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1 **Improved processing for the production of cereal-based fermented**
2 **porridge enriched in folate using selected lactic acid bacteria and a back**
3 **slopping process**

4
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18

19 **Abstract**

20 The objective was to study the effect of two selected lactic acid bacteria, *Lactobacillus*
21 *plantarum* 6.2 and *Lactobacillus fermentum* 8.2, on folate production in a cereal-based
22 fermented porridge called *ben-saalga*. We profited from previous improvements in
23 processing to produce porridges with higher energy content, by including a combination of
24 precooking and inoculation with amylolytic strains (*Lactobacillus plantarum* A6 or
25 *Lactobacillus fermentum* MW2), which we combined with the folate producing strains. For
26 comparison with the action of natural microbiota, fermentation was performed by traditional
27 and back slopping process. Folate contents were determined microbiologically. Porridges
28 prepared with starter cultures *L. plantarum* 6.2 + *L. fermentum* MW2 or *L. fermentum* 8.2 +
29 *L. plantarum* A6 had significantly higher ($p < 0.05$) folate contents (7.1 and 7.3 $\mu\text{g}/100$ g
30 fresh matter respectively) than the porridge prepared using the traditional process (4.2 $\mu\text{g}/100$
31 g fresh matter). Back slopping also enabled an interesting increase in folate contents (6.1
32 $\mu\text{g}/100$ g fresh matter, $p < 0.05$). Five minutes of cooking had no significant impact on folate
33 contents of the porridges. These results underline the feasibility of new ways to produce
34 folate rich foods available to the poorest populations using local materials with slight
35 modification of the processes.

36

37 **Keywords:** Cereal, Fermentation, Folate, *Lactobacillus fermentum*, *Lactobacillus*
38 *plantarum*

39

40 **1. Introduction**

41 Folate, or vitamin B9, is a crucial component in maintaining the individual's health over
42 the course of life (Iyer & Tomar, 2009). Folate is necessary for basic cellular functions
43 involved in providing one-carbon groups for several essential reactions related to amino acid
44 metabolism, nucleotide biosynthesis, and methylation cycle (Iyer & Tomar, 2009). Unlike
45 plants and micro-organisms, humans do not have a metabolic system able to synthesize *de*
46 *novo* folate, which must be acquired entirely from dietary sources (Zhao, Matherly, &
47 Goldman, 2009). Insufficient dietary intake of folate is associated with folate deficiency,
48 which can cause megaloblastic anaemia and foetal neural tube defects (Iyer & Tomar, 2009).
49 Folate deficiency is very frequent in many countries worldwide (McLean, de Benoist, &
50 Allen, 2008; Youngblood et al., 2013). In Africa, folate deficiency is related to the low
51 dietary diversity and the poor energy and nutrient density of complementary foods for
52 infants (Dewey & Brown, 2003; McNulty, 1995). Indeed, in many African countries, the
53 most common foods used as complements to breast feeding are traditional cereal-based
54 porridges (Dop & Benbouzid, 1999) which rarely fulfil the infant's nutritional needs as they
55 have very low energy and nutrient densities (Mouquet-Rivier et al., 2008). The porridges can
56 also be consumed after fermentation, which can improve their overall nutritional quality
57 (Singh, Rehal, Kaur, & Jyot, 2015). Most fermentation are accomplished by lactic acid
58 bacteria (LAB), which have positive effect on food quality, for example thanks to synthesis
59 of compounds of interest including vitamin B9 (Blandino, Al-Aseeri, Pandiella, Cantero, &
60 Webb, 2003).

61

62 *Ben-saalga* is a traditional fermented cereal-based porridge produced from pearl-millet
63 (*Pennisetum glaucum*) at a small scale in informal micro-enterprises in West African
64 countries particularly in Burkina Faso (Tou et al., 2006). Forty-five percent of the 6-23

65 month-old children in Ouagadougou eat this porridge daily (Mouquet-Rivier et al., 2008). Its
66 energy content is on average 41 kcal/100 g dry matter (DM), which is very low (Mouquet-
67 Rivier et al., 2008). Its folate content is also extremely low: between 0 and 3.3 µg/100 g fresh
68 matter (FM) (Saubade, Humblot, Hemery, & Guyot, 2017b). Given how frequently it is
69 consumed by young children, different attempts have been made to improve its nutritional
70 quality using LAB selected for their functional properties. A modification of the recipe by
71 including a pre-cooking step, and the addition of amylolytic bacteria, has already been
72 successfully explored. The resulting porridge had increased DM content: 15.3 to 17.4 g
73 DM/100g, and consequently higher energy density: 75.8 to 84.7 kcal/100 g DM (Songré-
74 Ouattara et al., 2009, 2010). Using folate producing LAB to increase the folate content of
75 fermented cereal foods has been reported to be a potentially useful tool (Saubade, Hemery,
76 Guyot, & Humblot, 2017a). Most investigations to date have focused on fermented rye and
77 oat sourdoughs consumed in developed countries (Kariluoto et al., 2006, 2014) and few
78 studies of the increase in the folate content of fermented complementary foods for young
79 children in developing countries have been conducted using folate producing LAB. *In situ*
80 fortification of pearl-millet porridges fermented by two strains of *Lactobacillus fermentum*
81 selected for their ability to produce high levels of folate in culture media, resulted in a
82 successful folate production in fermented gruel, which peaked around 7 µg/100g FM (Greppi
83 et al., 2017b).

