic amines are presented and discussed
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33 49 to give insights into the succession of important reactions to control the sensory and
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35 50 nutritional quality of fermented ALBS. This paper shows that a lot can be done to better
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37 51 understand and control the quality of FALBS but also similar protein-rich legumes to modulate
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39 52 the quality of the final products.
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45 56 **Introduction**6
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8 58 Legumes are a sustainable source of protein and other bio-active compounds that are
9 59 sensitive to temperature. Fermentation is a non-thermal preservation process that can be
10 60 applied to legumes. In addition, previous studies showed that due to microbiological activity,
11 61 the fermentation step increased certain B-group vitamins and decreased anti-nutritional
12 62 factors widely found in legumes such as alpha-galactosides or phytates (Adeyemo & Onilude,
13 63 2013; Granito et al., 2002). Some fermented products are traditionally made from legumes
14 64 such as soybean, locust bean seed, chickpea, black gram or peanut (Reddy, 2018). A variety of
15 65 microorganisms are involved: lactic acid bacteria, yeasts, fungus or *Bacillus* producing a variety
16 66 of tastes, aromas, textures and flavors. The fermented legumes are widely used for flavoring
17 67 in Asia or West Africa (Odunfa, 1988).

18 68 Among them, a typical and popular West African condiment, called *soumbala*, *netetu*, *afitin*,
19 69 *iru* or *dawadawa* depending on the country, is made by *Bacillus* fermentation of a protein-rich
20 70 legume, the African locust bean seed (*Parkia biglobosa*). The traditional process of soumbala
21 71 production consists of three main steps: cooking the seeds in boiling water for 12 to 24h to
22 72 soften the hard outer shell and facilitate dehulling, cooking the cotyledons, spontaneous
23 73 fermentation, and final stabilization by drying (Dakwa et al., 2005; Odunfa, 1985; Yusuf &
24 74 Rahji, 2012). This production is still done on a small-scale and therefore hardly meets
25 75 consumer demand in terms of quantity as well as sanitary and organoleptic quality (Odunfa,
26 76 1985; Omafuvbe et al., 2004). Organoleptic attributes of fermented African locust bean seed
27 77 (FALBS) are the result of the production of hundreds of chemical compounds during
28 78 fermentation (Akanni et al., 2018; Ouoba et al., 2005). These numerous reactions are due to
29 79 the metabolism of *Bacillus* species that hydrolyzes proteins to free amino acids, ammonia and
30 80 other basic nitrogenous compounds. This results in an increase of pH and a so-called “alkaline
31 81 fermentation”, which contributes to the stability of the final product and also to its typical
32 82 ammoniacal flavor (Akanni et al., 2018; Amoa-Awua et al., 2014; Ouoba et al., 2003). *Bacillus*
33 83 not only produce proteases but a diversity of other extracellular hydrolases that also react
34 84 with the seed carbohydrates and lipids which may contribute to the flavors and aromas
35 85 development (Odunfa, 1985).

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3 87 Volatile organic compounds (VOCs) have already been studied in FALBS (Akanni et al., 2018;
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5 88 Ouoba et al., 2005). A total of 116 to 125 VOCs were identified, belonging, by order of
6
7 89 quantities, to acids, pyrazines, nitrogen-containing compounds, aldehydes, alcohols, ketones,
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9 90 esters, alkanes, alkenes, benzenes, phenols, and sulfur-containing compounds. Depending on
10
11 91 the relative concentration of the different VOCs, some consumers may or may not appreciate
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13 92 the product (Amoa-Awua et al., 2014; Dakwa et al., 2005). Indeed, as for strong aromatic
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15 93 products such as cheese or wine, the balance of the different molecules released and their
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17 94 control is of major importance on the sensory quality and acceptance of the end-product. To
18
19 95 achieve this objective, knowledge of the impact of fermentation parameters on the
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21 96 production dynamic of the different volatiles is a first step. Akanni *et al.* (2018) and Ouoba *et*
22
23 97 *al.* (2005) worked on the influence of *Bacillus* strains on the typology of final aroma
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25 98 compounds. Akanni *et al.* (2018) found that *B. subtilis* and *B. licheniformis* contributed to
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27 99 volatile acids, *B. cereus* to aldehydes and alcohols, and *B. amyloliquafaciens* produced sulfur-
28
29 100 containing compounds. Another way to control VOCs release is to modify the processing
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31 101 conditions. Amoa-Awua *et al.* (2014) added humectant molecules like NaCl or glycerol during
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33 102 fermentation to limit *Bacillus* populations. They also tested the effect of steaming after
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35 103 fermentation. Both treatments applied significantly reduced the pungent odor because of a
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37 104 decrease in ammonia content. They found that the VOCs associated with the most undesirable
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39 105 sensory descriptors were ammonia, phenols, indoles, and volatile SCFA (Amoa-Awua et al.,
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41 106 2014; Delgado et al., 2011). On other fermented or ripened products such as cheese, authors
42
43 107 found that SCFA had a major sensory impact because of their low odor threshold (Delgado et
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45 108 al., 2011; Faccia et al., 2018; Noronha et al., 2008).

46
47 109 In addition to VOCs some non-volatile compounds may significantly contribute to the
48
49 110 organoleptic properties of FALBS. Indeed, previous studies showed that FALBS contained
50
51 111 significant amounts of free amino acids (AA) and FFA that can impact its sensory properties
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53 112 (Esse et al., 2021; Odunfa, 1985; Omafuvbe et al., 2004). Indeed, Zhang et al. (2020) showed
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55 113 that AA such as serine, aspartic and glutamic acids, glycine, alanine, proline, cysteine,
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57 114 methionine, and lysine, significantly and positively influenced the sensory descriptors of
58
59 115 chicken broth and muscle (Zhan et al., 2020). Other bioactive molecules that may come from
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116 AA are biogenic amines (BA). Although BA are present in living cells and contribute to different
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118 physiological functions, high consumption via food can cause intoxication. Secondly,
elevated levels of biogenic amines can contribute to the pungent or objectionable odor of

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3 119 fermented products (Cohen et al., 2014; Dekeirsschieter et al., 2009). The link between
4
5 120 biogenic amines and the sensory profile of fermented products was studied in wine. It was
6
7 121 proved that agmatine and β -phenylethylamine were correlated to a positive sensory profile,
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9 122 while putrescine, tyramine, histamine, cadaverine and spermidine were linked to negative
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11 123 descriptors (Ordóñez et al., 2017). Finally, FFA like myristic, palmitic, stearic, and eicosanoic
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13 124 acids can also affect the flavor of lipid-rich foods (Zhan et al., 2020).
14
15 125 Alkaline fermentation of legumes in general, and that of African legumes in particular, is poorly
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17 126 studied. However, the use of fermented legumes contributes to food sustainability and flavor
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19 127 diversity. Therefore, it is important to gain insight into their production and into the reactions
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21 128 that takes place during fermentation that impact their sensory and nutritional quality. Because
22
23 129 fermented African locust bean seeds is a condiment, the previous studies mainly focused on
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25 130 VOCs profile at the end of processing. However, a better understanding of volatile and non-
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27 131 volatile compounds release throughout the fermentation process would be very helpful for
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29 132 producers to better control the quality of fermented legumes. Therefore, the objective of this
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31 133 paper is to describe the kinetics of bioactive compound production during the spontaneous
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33 134 fermentation of ALBS.

34 136 **Material and methods**

38 138 **Plant material**

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40 139 The harvest of African locus bean seeds takes place from March to May. Seeds are dried and
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42 140 are available on local markets till the next harvest season. The seeds used for this study were
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44 141 bought in the region of Khorogo, where most Soumbala production is located, in the north of
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46 142 Côte d'Ivoire.

49 144 **Fermentation of African locust bean seeds**

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51 145 Before fermentation, ALBS were first put into boiling water at a ratio seed/water of 1/3 for
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53 146 24h over a traditional wood fire to prepare dehulling. The seeds were dehulled in a laboratory
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55 147 mortar. Dehulled raw seeds were cooked in boiling water at a ratio seed/water of 1/1 for 1
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57 148 hour. Spontaneous fermentation of 500 g of cooked cotyledons was done in jute bags placed
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59 149 in small baskets in a chamber regulated at 40 °C for 48 h. Samples were collected at 0, 12, 24,

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3 150 **36 and 48 h of fermentation**. To stabilize them, fermented seeds were dried in an oven at 50
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5 151 °C for 12 h. The whole process was repeated. A total of 17 samples were collected throughout
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7 152 the production process and more precisely: 3 samples of raw seeds, 4 samples of cooked seeds
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9 153 at time 0 of fermentation, 2 samples after 12, 24 and 36 h of fermentation and finally 4 at the
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11 154 end of 48 h of fermentation. All samples were dried and stored at -18 ° C.

