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**Total folate in West African cereal-based fermented foods:  
bioaccessibility and influence of processing**

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1 **Abstract:**

2 Cereal-based fermented foods (CBFF) are frequently consumed by young children in West-  
3 Africa. Seven CBFF types (gelatinized doughs, porridges, dumplings, and fritters, made from  
4 corn, sorghum or pearl millet) were produced according to the commonly used methods in  
5 traditional production units in Burkina Faso. Their total folate content, analyzed using a  
6 microbiological method, was between 1.8 and 31.3 µg/100g fresh weight. Folate degradation  
7 occurred at several steps during the cereal grain processing into CBFF, for instance during  
8 debranning (-51%), soaking (-20% to -49%) and wet-milling (-39% to -59%). Conversely,  
9 fermentation increased the folate content of some CBFF (+9 to +27%). Folate  
10 bioaccessibility, assessed using a static *in vitro* digestion model, ranged from 23% to 81%.  
11 The bioaccessible folate content was influenced by the total folate content, the food matrix  
12 structure, and folate stability. CBFF contribution to the reference folate intake for young  
13 children was at most 23%. This work generated new information on the influence of  
14 traditional processing on the vitamin B9 content of CBFF, and the first data on folate  
15 bioaccessibility in West African CBFF.

16

17 **Key-words:**

18 Folate, processing, cereal, digestion, fermentation, Africa, infant food, complementary feeding,  
19 food analysis, food composition

20

21 **Abbreviations:**

22

23 ANOVA: Analysis of variance

24 AOAC: Association of Official Analytical Chemists

25 CBFF: Cereal-based fermented food

26 CFC: Cereal-based fermented food folate contents

27 DF: Daily frequency

28 DFI: Daily folate intake

29 DFI-OFP: Daily folate intake needed from other food products

30 DM: Dry matter

31 FAO: Food and Agriculture Organization of the United Nations

32 FW: Fresh weight

33 LAB: Lactic acid bacteria

34 PS: Portion size

35 RNI: Recommended nutrient intake

36 SGF: Simulated gastric fluid

37 SIF: Simulated intestinal fluid

38 SSF: Simulated salivary fluid

39 TPU: Traditional production unit

40 WAFCT: West African food composition table

41 WHO: World Health Organization

42

## 43 **1. Introduction**

44 Folate (vitamin B9) is essential for human metabolism, because it is involved in cell division,  
45 DNA replication, methylation, nucleotide biosynthesis, and amino acid metabolism (McNulty  
46 and Scott, 2008; Preedy, 2013). Folate deficiency is a global health issue, in both developed  
47 and low-income countries (McLean et al., 2008; Youngblood et al., 2013), and leads to many  
48 health disorders, such as neural tube defects, megaloblastic anemia, and colorectal cancer  
49 (Bailey et al., 2015; FAO/WHO, 2004). Recent studies carried out in West African countries  
50 (Burkina Faso, Ivory Coast, Mali and Senegal) showed that folate deficiency is a severe  
51 public health problem among women of reproductive age and children (Arsenault et al., 2014;  
52 Bationo et al., 2019a; Fiorentino et al., 2016; Kennedy et al., 2010; Rohner et al., 2014). For  
53 instance, in 2010, the folate dietary intake was adequate only in 12% of women of  
54 reproductive age in Burkina Faso (Becquey and Martin-Prevel, 2010).

55 Cereals are the main crops in Africa (45% of arable land), and make an important contribution  
56 to the human diet in this continent, due to the high consumption of cereal-based products  
57 (Galati et al., 2014). They cannot be eaten raw, and are processed before consumption.  
58 Fermentation is widely used in many West African countries to transform cereals (sorghum,  
59 maize and millet) into edible products (Guyot, 2012). Traditional cereal-based fermented  
60 foods (CBFF), such as porridges or gelatinized doughs, are frequently consumed by young  
61 children in West Africa (Greffeuille et al., 2010; Hama, 2012). Fermentation is one of the  
62 oldest ways to preserve food products, and to enhance their sanitary and nutritional quality  
63 through the production of numerous nutritional and anti-microbial substances by fermenting  
64 microorganism (Nout, 2009). Fermentation is also considered as a good way to increase the  
65 folate content of food products, because yeast and many lactic acid bacteria (LAB) can  
66 synthesize folate during fermentation (Moslehi-Jenabian et al., 2010; Saubade et al., 2017a).  
67 Previous works have reported the isolation of folate-producing strains from different cereals  
68 (Capozzi et al., 2012; Salvucci et al., 2016). Moreover, studies on CBFF that are commonly

69 consumed in industrialized countries, such as rye, wheat and oat sourdoughs, have shown that  
70 fermentation can improve their folate content (Kariluoto et al., 2004, 2006a, Laiño et al.,  
71 2013, Jägerstad et al., 2005).