84

85 In the present study, taking into account that the DM content and the energy density of the
86 traditional *ben-saalga* have already been optimized using a modified processing method
87 including the use of amylolytic LAB (Songré-Ouattara et al., 2009; 2010), we made use of
88 this modified processing and aimed to increase the folate content of the porridge using
89 selected productive strains. To this end, porridges were prepared using a traditional process

90 (spontaneous fermentation of uncooked paste) as control, and by including a precooking step
91 followed by inoculation by back slopping from the spontaneous fermentation, or inoculation
92 by two combinations of folate-producing LAB and amylolytic LAB.

93

94 **2. Material and methods**

95 **2.1. Starter cultures and preparation of the inoculum**

96 Based on the study performed by Greppi, Hemery, Berrazaga, Almaksour, & Humblot
97 (2017a) to identify folate-producing and folate-consuming LAB among 151 LAB strains
98 previously isolated from *ben-saalga*, two LAB strains namely *L. fermentum* 8.2 and *L.*
99 *plantarum* 6.2 were selected for their efficiency. Those strains belong to the LAB species (*L.*
100 *fermentum* and *L. plantarum*), the most frequently involved in pearl-millet fermentation
101 (Turpin, Humblot, & Guyot, 2011). *L. plantarum* A6 and *L. fermentum* MW2 were used as
102 amylolytic LAB because of their proven efficiency in hydrolysing starch in various food
103 matrices (Agati, Guyot, Morlon-Guyot, Talamond, & Hounhouigan, 1998; Giraud, Brauman,
104 Keleke, Lelong, & Raimbault, 1991). In order to follow the two species growth in the food
105 matrix, we chose a combination of one *L. plantarum* and one *L. fermentum* to easily allow
106 their detection in the food matrix using real time PCR.

107

108 Each starter culture was grown overnight in de Man, Rogosa and Sharpe (MRS) broth
109 medium (BD Difco, Sparks, MD, USA) at 30 °C. To inoculate the gelatinized pearl-millet
110 slurry, the cultures were centrifuged at 8000 × g for 10 min at 4 °C, and the pellets obtained
111 were washed in sterile 0.9% NaCl centrifuged in the same conditions and suspended in sterile
112 0.9% NaCl to form a cell suspension, which constituted the inoculum. It contained on
113 average $7.2 \pm 0.6 \log_{10}$ CFU/ml for *L. fermentum* 8.2; $6.8 \pm 0.3 \log_{10}$ CFU/ml for *L.*

114 *plantarum* 6.2; $6.9 \pm 0.2 \log_{10}$ CFU/ml for *L. plantarum* A6 and $5.4 \pm 0.3 \log_{10}$ CFU/ml for *L.*
115 *fermentum* MW2.

116

117 **2.2. Ben-saalga processing and inoculation conditions**

118 All the porridges used in this study were prepared from raw pearl-millet grains purchased
119 in a local market in Ouagadougou. Two types of porridge were prepared at laboratory scale
120 (Figure 1). The first type was prepared according to the traditional process described by Tou
121 et al. (2006) with 700 g of pearl-millet grains. The second was prepared according to a
122 process based on the traditional process described by Songré-Ouattara et al. (2010) with one
123 modification: to be consistent with what is done in TPUs in Ouagadougou, the milling step
124 was wet, i.e. water was added to the soaked grains during milling in a public mill, and the
125 resulting product was a paste of milled soaked grains.

126

127 Briefly, the modified process consisted of the following steps: the pearl-millet grains (700
128 g) were soaked in tap water (1:1.5 w/v) for 16 h, washed (1:2 w/v), drained for 30 min, wet
129 milled, water was added and the grains were sieved (1:3.5 w/v) through muslin. The resulting
130 dough was boiled for 10 min to gelatinize the starch (3000 g), and then cooled to around 30
131 °C. The inoculation was carried out with one folate producing strain (10%, v/w) and one
132 amyolytic strain (1%, v/w). After inoculation, the resulting samples were named *L.*
133 *plantarum* 6.2 + *L. fermentum* MW2 (6.2 + MW2) and *L. fermentum* 8.2 + *L. plantarum* A6
134 (8.2 + A6).

135

136 In addition, as a control of spontaneous fermentation after precooking, the back slopping
137 was used. It consisted in the addition of a small amount (10 % v/w) of fermented paste from a
138 previous successful prepared using traditional process fermentation (Figure 1). This

139 fermented paste of semi liquid form was collected during the fermentation step of traditional
140 processing. It contained on average $7.6 \pm 0.2 \log_{10}$ CFU/ml (total count). It can be considered
141 as a natural selection of the microorganisms the most adapted to the pearl-millet substrate. In
142 addition, it promotes better hygienic conditions by favouring an immediate dominance of
143 LAB, and this technique is particularly suitable for household and TPUs with only
144 rudimentary facilities, taking place in, e.g., developing countries (Nout, Rombouts, &
145 Havelaar, 1989).

146

147 All fermentation took place at ambient temperature (around 30 °C) for 24 h. After
148 fermentation, the porridges were prepared by cooking the fermented paste for 5 min after
149 reaching boiling point. Experiments were performed in triplicate for each fermentation
150 condition.