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14 156 **Proximate composition**

16 157 **The dry matter content was determined by oven drying at 105°C to a constant weight. The**
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18 158 **nitrogen content of all samples was determined using the Kjeldhal method and a conversion**
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20 159 **factor of 5.85 was used according to AOAC 979.09. Lipid content was done by Soxhlet**
21
22 160 **extraction according to AOAC 963.15. Total free fatty acids of the lipids obtained by Soxhlet**
23
24 161 **extraction was determined by titrimetry according AOAC Cd 3d-63. Ashes were obtained**
25
26 162 **gravimetrically after incineration of the sample at 550°C for approximately 7h according AOAC**
27
28 163 **923.03. Total carbohydrates were estimated by subtracting the measured protein, fat, ash,**
29
30 164 **and water from the total weight.**

31 165

34 166 **Free and Total Amino Acid**

36 167 **Free amino acids were analyzed following the method used by (Moore et al., 1958), with**
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38 168 **modifications. Briefly, 15 mg of samples were weighed and placed in a sealable test tube. To**
39
40 169 **this, 50 µl of internal standard Norleucine (25 µM) and 4.95 mL of citrate buffer (pH 2.2) were**
41
42 170 **added. The solution was mixed for 1 h on a rotational shaker. For total amino acid**
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44 171 **determination, 10 to 20 mg of sample were weighed in a Schlenk tube and 50 µl of 25 µM**
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46 172 **norleucine and 450 µL of 4 M methanesulfonic acid were added. The tube was flushed with**
47
48 173 **nitrogen, closed and heated at 150°C for 2 h. After cooling, 450 µL of 4 M NaOH was added to**
49
50 174 **the hydrolysate, which was diluted to 5 mL with a loading buffer (citrate buffer at pH 2.2).**
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52 175 **Sample extracts for free and total amino acid analysis were filtered using a 0.45 µm membrane**
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54 176 **filter and injected into the amino acid analyzer (Biochrom 30+, Biochrom, France), using a**
55
56 177 **lithium cation exchange resin column and ninhydrin as a detection compound. All total and**
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58 178 **free amino acid contents were determined except total tryptophan, which is the most fragile**

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3 179 amino acid and is destroyed by the extraction procedure. Amino acid standards were also run
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5 180 in a similar way as the samples.
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10 182 **Biogenic amines**

12 183 Analysis of biogenic amines was carried out based on the procedure described in (Yoon et al.,
13 184 2015). Briefly, 20 mL of 0.4 M perchloric acid were added to 5 g of the samples, and the
14 185 mixture was homogenized using a vortex mixer (Vortex-Genie, USA), reacted in a cold
15 186 chamber at 4 °C for 2 h, and centrifuged at 3000 ×g at 4 °C for 10 min. The supernatant was
16 187 collected, and the residue was extracted again with an equal volume of 0.4 M perchloric acid.
17 188 Both supernatants were combined, and the final volume was adjusted to 50 mL with 0.4 M
18 189 perchloric acid. The extract was filtered then, derivatization of biogenic amines was carried
19 190 out as follows: one mL of extract (or standard solution) was mixed with 200 µL of 2 M sodium
20 191 hydroxide and 300 µL of saturated sodium bicarbonate solution. Two mL of dansyl chloride
21 192 solution (10 mg/mL) prepared in acetone were added to the mixture, which was then
22 193 incubated at 40 °C for 45 min. Residual dansyl chloride was removed by adding 100 µL of 25%
23 194 ammonium hydroxide. After incubation for 30 min at 25 °C, the volume of mixture was
24 195 adjusted with acetonitrile to 5 mL. Finally, the mixture was centrifuged at 3000 × g for 5 min,
25 196 and the supernatant was filtered through 0.2 µm-pore-size filters (Millipore Co., USA). The
26 197 filtered supernatant was kept at -25 °C before HPLC injection. An HPLC unit equipped with a
27 198 UV-Vis detector (Dionex Ultimate 3000, France) was employed. An ACE C18 column (5µm
28 199 particle size, 250 mm × 4.6 mm, ACE, France) was used for the separation of the biogenic
29 200 amines, and the samples were eluted with a step gradient of ammonium acetate (0.1 M
30 201 solvent A) and acetonitrile (solvent B) as the mobile phases at the flow rate of 1 mL/min. The
31 202 program was set for a linear gradient starting from 50% of solvent B to reach 90% of the
32 203 solvent at 19 min. The sample volume injected was 10 µL and monitored at 254 nm. Standard
33 204 stock solutions of biogenic amines, putrescin, cadaverine, histamine and β-phenylethylamine
34 205 were separately prepared at 10 mg/L concentration in distilled water.
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207 **Volatile compound**

208 Firstly, 1.5 g of ground sample was placed and sealed in 10 mL headspace vials with 1.4 mg of
209 the internal standard trans-2-octenal and extracted by headspace solid phase micro extraction
210 (HS-SPME) (Corrales et al., 2017). The extraction was performed at 60°C with 15 min
211 incubation and 30 min trapping and shaking using a polydimethylsiloxane/divinylbenzene
212 fiber (PDMS/DVB 65 mm, SUPELCO, Bellefonte, PA, USA). The volatile compounds were
213 identified using gas chromatography/mass spectrometry. A tandem gas chromatograph
214 6890/MSD 5973 N (Agilent Technologies, Palo Alto, USA) and a Gerstel autosampler MPS-2
215 were used. A non-polar capillary column DB-5MS (5% diphenyl, 95% dimethyl siloxane, 30 m
216 × 0.250mm × 0.25 µm) and a polar capillary column DBWax UI (polyethylene glycol 30 m ×
217 0.25mm × 0.25 µm), both from J&W Scientific (Folsom, CA, USA), were used with a carrier gas
218 hydrogen flow rate of 1.2 mL/min. Then, fiber was desorbed at 250 °C in splitless mode and
219 was eluted with the following temperature program: 3 °C per min from 40 °C to 170 °C, then
220 10 °C per min up to 240 °C and held for 10 min. Mass spectrum were recorded in EI+ mode at
221 70 eV within a range of 40 to 350 Da. Analyzer and source temperatures were 150°C and
222 250°C, respectively. Data were analyzed with Masshunter version B. 06.00 (Agilent
223 Technologies, Palo Alto, USA). The identification of peaks was performed by comparing their
224 mass spectra with those from the NIST 2011 (National Institute of Standard Technology)
225 database. Co-injection on both columns of alkanes from C8 to C20 (Sigma-Aldrich, St. Louis,
226 MO, USA) was used to calculate Kovats retention index (RI) for comparison with those found
227 on the Flavornet, Pherobase and NIST websites.

228

229 **Statistical analysis**

230 One-way ANOVA was used to analyze the effect of fermentation time on the macronutrient
231 composition in FALBS. If significantly different, means were further compared using Tukey's
232 test. One-way ANOVA was also done to select discriminant volatile compounds before
233 principal component analysis (PCA). Statistical analyses were performed with Statistica®
234 (StatSoft Inc., USA).

235

236 **Kinetic modelling**

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Microbiological growth during fermentation is assumed to be logistic and that production of different bioactive compounds produced by microorganisms is often linearly correlated to population, therefore we chose the usual Verhulst logistic function to represent production (Peleg et al., 2007; Yang et al., 2011):

$$[X](t) = [X]_{\infty} \times \frac{1}{1 + L \times \exp^{(-k \times t)}} \quad \text{eq. (1)}$$

Where $[X]$ is the bioactive compound in g or mg.kg⁻¹ (DB) at the time t (h) of fermentation, $[X]_{\infty}$ is the asymptotic value of the curve that can be assimilated to the maximal concentration of X at the end of fermentation, k is the logistic growth rate or steepness of the curve while L refers to its sigmoid shape.

For each compound, the constants $[X]_{\infty}$, L and k were identified by non-linear regression thanks to the Levenberg-Marquardt minimization procedure using the Matlab® software (The Mathworks Inc., Natick, Mass, USA).

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251 **Parameter standard deviation**

The standard deviations on identified parameters were determined via the method of bootstrapping simulations (Huet et al., 1992). The principle is the generation of a high number (500) of resamples of the observed dataset (and of equal size to the observed dataset) added with a Gaussian perturbation within the experimental standard deviations. The mean and standard deviations were estimated for each constant rate from the 500 values.

257

258 **Results**

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260 **Macro-composition evolution during fermentation**

Raw ALBS were composed of 5% ashes, 25 % total carbohydrates, 25 % lipids and 45 % proteins, on a dry basis (DB). The cooking step resulted in significant leaking of carbohydrates that decreased from 25 to 15% DB while proportions of lipids increased to 36% and proteins remained stable. The cooking step also resulted in hydration of the dry seeds up to 60 to 70%, which was necessary for fermentation (Esse et al., 2021).