72 Other processing steps used in cereal processing, such as milling, debranning, soaking and  
73 cooking, also can influence the folate content of cereal-based foods (Arcot et al., 2002b;  
74 Kariluoto et al., 2006b; Witthöft et al., 1999). However, data on the influence of cereal  
75 processing on folate content in African cereal-based foods are very scarce. One recent study  
76 (Saubade et al., 2018) assessed the influence of traditional processing steps (soaking, wet-  
77 milling, sieving, and fermentation) on the final folate content of a porridge commonly eaten in  
78 Burkina Faso. Three other studies have shown that folate can be produced by selected folate-  
79 producing microorganisms during fermentation of African maize-based or pearl millet-based  
80 porridges (Greppi et al., 2017; Hjortmo et al., 2008; Saubade et al., 2017b).

81 Moreover, the absorption of dietary folate mainly depends on its bioaccessibility (i.e., the  
82 fraction of a nutrient that is released from the food matrix and is available for intestinal  
83 absorption) (Parada & Aguilera, 2007). Folate bioaccessibility can be affected by many  
84 factors, including the action of gastrointestinal enzymes and pH during digestion, the presence  
85 of inhibitors, and the matrix effects (Arkbåge et al., 2003; Parada and Aguilera, 2007;  
86 Ringling and Rychlik, 2017). Very few studies have evaluated folate bioaccessibility from  
87 different food types, and to our knowledge, none focused on folate bioaccessibility in  
88 traditional African cereal-based foods. However, to improve folate intake from African  
89 CBFF, more accurate information on traditional cereal processing methods and their influence  
90 on folate content is required, as well as on the folate bioaccessibility of these commonly eaten  
91 food products.

92 Therefore, the aim of this study was to provide information on the influence of processing  
93 methods of African CBFF on their total folate content and folate bioaccessibility. It also  
94 allowed evaluating the potential contribution to the recommended folate intake of young

95 children of these CBFF that are frequently consumed in Burkina Faso and other West African  
96 countries (*i.e.*, porridges, fritters, gelatinized doughs, dumplings).

97

## 98 **2. Material and methods**

### 99 **2.1. Materials**

#### 100 **2.1.1. Plant materials**

101 The cereals used for the production of CBFF were maize (*Zea mays*), sorghum (*Sorghum*  
102 *bicolor*), and pearl millet (*Pennisetum glaucum*). These cereals were purchased by the  
103 producers from their usual suppliers at local markets in Ouagadougou (Burkina Faso).

104

#### 105 **2.1.2. Sample collection**

106 Seven CBFF types were investigated in this study: *akassa* and *doncounou* (made from corn),  
107 *kaffa* (made from sorghum), and *massa*, *fura*, *ben-kida*, and *ben-saalga* (made from pearl  
108 millet). *Akassa*, *doncounou* and *kaffa* are gelatinized doughs, *massa* are fritters, *fura* are  
109 dumplings, and *ben-kida*, and *ben-saalga* are porridges. Photographs of these CBFF are  
110 presented in Figure 1. These food products were sampled in 20 traditional production units  
111 (TPUs) in Ouagadougou, Burkina Faso. The processing method used for the production of  
112 each CBFF is described in Figure 2, and in supplementary material.

113 The processing of *ben-saalga*, the most commonly eaten CBFF, was monitored in detail at four  
114 different TPUs (three different days for each TPU). The parameters implemented at each steps  
115 were precisely recorded (e.g., material used, time, temperature, pH, and weight of the different  
116 fractions and by-products). Samples of pearl millet grains (raw material), intermediate/final  
117 products and by-products were also collected.

118 For five other CBFF (*akassa*, *doncounou*, *massa*, *fura*, and *ben-kida*), samples of raw materials  
119 (*i.e.*, grains of pearl millet, or corn) and final products (fermented foods, as eaten by  
120 consumers) were collected in three different TPUs for each food type. Moreover, in each

121 TPU, samples were collected on three different days. The process steps were not followed in  
122 details, but were listed by recording the producers' declarations, including estimations of the  
123 fermentation and cooking times. For *kaffa*, the least commonly eaten CBFF, samples of raw  
124 material (sorghum) and final product were collected on three different days from only one  
125 TPU.