151

152 **2.3. Sampling**

153 Samples for folate analysis and quantification of LAB were collected immediately after
154 inoculation (0 h), at the end of fermentation (24 h) and after 5 min of cooking corresponding
155 to the ready-to-eat porridge. The viable counts of microorganisms and the DM content were
156 determined immediately in all samples. DM content was determined by drying the samples at
157 105 °C in open dishes to constant weight. Aliquots were stored at -20 °C until real-time PCR
158 counts of specific LAB, and folate analysis.

159

160 **2.4. Counts of viable microorganisms**

161 For all experiments, 10 g of sample, taken at 0 h and 24 h during processing and after 5
162 min of cooking, were homogenized with 90 ml of sterile peptone water solution 0.1%
163 peptone 0.85% NaCl (Difco), using a stomacher 400 (Type BA 7021, Seward England) for 2

164 min. Presumptive aerobic mesophilic bacteria (total count), LAB, and yeasts were
165 enumerated on the following media: Plate Count Agar (PCA), MRS, and Sabouraud
166 Chloramphenicol Agar (all from BD Difco, Sparks, MD, USA), respectively. MRS plates
167 were incubated at 30 °C for 48 h, PCA plates at 37 °C for 48 h and Sabouraud plates at 30 °C
168 for 3 days. Enumerations were done in triplicate for each sample. Results are expressed in
169 log₁₀ CFU/g of sample.

170

171 **2.5. DNA extraction and real-time PCR for the quantification of LAB**

172 Total DNA extraction from samples and real-time PCR were performed as previously
173 described in detail (Saubade, Humblot, Hemery & Guyot., 2017b). Briefly, samples (20 g)
174 collected at 0 h and 24 h of fermentation were extracted after serial centrifugation to
175 eliminate starch using the Wizard genomic DNA purification kit (Promega, Charbonnières,
176 France) with an additional lysis step using an amalgamator with zirconium beads (VWR,
177 Fontenay-sous-Bois, France). The concentration and purity of the DNA were estimated by
178 measuring absorbance at 260 and 280 nm (NanoVue™, GE Healthcare, Uppsala Sweden).
179 The DNA samples were then stored at -20 °C until real time PCR analysis.

180

181 Selected primers corresponding to the species (*L. fermentum*, *L. plantarum*) used to
182 inoculate the dough were used for the quantification of LAB by real-time PCR. We included
183 the primers corresponding to the genera *Leuconostoc* and *Weissella*, as they are also involved
184 in the spontaneous fermentation of *ben-saalga* (Humblot & Guyot, 2009; Saubade, Humblot,
185 Hemery & Guyot, 2017b). The selected primers are listed in Table 1. The specificity of each
186 previously selected primer was checked by real-time PCR using the DNA extracted from the
187 reference strains (*L. fermentum* ATCC14931, *L. plantarum* ATCC14917T, *Leuconostoc*
188 *mesenteroides* ATCC10880 and *Weissella confusa* ATCC10881T) and sterile water as a

189 negative control. The same strains were used to generate quantification matrixes, by
190 extracting DNA from pure culture with different known bacterial concentration. The real-
191 time PCR conditions were the same as those described in Saubade, Humblot, Hemery &
192 Guyot (2017b).

193

194 **2.6. Folate analysis**

195 Total folate content was determined in triplicate using the microbiological assay described
196 by Kariluoto and Piironen (2009), and adapted as described by Saubade, Hemery, Rochette,
197 Guyot, & Humblot (2018). Briefly, the thawed samples were diluted in the extraction buffer
198 and heated at 100 °C for 10 min, after which, the pH was adjusted to 7.2 and a tri-enzymatic
199 extraction with amylase (Sigma-Aldrich A9857, 20 mg/ml in 1% sodium ascorbate), chicken
200 pancreatin (γ -glutamylhydrolase) (R-Biopharm P2002, 100 mg/ml in Milli-Q water) and
201 protease (Sigma-Aldrich P5147, 3 mg/ml in 1% sodium ascorbate), was used to release folate
202 from the food matrix and to hydrolyse the polyglutamate forms of folate to mono- or
203 diglutamate. After appropriate dilutions of the centrifuged extracts, the concentration of folate
204 was determined on microtiter plates with *Lactobacillus rhamnosus* ATCC 7469 as the growth
205 indicator, folic acid (Sigma-Aldrich F7876) as the calibrant, and Folic Acid Casei Medium
206 (Difco, Sparks, MD, USA) as the assay medium. The performance of the method was
207 confirmed by analyzing a certified reference material (BCR 121 Wholemeal flour, IRMM,
208 Geel, Belgium). A blank sample and the reference sample were analysed in each series of
209 samples.

210

211 **2.7. Statistical analysis**

212 Data were analysed using SPSS version 20.0 (International Business Machines
213 Corporation, Armonk, New York, USA) and XLSTAT software (v. 2014.5.03). Statistical

214 analyses were performed using one-way ANOVA followed by Newman-Keuls post-hoc tests,
215 and differences were considered statistically significant at $p < 0.05$.