Fig. 1 represents the evolution of these macro-constituents on a DB during fermentation. In general, the composition of dry matter was quite stable. The major constituent being proteins,

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2
3 268 then lipids and finally the rest of dry matter is **composed mainly of** carbohydrates and
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5 269 minerals. A slight change came from an increase of lipids from 35 to 42%. This increase was
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7 270 due to the preferential consumption of carbohydrates and proteins by microorganisms during
8
9 271 fermentation and was already observed in other studies about ALBS fermentation (Azokpota
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11 272 et al., 2006; Esenwah & Ikenebomeh, 2008). However, within each nutrient category,
12
13 273 especially proteins, transformations occurred because of microorganism actions.
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15 274

16 275 **Main changes of biological components during fermentation**

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18 276 Figure 2 a) b) and c) show the release of free amino acids (AA), ammonium and free fatty acids
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20 277 (FFA) respectively. For the three indicators, the logistic model of Verhulst fitted well the
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22 278 experimental data. Therefore, we can assume that their production was linked with the usual
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24 279 shape of microorganism growth of *Bacillus*. Production of AA, NH_4^+ and FFA started after about
25
26 280 10 h of fermentation. After an exponential increase, the maximal concentration was achieved
27
28 281 after about 25 h for FFA, and 35 h for NH_4^+ and FFA. AA and NH_4^+ are released by protein
29
30 282 degradation, while FFA comes from triglyceride hydrolysis. The maximal value of AA was
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32 283 almost 12 % which represented one third of the proteins. In their experiments, (Odunfa, 1985)
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34 284 found a level of 8% of AA at the end of fermentation. FFA reached 7% which represented 20%
35
36 285 of the total lipids. The equation parameters of Verhulst are presented in Figure 3. The k values
37
38 286 (equation 1) were higher for AA, then ammonium and lastly FFA which is consistent with our
39
40 287 observations of proteins being consumed before lipids. The sigmoid curvature of free amino
41
42 288 acids and ammonium release (L) was also higher than that of FFA. Therefore, we can say that
43
44 289 during ALBS fermentation, the release of free amino acids occurred first followed by
45
46 290 ammonium because of protein and amino acid catabolism and in a second time lipid
47
48 291 catabolism occurred with the release of FFA. Odunfa (1985) showed that protease activity was
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50 292 higher than that of lipase. He also represented amino acid release of which the trend appeared
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52 293 logistic as well. Release of AA, NH_4^+ as well as FFA influence the flavor of fermented ALBS.
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54 294 Indeed, some AA can contribute to the umami taste (Lioe et al., 2010), while NH_4^+ induces the
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56 295 strong ammonia smelling characteristic of FALBS (Amoa-Awua et al., 2014). At last, FFA are
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58 296 major contributors to the flavor produced from lipolysis (Noronha et al., 2008). FFA and
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60 297 particularly palmitic, stearic and eicosanoic acids that are present in FALBS can produce a fatty
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299 298 flavor (Zhan et al., 2020). Previous studies showed that these three fatty acids were the most
important after linoleic and oleic acids in ALBS (Esse et al., 2021). In addition to this direct

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3 300 contribution to the sensory properties of FALBS, AA and FFA are precursors of other bioactive
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5 301 compounds shown in Figure 2 d) and e) which are biogenic amines (BA) and short chain fatty
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7 302 acids (SCFA). Those two compounds also behaved according to the Verhulst model and were
8
9 303 produced after amino acid release. Indeed, BA and SCFA growth parameters were close with
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11 304 k equal to 0.16 and 0.15 respectively. Therefore, they could be considered as secondary
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13 305 products of amino acids. Mohamed et al. (2001) also showed that BA were produced after AA,
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15 306 which may be interesting as a tool to control fermentation or ripening time of Egyptian salted-
16
17 307 fermented fish (Mohamed et al., 2001; Rabie et al., 2009). SCFA are fatty acids that have one
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19 308 to seven carbons. SCFA maximal content at the end of fermentation was 1.7%. SCFA could
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21 309 come from sugars, proteins and lipids (Akanni et al., 2018; Dursun et al., 2017). These
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23 310 molecules confirm the proteolysis and lipolysis that occur during ALBS fermentation and are
24
25 311 known to contribute significantly to the acidic, sour, and pungent flavor of FALBS (Odunfa,
26
27 312 1985; Omafuvbe et al., 2004). In cheese, a rich protein and lipid product, SCFA contribute
28
29 313 greatly to the flavor and its typicality and could be produced by anaerobic bacterial
30
31 314 fermentation linked to the metabolism of *Bacillus* (Pasvolosky et al., 2014).

315 316 **Release Kinetics of amino acids**

317 The composition in amino acids of ALBS was presented in a previous article (Esse et al., 2021).
318 The major amino acids were glutamic acid (Glu) at 20 %, followed by aspartic acid (Asp) at
319 10%, leucine (Leu) and lysine (Lys) at both 7%, arginine (Arg) and valine (Val) at 6%, alanine
320 (Ala), proline (Pro), glycine (Gly), phenylalanine (Phe) at 5%, serine (Ser), isoleucine (Ile),
321 tyrosine (Tyr), histidine (His), threonine (Thr) at 2 to 4%, and cysteine (Cys), methionine (Met)
322 and ornithine (Orn) at concentrations <2%. Figure 4 shows the release of the different free
323 amino acids. Among all amino acids of the legume, only 13 were significantly released and/or
324 observable during fermentation. To be easily represented, the amino acids were presented on
325 three different figures 4 a), b) and c) in g/100g DB. For all amino acids, the Verhulst function
326 fitted the experimental data well which is consistent with previous observations (Odunfa,
327 1985). Among acid AA, Glu was the most produced reaching nearly 1.5 % DB which is
328 consistent with the initial composition of the seed. This observation was already done and
329 Odunfa (1985) stated that the release of Glu was particularly interesting in FALBS since Glu is
330 a flavor enhancer. The author explained this high release by the ability of *Bacillus* species to
331 accumulate Glu extracellularly. Cyst and Met were among the least produced which is

1
2
3 332 coherent with the low values of sulfur AA in legumes (Krishnan, 2005). However, Asp release
4
5 333 was as low as the sulfur AA which was not consistent with the initial composition of the seed.
6
7 334 Çalik et al. (2000) showed that *Bacillus licheniformis* was able to express the protease activity
8
9 335 differently as a function of the oxygenation condition. In addition, the types of amino acids
10
11 336 that were excreted to the fermentation medium also depended on the oxygenation
12
13 337 conditions. The least release of amino acids of the aspartic acid-group could be explained by
14
15 338 the need of this AA in *Bacillus* cells and a low asparagine synthetase expression (EC. 6.3.1.4)
16
17 339 or high-aspartate kinase (EC. 2.7.2.4) activity (Çalik et al., 2000). Another non-observable AA
18
19 340 was Arg, although it was the fifth in terms of initial quantity. However, Çalik et al. (2000) did
20
21 341 not observe its non-excretion. With Cys (Fig. 4a), Phe and Lys (Fig 4c), Leu, Ile, Val, and Trp (fig
22
23 342 4 b) are essential amino acids. Leu, Val, Phe, and Lys releases were close to 1% at the end of
24
25 343 fermentation. As their initial content in the seed was about 3% DB, nearly 30% of the amino
26
27 344 acids were in their free form at the end of fermentation which may be beneficial for a good
28
29 345 assimilation of these essential amino acids (Ito et al., 2018; LU et al., 2017). Odunfa (1985)
30
31 346 also observed a high release of valine. Recommended daily amounts for these essential AA
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33 347 are 25 to 40 mg/kg/day as a function of AA (FAO/WHO/UNU, 2007). Therefore, the need for
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35 348 people weighing 60-80 kg is 1.5 to 3 g/day. Assuming one portion of this condiment weighs 20
36
37 349 g, a meal can provide 20 to 30% of recommended daily intake. The non-essential AA Tyr also
38
39 350 reached 1% while His and Orn reached 0.5%. At the end of fermentation, these AA were at 80,
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41 351 40 and 60 % in their free form respectively. Therefore, these compounds may be not crucial
42
43 352 for *Bacillus* cell metabolism or were favorably excreted.

353

354 **Release Kinetics of biogenic amines**

45 355 The main BA found and presented in Fig. 4d were by order of importance spermidine (Sper),
46
47 356 β -phenylethylamine (Phen), histamine (Hist), putrescine (Put), and cadaverine (Cad). Sper
48
49 357 comes from from Arg, Phen from Phe, Hist from His, Put from Arg and Cad from Lys. The final
50
51 358 levels were 200, 90, 65, 30, 40 ppm respectively leading to a total close to 400 ppm (Fig. 4).
52
53 359 This total value reached the limit defined by the European regulation for products having
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55 360 undergone an enzymatic maturation treatment. Therefore, BA production must be monitored
56
57 361 carefully during ALBS fermentation. The high content of Sper may be due to the high
58
59 362 conversion from Arg, and may explain the absence of this AA in Fig 4. An intermediary
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61 363 compound of this conversion can be Put. In cured ham, Arg content was low due to its

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2
3 364 hydrolysis to ammonia and Orn, followed by decarboxylation to Put that was most largely
4
5 365 present in aged hams (Virgili et al., 2007). Also, it was suggested that the amounts of Put and
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7 366 other BA in hams could be inversely related to the levels of their amino acid precursors and
8
9 367 that some BA generation may be regarded as the last step of the decarboxylation-deamination
10
11 368 mechanism starting with Arg (Alfaia et al., 2004). For the kinetic aspect, the most rapidly
12
13 369 produced free AA were Phe, His, Met, Glu, and Ile with $k > 0.5$ (Figure 3). Regarding BA, the
14
15 370 most rapidly produced were Hist, Cad, and Put respectively and their maximal concentration
16
17 371 was reached at the early stage of fermentation, that is to say after about 10 hours. Hist
18
19 372 production was highly correlated to Hist release. Cad was produced more rapidly than its
20
21 373 precursor but rapidly reached a maximum. Put was probably produced very rapidly from Arg,
22
23 374 but the quantity of this BA was low, probably due to its rapid conversion to Sper. Phen and
24
25 375 Sper were produced in the second part of fermentation and their maximal value was reached
26
27 376 after 30 hours of fermentation. It is important to remark that Sper and Hist did not fit Verhulst
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29 377 model after reaching their maximal content. Indeed, their content decreased suggesting a
30
31 378 potential degradation. This reaction may be conducted by microorganisms including certain
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33 379 strains of *Bacillus* (Alvarez & Moreno-Arribas, 2014; Lee et al., 2016).

380

381 **Release Kinetics of Short chain fatty acids**

382 The most odorant SCFA are present in Fig 5a. They were composed of formic (C1), acetic (C2),
383 butyric (C4) and the branched 3-methylbutanoic acid also called isovaleric acid (C5). Their
384 respective odor threshold in air in ppm is $8, 5 \cdot 10^{-3}, 3 \cdot 10^{-3}$ and $8 \cdot 10^{-5}$ respectively. Formic acid
385 odor is described as sharp acetic and fruity fermented, that of acetic acid, sour vinegar, while
386 butyric acid odor is cheesy, rancid and that of isovaleric acid, pungent. Less odorant SCFA are
387 on Fig 5b with lactic, oxalic, malic, tartaric, and citric acids (C2, C3, C4 and C6 respectively). In
388 addition to carboxylic function, lactic, malic, and tartaric acids have one or two alcohol
389 functions and oxalic and citric acids are respectively di- and tri- acids which may contribute to
390 decreasing their volatility and therefore increasing their odor threshold. An interesting point
391 is that the odorant SCFA were produced during fermentation while the others decreased
392 (tartrate and citrate being the most abundant in the raw seeds) or increased and then
393 decreased (oxalate, malate, and lactate). SCFA may have different origins. Butyric acid may
394 come from FFA like in cheese (Faccia et al., 2018; Poveda, Justa María et al., 2008). Acetic acid
395 may come from the oxidation of ethanol during fermentation while Isovaleric acid (3-

1
2
3 396 methylbutanoic acid) from branched amino acids like valine, leucine, and isoleucine. Among
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5 397 volatile acids, 2-methylbutanoic and 3-methylbutanoic acids were found to contribute largely
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7 398 to the typical aroma of cheese or fermented ALBS (Akanni et al., 2018; Poveda, Justa María et
8
9 399 al., 2008). Also, the Verhulst function described odorant SCFA release well. The first acids
10
11 400 produced were acetic and formic acids at a slow rate followed by isovaleric and then butyric
12
13 401 acids that exhibited a more logistic shape (higher L parameter) and slightly higher rates of
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15 402 formation (Figure 3). This order of release is concomitant with the successive consumption of
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17 403 carbohydrates, proteins and lipids as substrates for *Bacillus*. Therefore, SCFA are prevalent
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19 404 compounds released throughout ALBS fermentation but their individual prevalence could be
20
21 405 modulated by the macro-composition of ALBS and by fermentation time. This modulation
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23 406 could markedly influence odor but also taste of FALBS.
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25 408 **Evolution of the different VOCs families**

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27 409 A total of 111 volatile organic compounds (VOCs) were collected from the different samples:
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29 410 raw seeds, and cotyledons during different times of fermentation which is consistent with the
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31 411 number of 116-125 found in previous studies on similar products (Akanni et al., 2018; Ouoba
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33 412 et al., 2005). A total of 13 pyrazines, 9 aldehydes, 11 ketones, 15 esters, 16 acids, 15 alcohols,
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35 413 1 alkene, 1 amine, 2 pyridines, 8 benzenes, 2 phenols, 2 sulfur compounds, 6 amides, 1 furan
36
37 414 and 9 others were found. 60 of the most frequent VOCs constituting the 'volatolom' of our
38
39 415 samples of FALBS are presented in Table 1. These compounds were found in other protein-
40
41 416 rich products like beans, cheese, milk, shrimp, or cured fish such as anchovies or fermented
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43 417 products such as coco beans, wine, or free amino acid-rich products such as mushrooms
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45 418 (Aisala et al., 2019; Akanni et al., 2018; Amoa-Awua et al., 2014; Dehaut et al., 2014; Dursun
46
47 419 et al., 2017; Kouakou-Kouamé et al., 2020). During cocoa bean fermentation, there is a
48
49 420 succession of VOCs families as a function of fermentation time due to microbial succession of
50
51 421 a wide range of yeasts, lactic acid and acetic acid bacteria (Kuhnert et al., 2020; Schwan, 1998).
52
53 422 In the case of ALBS fermentation, flora is supposed to be more uniform and mainly composed
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55 423 of *Bacillus* conducting an alkaline fermentation. Therefore, the study of the release kinetics of
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57 424 VOCs in FALBS may contribute to explaining the metabolism behavior of *Bacillus* and its
58
59 425 relationship with the final aroma.
60
61 426 Figures 6 and 7 present the evolution of the different families of VOCs during production of
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63 427 fermented ALBS. The main constituents of raw seeds were alcohols (~30%), acids and esters.

1
2
3 428 After cooking, a marked change occurred with an increase of pyrazines from 10 to nearly 60
4
5 429 % of VOCs. This increase was certainly due to the Maillard reactions during cooking but also
6
7 430 the leaking of some hydrophilic or thermosensitive compounds like alcohols and esters while
8
9 431 the acids remained stable. After fermentation, a second marked change was observed with
10
11 432 the prevalence of acids that account for more than 45% of the VOCs. Pyrazines still remained
12
13 433 at a high concentration as can be observed in Figure 5a). Their production was not
14
15 434 monotonous. Indeed, their content increased at the beginning of fermentation but decreased
16
17 435 after 12 h so their final quantity was not significantly different from that at the beginning. The
18
19 436 total of VOCs increased significantly during fermentation ($p < 0.05$) and were strongly positively
20
21 437 correlated to the production of acids ($p < 0.05$). As can be seen in Fig 5b), another category of
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23 438 product that rose markedly during fermentation were amides ($p < 0.05$). The other compounds:
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25 439 alcohols, aldehydes, esters, ketones, and furans, decreased at the beginning of fermentation
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27 440 (specially for aldehydes, esters and ketones) but were produced after 24 h. However, their
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29 441 final content was still inferior or not significantly different to $t=0$ h. Sulfur compounds were in
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31 442 small quantity and did not evolve significantly.

32 443

32 444 **Succession of VOCs during fermentation**

34 445 To be more specific about the different molecules that evolved significantly during FALBS
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36 446 production, ANOVA analysis was carried out for all VOCs followed by PCA only on the
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38 447 molecules that discriminated the fermentation time. The results of PCA are presented in Fig.
39
40 448 8. The projection of different samples showed that PCA enabled the discrimination of raw
41
42 449 seeds (RS) from the cooked cotyledons before fermentation (0 H). The typical VOCs that were
43
44 450 positively correlated with the raw seeds were: ethanol, hexanol, carbon disulfide, butanal and
45
46 451 4,6,6-trimethylbicyclo[3.1.1]hept-3-en-2-one. This last molecule is also called verbenone and
47
48 452 has a camphoreous odor (Table 1). Alcohols are typical of the raw seeds and among them 1-
49
50 453 hexanol which is responsible for green notes than can be found in peas for instance (C. Zhang
51
52 454 et al., 2020).

52 455 These compounds were lost significantly during cooking and may therefore contribute only
53
54 456 slightly to the flavor of the final product. However, some VOCs were positively correlated with
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56 457 $t=0$ hours and therefore may have appeared during cooking. Among them, one pyrazine, 2,3-
57
58 458 Dimethyl-5-ethylpyrazine that has a strong roasted aroma, could have come from the long
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60 459 cooking period (Qian & Reineccius, 2002). We also found xylene which is an aromatic

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2
3 460 hydrocarbon and may be considered as contamination arising from wood-cooking (Ciecierska,
4 461 2020). Lastly, 4-methanol-himidazole is a late Maillard compound (Licht et al., 1992). These
5 462 two last compounds are cyclic neo-formed compounds produced because of the very long
6 463 cooking time that may have an adverse effect on health at high concentrations.