216

217 **3. Results**

218

219 **3.1. Changes in the microbial population during fermentation**

220 At the beginning of fermentation, the number of LAB and mesophilic aerobic bacteria
221 were quite similar under all experimental conditions (Figure 2). With traditional and back
222 slopping fermentation, respective yeast counts of $4 \log_{10}$ CFU/g and $3 \log_{10}$ CFU/g were
223 found. No yeasts were found in samples inoculated with starter cultures. After 24 h of
224 fermentation, LAB counts increased at a rate of $9 \log_{10}$ CFU/g in all conditions. In samples
225 resulting from spontaneous fermentation, LAB became the dominant microbiota. After five
226 minutes of cooking, which lead to pockets of low temperature due to the amount of porridge
227 cooked, all LAB were still present at detectable levels in the samples in all experimental
228 conditions, while yeasts counts decreased or were undetectable.

229

230 **3.2. Quantification of targeted LAB by real-time PCR**

231 At the beginning and after fermentation, *L. fermentum* and *L. plantarum*, i.e. the species
232 used for inoculation, were detected in samples 8.2 + A6 and 6.2 + MW2. In the 8.2 + A6
233 samples, *L. fermentum* and *L. plantarum* increased significantly ($p < 0.05$) from $6 \log_{10}$ CFU /
234 g to $7 \log_{10}$ CFU / g of porridge, and from $4 \log_{10}$ CFU / g to $6 \log_{10}$ CFU / g of porridge,
235 respectively (Figure 3). In the 6.2 + MW2 samples, *L. fermentum* and *L. plantarum* increased
236 significantly ($p < 0.05$) from $5 \log_{10}$ CFU / g to $7 \log_{10}$ CFU / g of porridge, and from $5 \log_{10}$
237 CFU / g to $6 \log_{10}$ CFU / g of porridge, respectively. Both *L. plantarum* and *L. fermentum*
238 were detected in samples from traditional or back slopping processes, but *L. fermentum* was

239 the dominant microorganism. The genus *Weissella* and *Leuconostoc* were detected in all
240 porridges and underwent a significant increase ($p < 0.05$) after fermentation in samples from
241 traditional and back slopping process. In samples 6.2 + MW2, *Leuconostoc* showed a
242 significant increase ($p < 0.05$) after fermentation.

243

244 **3.3. Dry matter contents**

245 Table 2 lists the DM contents of porridges under all fermentation conditions. At the
246 beginning of fermentation (0 h), pearl-millet doughs made using traditional and back
247 slopping processes had significantly higher DM ($p < 0.05$) than those inoculated with the two
248 combinations of strains 8.2 + A6 or 6.2 + MW2. After fermentation and cooking, DM
249 contents were not modified whatever the fermentation conditions.

250

251 **3.4. Folate contents in porridges prepared following the four processes**

252 Table 3 shows the folate contents of fermented pearl-millet porridges. At the beginning of
253 fermentation, folate contents were similar in all the pearl-millet doughs (ranging from $2.6 \pm$
254 $0.5 \mu\text{g}/100 \text{ g FM}$ to $3.4 \pm 0.4 \mu\text{g}/100 \text{ g FM}$) with the exception of the sample prepared using
255 the back slopping process, which had lower folate content ($1.8 \pm 0.4 \mu\text{g}/100\text{g FM}$). After 24 h
256 of fermentation, folate contents remained stable ($3.6 \pm 0.5 \mu\text{g}/100\text{g FM}$) in doughs prepared
257 using the traditional processing method. In contrast, folate contents increased when the
258 precooked batter was inoculated by back slopping or by both the LAB combinations.
259 Whatever the fermentation conditions, five minutes of cooking had no significant effect on
260 the folate contents of the porridges.

261

262 The overall gains in folate content in the cooked foods compared to the initial folate
263 contents were 134% for fermentation performed with the combined inoculation with 8.2 +

264 A6, 184% for fermentation performed with co-inoculation with 6.2 + A6, and more than
265 200% with back slopping.

266

267 **4. Discussion**

268 The nutritional quality of traditional *ben-saalga* is very poor since its energy density and
269 its folate content are very low (Mouquet-Rivier et al., 2008; Saubade, Humblot, Hemery &
270 Guyot, 2017b). In this work, we used a combination of precooking and inoculation with
271 selected strains, and back slopping, to improve the nutritional quality of the porridge.

272

273 The agents of fermentation in the samples obtained using traditional and back slopping
274 fermentation processes were similar to those prepared with the spontaneous fermentation
275 pattern of pearl-millet dough previously described, as mesophilic aerobic bacteria, yeasts, and
276 lactic acid bacteria were found to be present (Humblot & Guyot, 2009; Tou et al., 2006).
277 High microbial count variability was observed, mainly after cooking (for 5 min). This
278 variability may be due to the difficulty to control the temperature in such amount of porridge,
279 in conditions similar to the one done in TPUs, thus leading to pockets of lower temperature. It
280 can be also due to the microbial composition of the back-slopping inoculum, which can differ
281 from one fermentation to another. The fermented dough prepared using a combination of
282 strains inoculated after precooking contained a high concentration of LAB. Real-time PCR
283 targeting specific species or genera showed that the species corresponding to the starter
284 cultures used were present at the beginning and at the end of the fermentations. But using this
285 methodology it was not possible to state that the cultures present at the beginning and end of
286 the fermentation were the same strains. They belong to the same species and further studies
287 would be needed to confirm their identities. What is more, they were not out-competed by
288 other species although little growth of *Leuconostoc* and *Weissella* genus was observed in the

289 samples inoculated with the selected strains after 24 h of fermentation. As the experiments
290 were conducted in a non-sterile environment, this is probably explained by the reintroduction
291 of this genus after precooking (air, dust etc.).