7 464 The compounds positively correlated with the first hours of fermentation were aldehyde
8 465 (benzaldehyde and 2,5-dihydroxybenzaldehyde), esters (ethyl acetate), ketones (acetoin, 2,3
9 466 butadione, 2-decanone and 2-octanone), 2-pentyl-thiophene and 2-pentyl-Furan. 2-
10 467 pentylfuran and 1-octen-3-ol compounds are known for contributing to the green and beany
11 468 aroma of cooked beans (Owens et al., 1997). They were also found in sugar-rich fermented
12 469 products such as grape wine, alcoholic beverages made from sorghum and particularly fruit
13 470 wines involving yeasts (Feng et al., 2015; Song et al., 2019; H. Zhang et al., 2021). Therefore,
14 471 these compounds may come from the cooking process and the early stage of fermentation
15 472 when *Bacillus* consume sugars and provide fruity notes. Owens et al. (1997) reported that
16 473 *Bacillus subtilis* when grown in sugars excrete alpha-acetolactate that is later decarboxylated
17 474 to acetoin which is consumed when carbohydrate depletion occurs. He also noticed that the
18 475 other ketones produced may be the result of lipid and/or amino acid degradation. However,
19 476 these compounds have a relative high odor threshold so they may only contribute slightly to
20 477 the aroma of FALBS.

21 478 The other group that appeared during fermentation were pyrazines. Their maximum content
22 479 was achieved between 10 to 15 h of fermentation. The main pyrazines by order of quantity
23 480 were 2,5-dimethyl-pyrazine, methyl-pyrazines, ethyl-pyrazine, 2-ethyl-6-methyl-pyrazine, and
24 481 2,6-dimethyl-pyrazine. Pyrazines in general and 2,5-dimethyl-pyrazine in particular were also
25 482 found during *Bacillus* fermentations (Akanni et al., 2018; Besson et al., 1997; Ouoba et al.,
26 483 2005; Owens et al., 1997). Owen et al. (1997) also noticed that the free amino acids, the
27 484 alkaline pH and the incubation temperature (40 °C) favored the production of pyrazines.
28 485 Ouoba et al. (2003), during fermentation in controlled conditions in the laboratory with
29 486 different *Bacillus* starters, found that the profile of pyrazines, ketones, aldehydes and esters
30 487 was dependent on the *Bacillus* species. In our case, we observed that the amount of pyrazine
31 488 significantly decreased at the end of fermentation until it reached the initial level just after
32 489 cooking. This decrease was also noticed but not explained by Owen et al. (1997). Therefore,
33 490 the roasted notes of the final product due to pyrazines may depend on the fermentation time.

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3 491 Contrary to pyrazines, acids were released constantly throughout fermentation. The main acid
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5 492 in quantity was 3-methyl-butanoic acid (isovalerate) as observed previously, followed by 2-
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7 493 methyl-butanoic acid. In addition, with these two volatile acids, we found 2-methyl-2-butenic
8
9 494 acid also called tiglic acid. It is supposed to be formed from isoleucine and presents ripe fruit
10
11 495 notes (Y. Zhang et al., 2018). Simultaneously with acids, esters were formed. Akanni et al.
12
13 496 (2018) noticed that esters have been known to constitute a major volatile compound in
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15 497 African fermented condiments. The esters are presumably the consequence of chemical
16
17 498 reactions between acids and alcohols and produce floral and fruity notes. Logically, the main
18
19 499 esters found were that of the main acids, i.e., 3-methyl-butanoic ester, 2-methyl-butanoic
20
21 500 ester, and acetic acid-methyl ester. The other secondary products released were amides that
22
23 501 were correlated to the last hours of fermentation. The main amides were acetamide,
24
25 502 propanamide, butanamide, 2-methyl-propanamide and 3-methyl-butanamide. These
26
27 503 molecules may come from the reaction between free amino acids and acids and were
28
29 504 observed in cocoa beans and wine (Kuhnert et al., 2020; Schueuermann et al., 2016).
30
31 505 Therefore, acids are involved in different reactions with alcohols and free amino acids which
32
33 506 may explain the slight decrease of certain acids like formate or malate at the end of
34
35 507 fermentation as observed in Fig 5a and b. Lastly, two other VOCs were found at the very final
36
37 508 stage, which were phenol, a degradation product of tyrosine, and 2-piperidinone found in
38
39 509 fermented bean (Kim & Chung, 2008; Mathus et al., 1995). Phenol is an irritant and considered
40
41 510 objectionable while 2-piperidinone, is ammoniacal and pepper-like (Maraval et al., 2008).
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43 511 Again, specific monitoring of the last hours of fermentation could be of interest to control the
44
45 512 concentration of these two last compounds.

513

514 Conclusion

515 This paper showed the release of different bioactive compounds during spontaneous FALBS
516 production that have sensory, nutritional and toxicity impacts. During fermentation, VOCs
517 number was multiplied by a ten-fold which confirm the very important impact of this step on
518 the final quality of FALBS. This is because ALBS is a legume rich in proteins and lipids and
519 because *Bacillus* species involved in this fermentation are able to metabolize them with their
520 proteases and lipases. The results also highlighted the important kinetic aspects of this
521 fermentation. The main early products are a result of protein catabolism and are free amino
522 acids with glutamic acid presenting the highest release and some biogenic amines like

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2
3 523 cadaverine, putrescine and histamine. In a second phase, SCFA and FFA releases occurred.
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5 524 Particularly, the cleavage of branched-chain amino acids produced isovaleric acid which was
6
7 525 the main acid produced. The odorant butyric acid was produced later and at a lower content
8
9 526 certainly due to triglyceride hydrolysis. Therefore, we can conclude that the first 20 hours of
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11 527 fermentation of ALBS lead to maximal contents in acids contributing significantly to the “sour,
12
13 528 pungent and cheesy” notes of FALBS. During this phase, pyrazines were also produced in high
14
15 529 certainly giving roasted notes to FALBS. Lastly, esters, amides and phenol were produced from
16
17 530 complex reactions between amino acids, alcohols and acids produced in the previous phases.
18
19 531 Maximal ammonia content was achieved during this phase increasing the final pH of FALBS.
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21
22 533 The perspectives of this research will be to work firstly on the raw material. Indeed, raw seed
23
24 534 composition and their pre-treatment (cooking) are important to monitor the proportion of
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26 535 carbohydrates, proteins and lipids that are the substrates of fermentation. Secondly, it would
27
28 536 be important to deepen the link between *Bacillus* species growth and the bioactive
29
30 537 compounds release during fermentation. To do so, a controlled fermentation should be done
31
32 538 and *Bacillus* population, enzyme activities, AA, BA, SCFA, and VOCs should be monitored as a
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34 539 function of fermentation time. Lastly, the link between fermentation conditions (temperature,
35
36 540 aeration) and these dynamics would be very crucial to make in order to understand the
37
38 541 possible levers of bioactive compounds modulation in the final product.
39

40

41 543 **Conflicts of interest statement**

42 544 The authors declare that they have no known competing financial interests or personal
43
44 545 relationships that could have appeared to influence the work reported in this paper.

45 546

46 547 **Figure and Table list**

47 548 **Figure 1. Evolution of macro-constituents during fermentation of ALBS**

48
49 549 **Figure 2. Release in g/100 g DB of a) free amino acids (AA), b) ammonium (NH₄⁺), c) free fatty
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51 550 acids (FFA), d) biogenic amines (BA), e) short chain fatty acids (SCFA) during ALBS
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53 551 fermentation. Error bars represent the standard deviation (n = 3) and lines represent the
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55 552 modelled data.**

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57
58 553 **Figure 3. Verhulst equation parameters for all compound releases during fermentation of**
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60 554 **ALBS**

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3 555 **Figure 4. Release of a), b), c) free amino acids and d) biogenic amines during ALBS**
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5 556 **fermentation. Error bars represent the standard deviation (n = 3) and lines represent the**
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7 557 **modelled data.**

8 558 **Figure 5. Release of a) odorant SCFA and d) less odorant SCFA during ALBS fermentation.**
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10 559 **Error bars represent the standard deviation (n = 3) and plain lines represent the modelled**
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12 560 **data.**

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14 561 **Figure 6. Evolution of the different VOCs according to the chemical family during fermented**
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16 562 **ALBS production**

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18 563 **Figure 7. Evolution of a) total VOCs, acids and pyrazines and b) other VOCs families during**
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20 564 **ALBS fermentation**

21 565 **Figure 8. PCA on molecules that evolve significantly during FALBS production (RS: raw seed)**

22
23 566 **Table 1. Most frequent VOCs produced during fermented ALB production**

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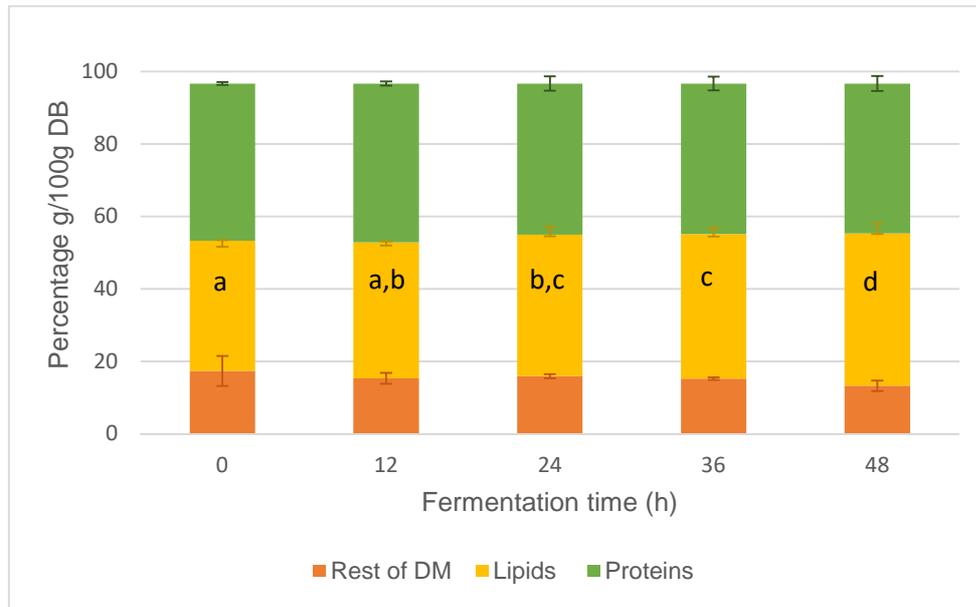
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Values bearing different letters for the same parameter are significantly different ($p < 0.05$).

Figure 1. Evolution of macro-constituents during fermentation of ALBS

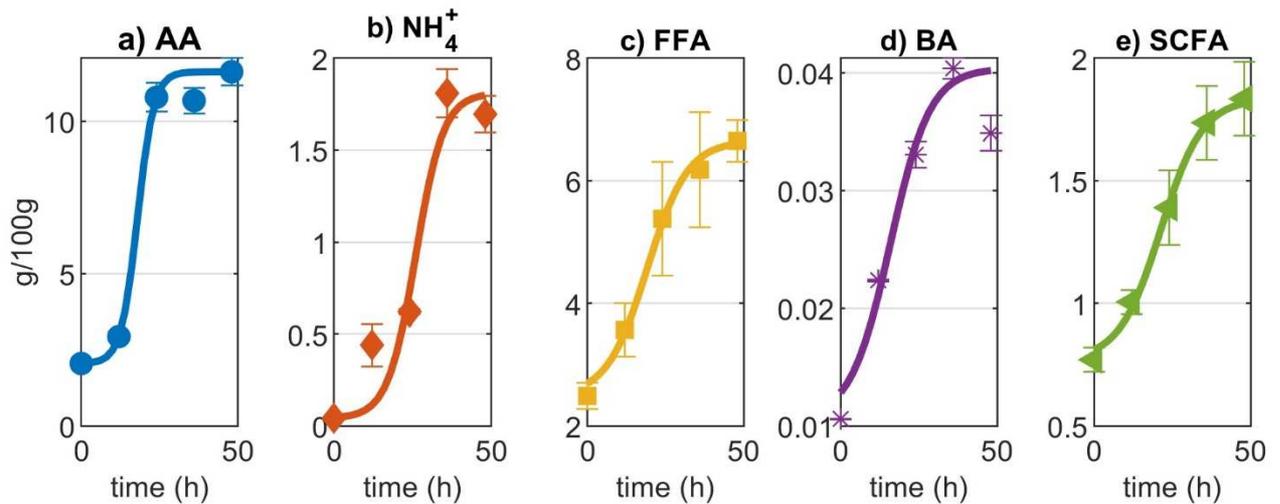


Figure 2. Release in g/100 g DB of a) free amino acids (AA), b) ammonium (NH₄⁺), c) free fatty acids (FFA), d) biogenic amines (BA), e) short chain fatty acids (SCFA) during ALBS fermentation. Error bars represent the standard deviation (n = 3) and lines represent the modelled data.

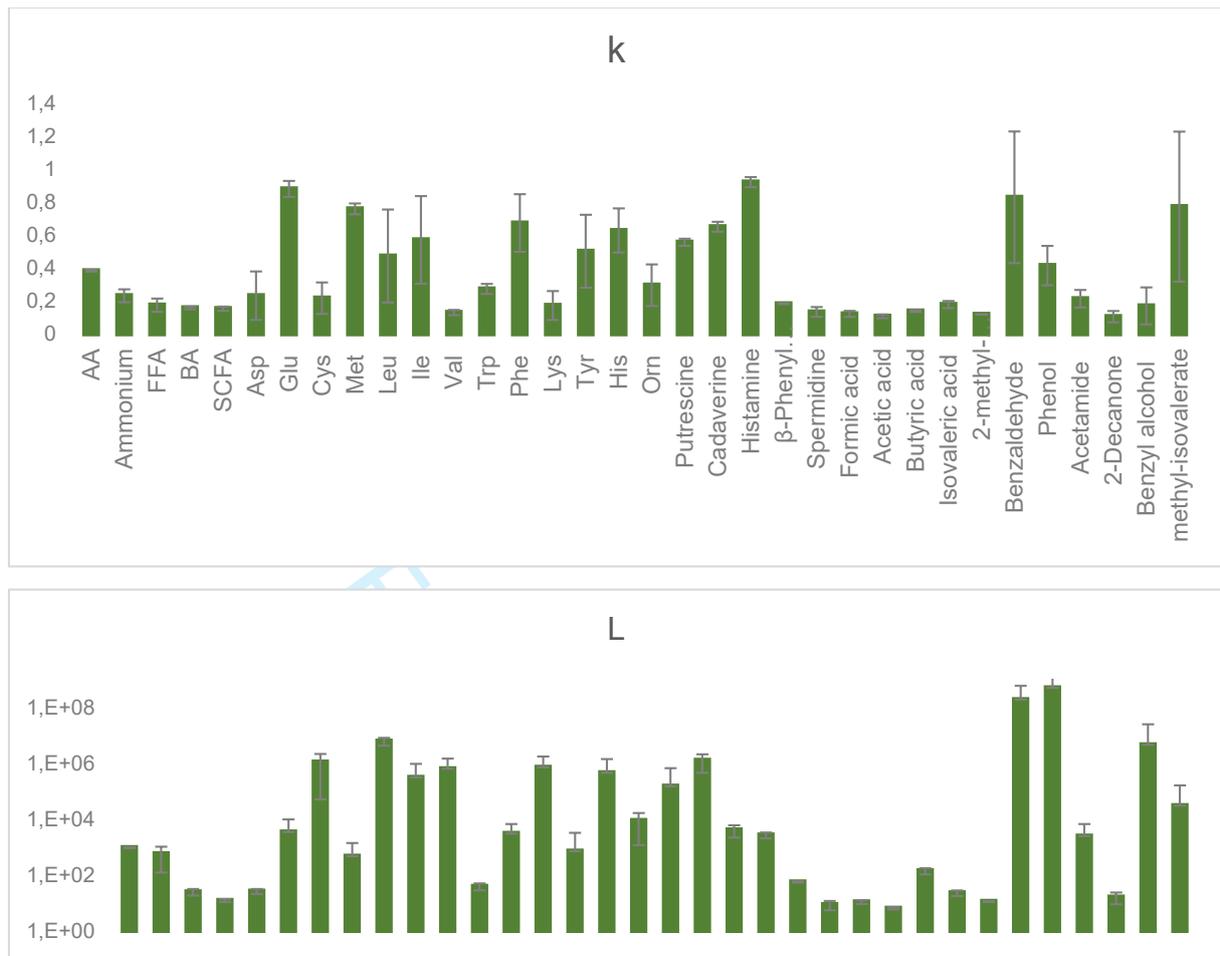


Figure 3. Verhulst equation parameters for all compound releases during fermentation of ALBS

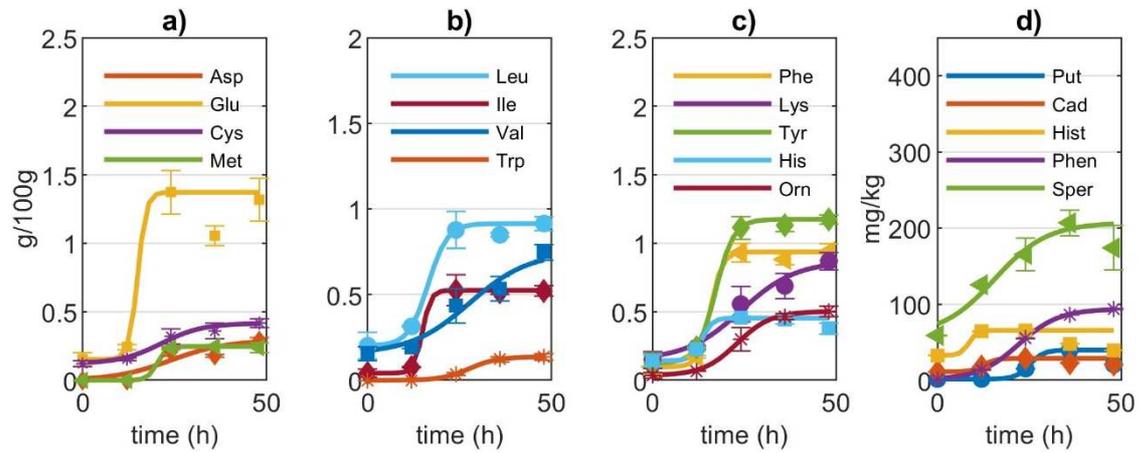


Figure 4. Release of a), b), c) free amino acids and d) biogenic amines during ALBS fermentation. Error bars represent the standard deviation (n = 3) and lines represent the modelled data.