292

293 In the present study, all fermentations with selected LAB strains led to a significant
294 increase in folate contents of the dough compared with both the unfermented (0 h) and the
295 traditional *ben-saalga*. This clearly revealed folate production by the *L. plantarum* 6.2 and *L.*
296 *fermentum* 8.2 used as folate producers. Folate contents of precooked paste inoculated with
297 the two combinations of strains were similar to that reported by Greppi et al. (2017b) using *L.*
298 *fermentum* 8.2 alone.

299

300 Back slopping fermentation led to a similar increase in the folate contents of the porridges
301 as in fermentation using starter cultures. This increase in folate content may be due to the
302 production of folate by naturally occurring yeasts and LAB present in the traditional
303 spontaneous fermentation and used as inoculum. Counts of microorganisms in the fermented
304 dough prepared using the back slopping process indeed showed the presence of yeasts and
305 LAB. Numerous studies have shown that yeasts can also significantly increase (up to 700%)
306 the folate content of cereal fermented products (Hjortmo, Hellström, & Andlid, 2008;
307 Kariluoto et al., 2014). In addition, a symbiotic relationship between yeasts and LAB
308 concerning folate production was recently reported by Greppi et al. (2017b) in fermented
309 pearl-millet porridge, since the use of the combination of selected microorganisms is more
310 efficient than folate producing LAB alone. The only difference between the two processes is
311 including a precooking step in the case of back slopping. Such a positive effect of precooking
312 alone was unexpected and further studies are necessary to explain it. Yet, spontaneous
313 fermentations have been shown several times to lead to highly variable products in terms on

314 nutritional quality and microbial composition, the choice of inoculation with selected
315 microorganisms would be preferred (Saubade, Hemery, Guyot, & Humblot, 2017a).

316

317 We deliberately used the conditions developed by Songré-Ouattara et al. (2009; 2010)
318 which target 15-17 g DM/100g using amylolytic LAB to ferment pearl-millet. However, we
319 obtained porridges with slightly less DM content than expected, due to the addition of starter
320 cultures in liquid form, which induced a slightly lower dry matter content, due to a dilution
321 effect. But during the inoculation by back slopping, the addition of a small amount of
322 fermented paste from a previous successful natural fermentation induced an increase of dry
323 matter content of the precooked paste.

324

325 Based on joint recommendations by the World Health Organization (WHO) and the Food
326 and Agricultural Organization (FAO) of the United Nations (UN), folate requirements,
327 expressed as recommended nutrient intakes (RNIs) of most of the population including
328 adolescents, adults and the elderly are 400 µg of folate per day (Food and Agriculture
329 Organization of the United Nation/World Health Organization, 2005). However, a dose of
330 65-300 µg of folate per day would satisfy the folate requirements of infants and young
331 children (Food and Agriculture Organization of the United Nation/World Health
332 Organization, 2005). A food is considered to be a good source of folate when it contributes at
333 least 15% of daily recommended intake (EC Regulation 1924/2006). Based on this definition,
334 traditional *ben-saalga* is far from being a good source of folate. In this study, one 200 g
335 portion of the porridges prepared using precooking and inoculation by back slopping or with
336 both combinations of LAB strains would represent 15% RNIs for infants aged 6-12 months.
337 This portion can be consumed by infants and young children owing to the functional gastric
338 capacity of 30 g / kg of body weight (BW) / meal (World Health Organization, 1998).

339 However, Mouquet-Rivier, Traoré, Soma, Kaboré, & Trèche, (2016) recently reported that
340 mean intakes of *ben-saalga* consumed by the 6-9-month-old and the 15-20-month-old
341 children ranged from 7.6 to 19.7 g/kg of BW / meal in Burkina Faso. This was below the
342 functional gastric capacity of 30 g/kg of BW/meal, suggesting that portions of fermented
343 porridges prepared with inoculation would cover far less than the 15% of daily recommended
344 intake of folate. The development of a cereal-based fermented porridges enriched in folate,
345 even though promising, needs to be further refined. For example, based on previous findings,
346 the use of co-cultures of folate producing LAB and amylolytic LAB to ferment pearl-millet,
347 combined with the use of legume seeds such as groundnuts or soybean, which contain folate
348 and other useful nutrients (protein, lipids, etc.) could be further explored (Songré-Ouattara et
349 al., 2009, 2010). The optimisation of the duration of fermentation should also be taken into
350 account.

351

352 **5. Conclusion**

353 This study showed that the use of two starter cultures previously isolated from pearl-millet
354 fermented slurry, and combined with amylolytic LAB strains, may increase the folate content
355 of cereal-based fermented porridges. Back slopping is a potentially advantageous alternative
356 way to improve the folate content of cereal based fermented foods.

357

358 **Disclosure statement**

359 The authors declare no conflict of interest

360

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Figure captions

Figure 1: Processing of pearl-millet into fermented porridges using a process based on the traditional process, or including precooking of the paste followed by inoculation with three different starters: back slopping using dough taken from the traditional fermentation process, a combination of *L. fermentum* 8.2 and *L. plantarum* A6, and a combination of *L. plantarum* 6.2 and *L. fermentum* MW2.

Figure 2. Microbial counts of fermented porridges obtained following a process based on the traditional process, or including precooking of the paste followed by inoculation with three different starters: back slopping using dough taken from the traditional fermentation, a combination of *L. fermentum* 8.2 and *L. plantarum* A6 and a combination of *L. plantarum* 6.2 and *L. fermentum* MW2. Samples were taken at the beginning of the fermentation (0 h : white bars), at the end of the fermentation (24h : black bars) and after cooking (for 5 min : grey bars). Values are expressed as means \pm standard error of the mean. For yeasts, means with different letters differ significantly between samples prepared according the different processes ($p < 0.05$).

Figure 3. Real-time PCR quantification of *L. plantarum* (white bars), *L. fermentum* (black bars), *Leuconostoc* (striped bars) and *Weissella* (grey bars) during the preparation of the porridges using the different processes at the beginning of the fermentation (0 h), at the end of the fermentation (24 h) and after cooking (for 5 min).

Values are expressed as means \pm standard error of the mean.

Means with different letters differ significantly ($p < 0.05$).

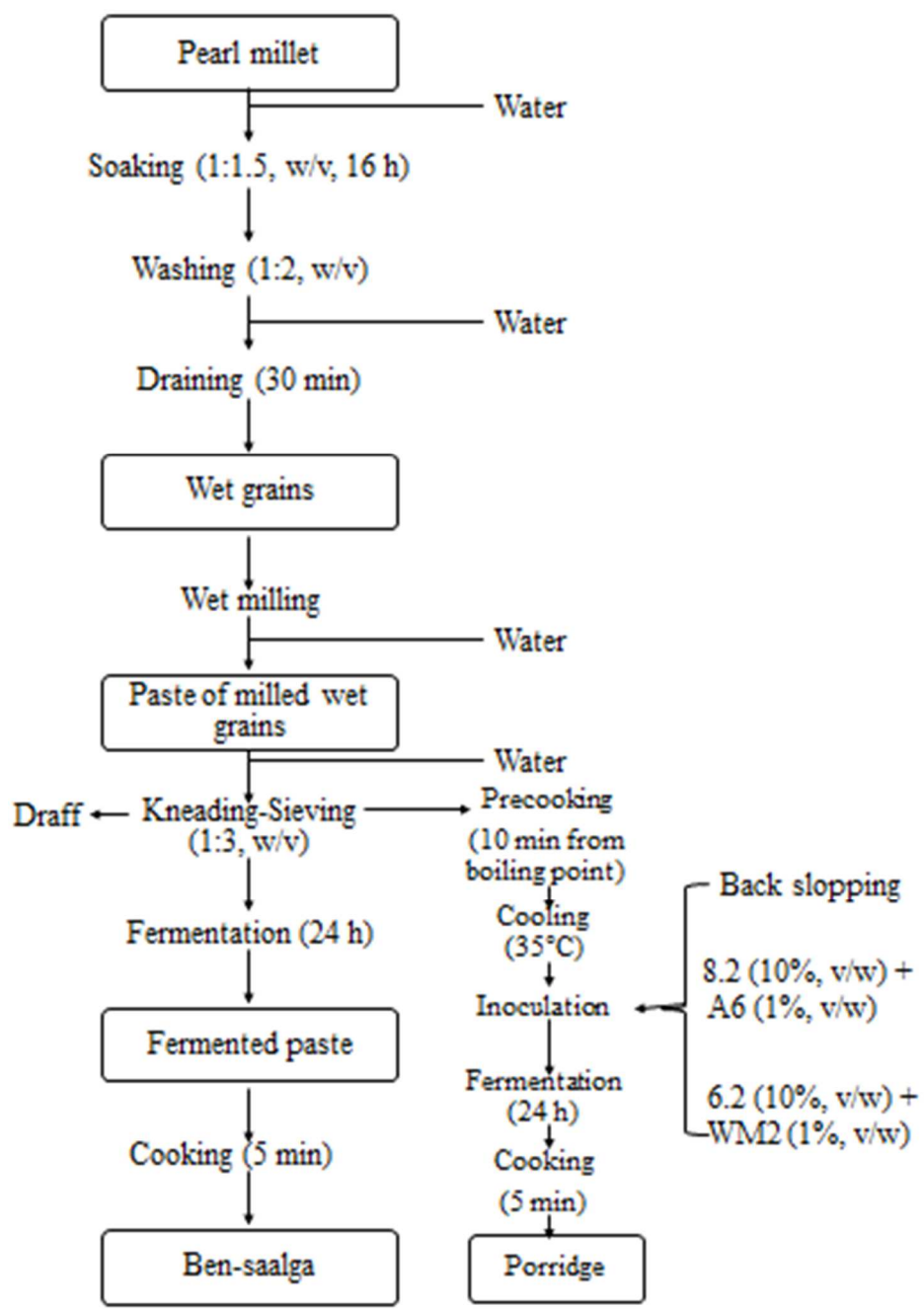


Figure 1.