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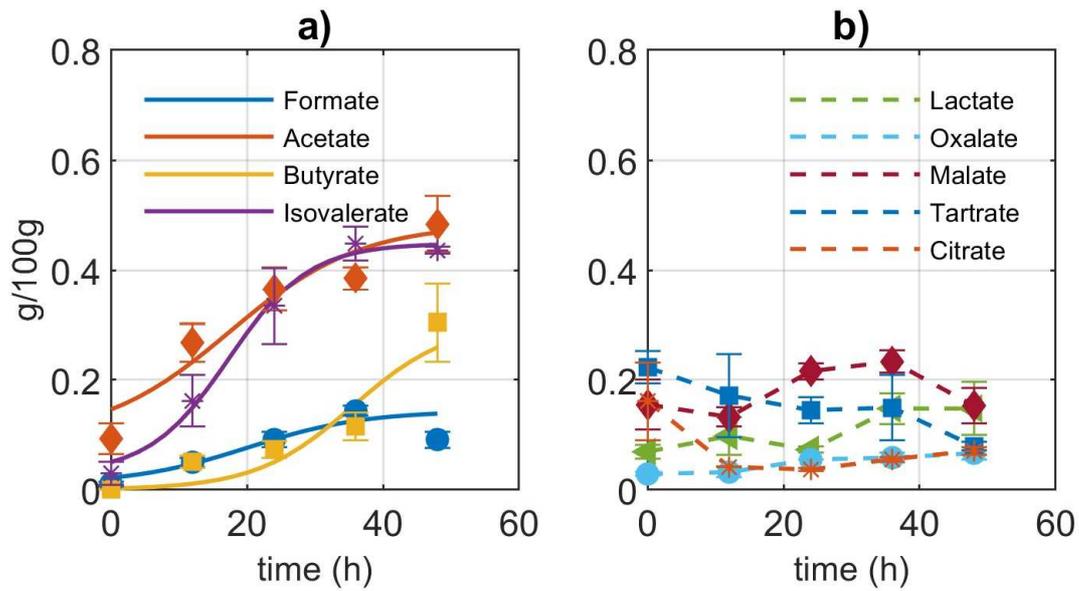


Figure 5. Release of a) odorant SCFA and d) less odorant SCFA during ALBS fermentation. Error bars represent the standard deviation (n = 3) and plain lines represent the modelled data.

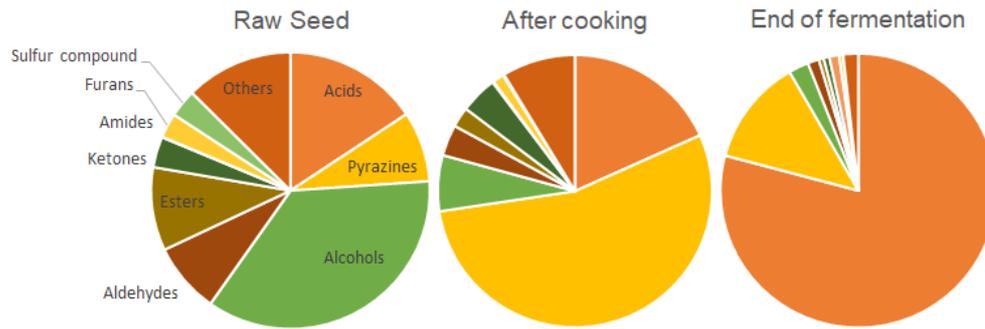


Figure 6. Evolution of the different VOCs according to the chemical family during fermented ALBS production

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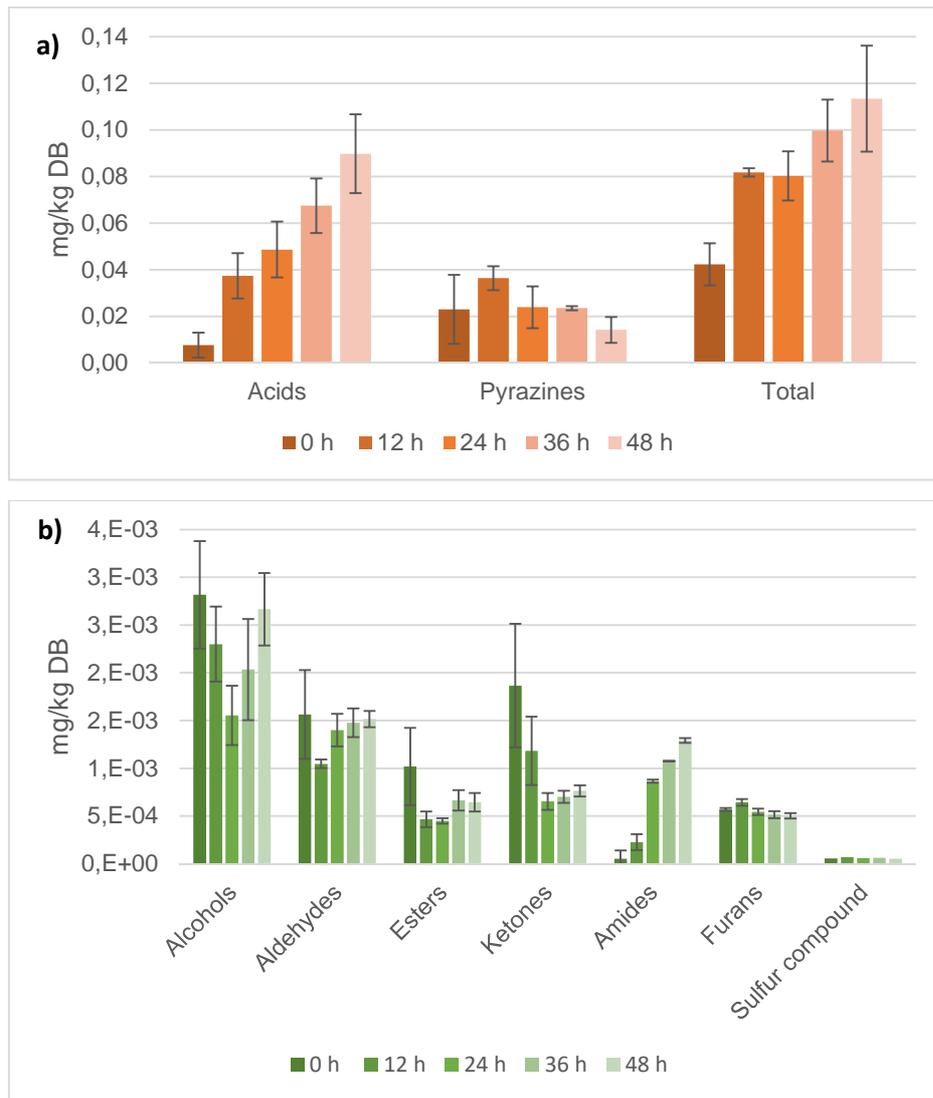


Figure 7. Evolution of a) total VOCs, acids and pyrazines and b) other VOCs families during ALBS fermentation