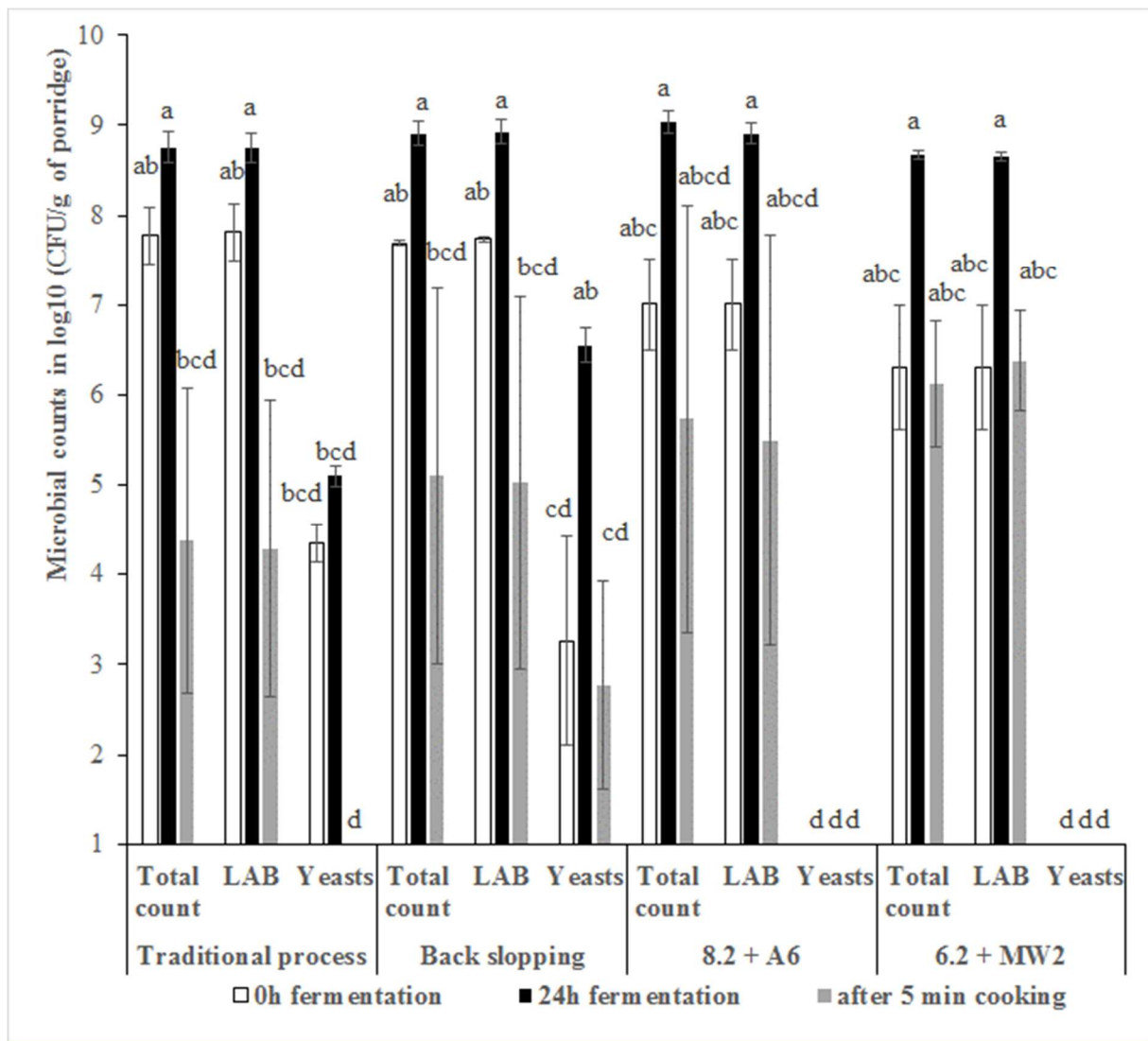


Figure 2.

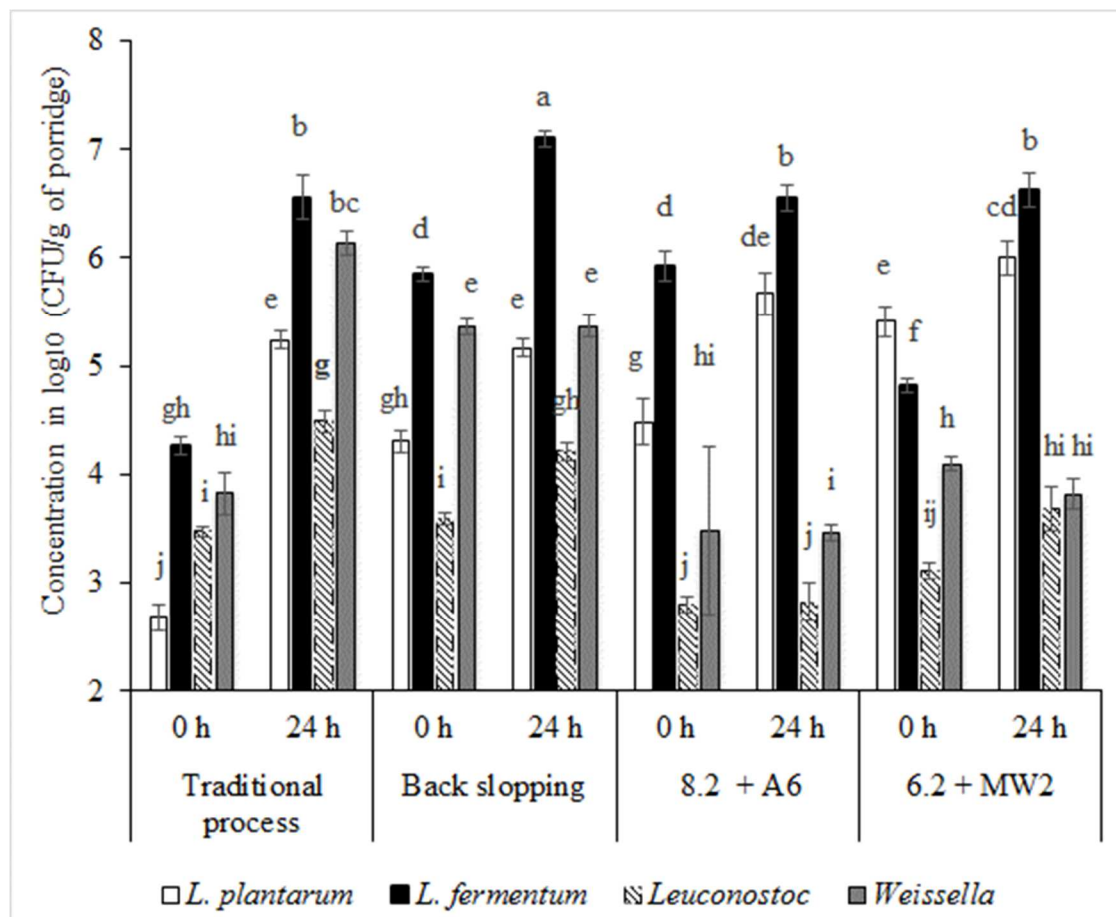


Figure 3.

Table 1. Primers used to quantify the genus or species of bacteria from *ben-saalga* samples using real-time PCR. The primers were designed to match the gene coding 16S rRNA.

Name	Sequences 5'-3'	Targets	References
Lferm72f*	CCTGATTGATTTTGGTCGC	<i>L. fermentum</i>	(Omar & Ampe, 2000)
Lpla72f	ATCATGATTTACATTTGAGTG	<i>L. plantarum</i>	(Chagnaud, Machinis, Coutte, Marecat, & Mercenier, 2001)
338r*	CTGCTGCCTCCCGTAGGAGT	Bacteria	(Muyzer, Dewaal, & Uitterlinden, 1993)
Leu200f*	CGGCGTCACCTAGAGATGGATC	<i>Leuconostoc</i>	(Omar & Ampe, 2000)
518r	ATTACCGCGGCTGCTGG	Universal	(Muyzer, Dewaal, & Uitterlinden, 1993)
S-G-Wei-0121-a-S-2	CGTGGGAAACCTACCTCTTA	<i>Weissella</i>	(Jang et al., 2002)
S-G-Wei-0823-a-A-18	CCCTCAAACATCTAGCAC	<i>Weissella</i>	(Jang et al., 2002)

*Primers from the literature were converted into their reverse complement.

Table 2: Dry matter content of samples taken during the preparation of the porridges using the different processes at the beginning of the fermentation (0 h), at the end of the fermentation (24 h) and after cooking (for 5 min.).

Fermentation conditions	Dry matter content (g DM/100 g of sample)		
	0 h	24 h	5 min
Traditional process	13.1 ± 0.3 ^b	12.2 ± 0.4 ^a	13.3 ± 0.2 ^{bc}
Back slopping process	14.1 ± 0.2 ^c	13.8 ± 0.3 ^{bc}	13.7 ± 0.3 ^{bc}
<i>L. fermentum</i> 8.2 + <i>L. plantarum</i> A6	11.6 ± 0.7 ^a	11.6 ± 0.6 ^a	12.0 ± 0.8 ^a
<i>L. plantarum</i> 6.2 + <i>L. fermentum</i> MW2	11.8 ± 0.8 ^a	11.7 ± 0.4 ^a	11.8 ± 0.4 ^a

Values are expressed as means ± standard error to the mean.

Means with no common letter are considered significantly different ($p < 0.05$).

Table 3: Folate contents of samples taken during the preparation of the porridges according to the different processes at the beginning of the fermentation (0 h), at the end of the fermentation (24 h) and after cooking (for 5 min.).

Fermentation conditions	Changes in folate contents ($\mu\text{g}/100\text{ g FM}$)			Folate gain (% of 0 h)
	0 h	24 h	5 min of cooking	
Traditional process	3.4 ± 0.4^b	3.6 ± 0.5^b	4.2 ± 0.9^b	22
Back slopping process	1.8 ± 0.4^c	7.1 ± 1.7^a	6.1 ± 0.8^a	237
<i>L. fermentum</i> 8.2 + <i>L. plantarum</i> A6	3.0 ± 0.6^b	6.5 ± 0.8^a	7.1 ± 1.0^a	134
<i>L. plantarum</i> 6.2 + <i>L. fermentum</i> MW2	2.6 ± 0.5^b	7.7 ± 1.4^a	7.3 ± 0.6^a	184

Results are expressed as mean \pm standard error of the mean.

Means with different letters in the same row differed significantly when compared at different stages of the same process ($p < 0.05$).