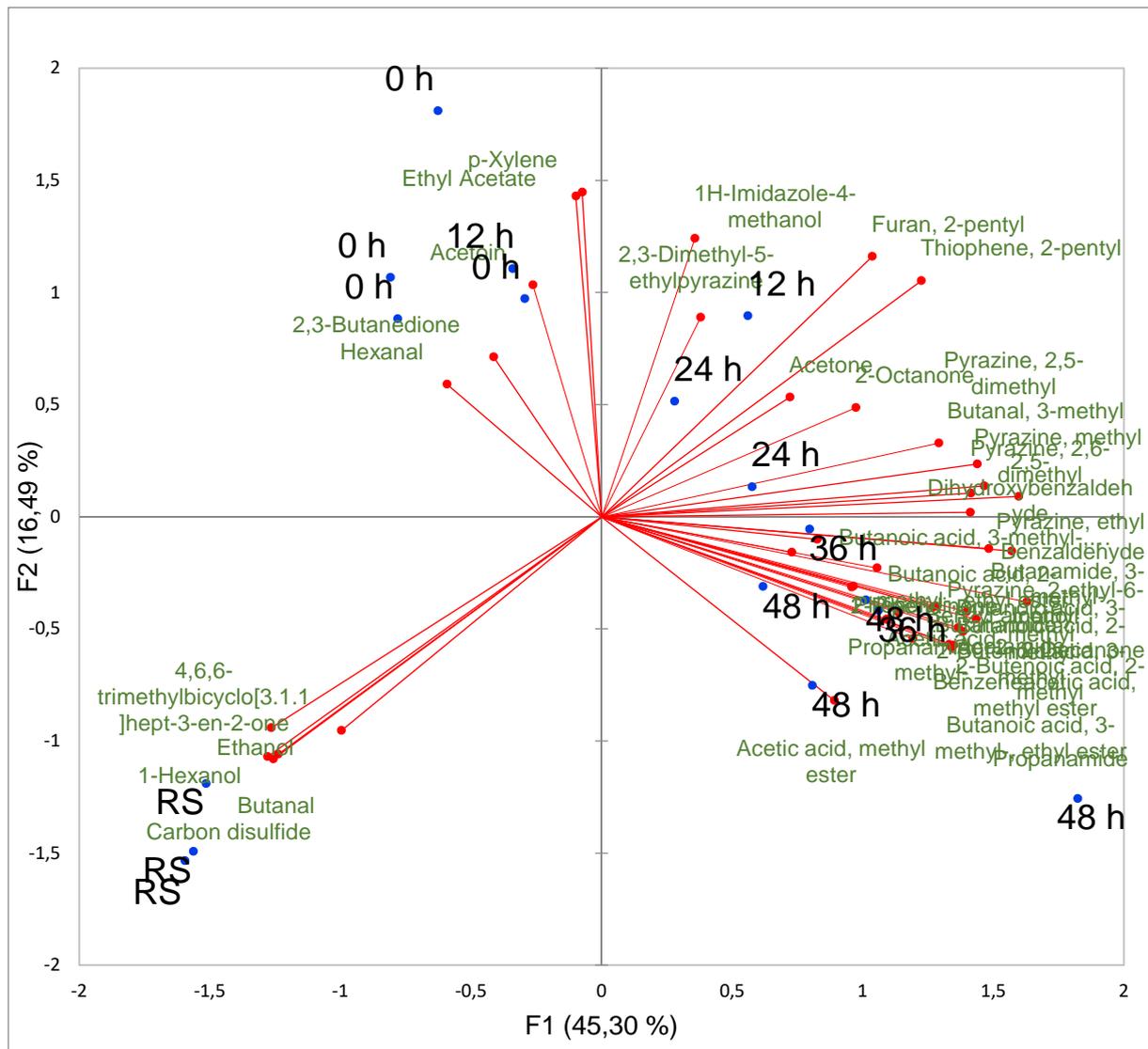


Figure 8. PCA on molecules that evolve significantly during FALBS production (RS: raw seed)

	<i>Odor</i>	<i>Assumed bioreaction origine</i>	<i>Odor threshold (µg/kg) in water</i>	<i>Product</i>	<i>References</i>	<i>Present in raw ALBS</i>	
<i>Acids</i>	3-methyl-butanoic acid (isovaleric acid)	acidic, sour, pungent,	produced from branched-chained amino acids such as valine, leucine, and isoleucine during fermentation	120	Bambara bean	Akanni (2018)	
	2-methyl-butanoic acid			100	Bambara bean	Akanni (2018)	
	2-methyl butenoic (tiglic acid)	Ripe fruit			Beverage fermented with shiitake	Zhang (2018)	
	2-methyl-propanoic acid (isobutyric acid)	Meat, cheese	-		Adjuevan	Kouakou-Kouame (2020)	
	Ethanoic acid (Acetic acid)	sour, vinegar note	Oxidation of ethanol		Bambara bean	Akanni (2018)	
	Butanoic acid (butyric acid)	rancid, cheesy			Cheese	Delgado (2011)	
	Propanoic acid (Propionic acid)	pungent acidic cheesy vinegar	hydrolysis triglycerides by lipases		Milk	Dursun (2017)	
<i>Pyrazines</i>	2,5-dimethyl-pyrazine	chocolate, roasted nut		800			
	trimethyl-pyrazine	roasted, burnt notes	from free amino acid liberation and heating	400	Bambara bean	Akanni (2018)	
	2,6-dimethyl-pyrazine	chocolate, roasted nut, fried potatoes		200			
	tetramethyl-pyrazine	nutty, musty, chocolate		1000			
<i>Alcohols</i>	1-Hexanol	Herbal, fusel oil, alcoholic	-	20000	*	yes	
	Phenylethyl Alcohol	Floral, rose, honey	-	20000	*		
	1-Octen-3-ol	mushroom and fermented-like odor	oxidation of linoleic acid		Bambara bean, Adjuevan	Akanni (2018), Kouakou-Kouame (2020)	
	1-Octanol	waxy, paint jar			mushrooms	Aisala (2019)	
	3-Octen-1-ol	fatty fruity herbal					
	Ethanol	Alcoholic	fermentation of sugars		bambara	Akanni (2018)	yes
	Benzyl alcohol	pleasant, fruity	-		bambara	Akanni (2018)	
	2-Octen-1-ol	Dust, cement, mushroom	reduction of aldehydes		Milk	Dursun (2017)	
<i>Aldehydes</i>	2,3-Butanediol	rubber, fruity, creamy, and buttery	produced from branched-chained amino acids		Bambara bean	Akanni (2018)	yes
	Benzaldehyde	pleasant, sweet, aromatic note	cysteine heating, heat generated compound, amino-acids catabolism	350	Bambara bean milk, anchovies	Akanni (2018), Dursun, Dehaut (2014)	
	Butanal (butyraldehyde)	Aldehydic, fruity	oxidative cleavage of lipids		African locust bean	Ouoba (2015)	yes
	Hexanal	green odor, fat		4.5	bambara, African locust bean, anchovies, Adjuevan	Akanni (2018), Ouoba (2015), Dehaut (2014),	yes

						Kouakou-Kouame (2020)
	3-methyl-butanal (isovaleraldehyde)	Malty, almond	oxidative cleavage of lipids, amino acid catabolism (L-Leucine)	1.1	African locust bean, anchovies	Ouoba (2015) Dehaut (2014)
<i>Esters</i>	Benzeneacetic acid methyl ester					
	Ethyl acetate					
	Acetic acid methyl ester					yes
	Methyl isovalerate - ester					
	Butanoic acid 2-methyl- ester	Floral and fruity notes	esters comes from chemical reactions between microbial acidic and alcoholic metabolites		Bambara , Adjuevan	Akanni (2018), Kouakou-Kouame (2020)
	Butanoic acid methyl ester					yes
	Acetic acid butyl ester					yes
	Butanoic acid butyl ester					yes
<i>Ketones</i>	Acetoin	butter-like aroma	Product of carbohydrates metabolism	800	bambara	Akanni (2018),
	2-Decanone	orange like floral*				
	2-Octanone	butter-like aroma*			Fruit wine	Feng (2015) yes
	Benzyl methyl ketone					
	Acetone		products of lipids and or amino acids degradation		ALBS	Ouoba (2015)
	Acetophenone Results	sweet and floral odors, almond			Adjuevan	Kouakou-Kouame (2020)
	2,3-Butanedione	toffee, butter, candy	Fermentation of sugars		anchovies, mushrooms	Dehaut (2014), Aisala (2019) yes
	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl	Camphoreous *				yes
<i>Amides</i>	Butanamide					
	Acetamide				Cocoa beans	Kuhnert (2020)
	N-(3-Methylbutyl) acetamide		reactions of peptides with Acids		Wine	Schueuermann (2016)
	Propanamide					
	2-methyl-propanamide,					
	3-methyl-butanamide					
<i>Others</i>	Phenol	sickening, sweet and irritating	heat-generated compounds		Milk	Dursun (2017)
	1-Butanamine					
	2-pentyl-Furan	fruity green earthy beany vegetable *			Baijiu	Zhang (2021) yes

Carbon disulfide	pungent and sulfury odors	oxidation of the reducing sulfur compounds	bambara, Milk	Akanni (2018), Dursun (2017)	yes
1,3-dimethyl-benzene,					yes
Toluene					yes
2-pentyl-thiophene,	Wine	thermal degradation of lipid oxidation products with amino acids and reducing sugars, cysteine	Model media	Song (2019)	
Oxime methoxy-phenyl		reaction aldehydes or ketones with a nitrogen-containing reducing agent in a weakly acidic medium	Milk	Dursun (2017)	yes
Ethylbenzene	gasoline				yes
3-Acetyl-1H-pyrroline	popcorn, basmati rice		Tomato pepper pomace, mushrooms	Aisala (2019)	
Glycerin					yes
Trimethyl-oxazole	Nutty, sweet, green				
Indole	manure, burnt		Adjuevan	Kouakou-Kouame (2020)	yes
2-Piperidinone	ammoniacal, pepper-like		mushrooms	Aisala (2019)	
D-Limonene	orange*				

*<http://www.thegoodscentcompany.com/data/rw1007681.html>

Table 1. Most frequent VOCs produced during fermented ALB production