126 For all seven CBFF, in each TPU, three 50g samples were collected per day, on 3 different  
127 days (*i.e.*, 9 samples collected per TPU). They were used for dry matter analysis (on site), and  
128 then individually stored at -20°C for total folate analysis and *in vitro* digestion.

129

### 130 **2.1.3. Chemicals and enzymes**

131 Porcine pancreatic  $\alpha$ -amylase (E-PANAA, #150402, 110.8 U/mg) was purchased from  
132 Megazyme (Bray, Ireland). Chicken pancreas  $\gamma$ -glutamylhydrolase (P2002) was obtained from  
133 R-Biopharm. Protease from *Streptomyces griseus* (P5147, 3.5 units/mg), *Aspergillus oryzae*  
134 alpha-amylase (A9857, #SLBM0836V, 50.4 U/mg), pepsin from porcine gastric mucosa (P7000,  
135 #BCBF9832V, 536 U/mg), bovine pancreatic trypsin (T0303, #SLBR6593V, 160.6 U/mg)  
136 and chymotrypsin (C4129, #SLBG2821V, 40 U/mg), and bovine and ovine bile extracts  
137 (B8381, #SLBH1720V, 881.3 g/mol) were purchased from Sigma-Aldrich (St Louis, MI,  
138 USA). All the enzymes were stored at -20 °C. All other used chemicals were of analytical  
139 grade.

140

## 141 **2.2. Methods**

### 142 **2.2.1. *Ben-saalga* mass balance assessment**

143 The traditional processing of pearl millet into *ben-saalga* has been described before (Tou et  
144 al., 2006). To follow the folate losses and gains during *ben-saalga* processing, and to monitor  
145 folate distribution in pearl millet fractions, a mass balance assessment was carried out at each  
146 TPU, as described by Saubade et al. (2018). Briefly, the weight of all utensils and sampled

147 products (samples taken for folate analysis, raw materials, intermediate products, by-products,  
148 and final products) was recorded at each step of the process. The dry matter (DM) content of  
149 all samples was measured, then each sample weight and DM content were combined with the  
150 folate content to calculate the amount of folate (in  $\mu\text{g}$ ) in the original pearl millet grains, and  
151 in the intermediate and final products. The folate content distribution in the intermediate and  
152 final products was expressed as the percentage of the initial folate quantity in pearl millet  
153 grains.

154

### 155 **2.2.2. Dry matter content and total lipid content**

156 The DM content of all samples was measured by drying the samples in an air-circulating oven  
157 at  $105^{\circ}\text{C}$  in open dishes until constant weight (overnight,  $\sim 16\text{h}$ ). Samples were then cooled in  
158 a desiccator and weighted, and the DM content was expressed as g DM/100 g sample (ICC  
159 Standard methods, 2003).

160 In *massa* samples, the lipid content also was measured because the frying step led to oil  
161 absorption that influenced their DM content. *Massa* samples were first lyophilized and  
162 ground, and then lipids were extracted with diethyl ether using a semi-automatic 2055 Soxtec  
163 system (Foss, Nanterre, France), according to the Association of Official Agricultural  
164 Chemists Official Methods 2003.05 and 2003.06 (AOAC, 2006). The defatted DM was  
165 calculated by subtracting the lipid content from the DM content, and was used to assess the  
166 influence of processing on *massa* folate content, on a dry matter basis.

167

### 168 **2.2.3. Determination of total folate content**

169 Total folate content was determined in triplicate using the microbiological assay described by  
170 Kariluoto & Piironen (2009) and adapted by Saubade et al. (2018). Briefly, thawed samples  
171 were diluted in extraction buffer and heated at  $100^{\circ}\text{C}$  for 10 min. After adjusting the pH to  
172 7.2, a tri-enzymatic extraction with  $\alpha$ -amylase (20 mg/ml in 1% sodium ascorbate), chicken

173 pancreas  $\gamma$ -glutamylhydrolase (100 mg/ml in Milli-Q water) and protease (3 mg/ml in 1% sodium  
174 ascorbate) was used to release folate from the food matrix. After appropriate dilutions of the  
175 supernatants, folate concentration was determined in microtiter plates with *Lactobacillus*  
176 *ramnosus* ATCC 7469 as the growth indicator organism, folic acid (Sigma-Aldrich F7876)  
177 as the calibration standard, and Folic Acid Casei Medium (Difco, Sparks, MD, USA) as the  
178 assay medium. The method performance was confirmed by analyzing a certified reference  
179 material (BCR 121 Wholemeal flour, IRMM, Geel, Belgium) for each incubation. The  
180 certified folate value for this reference material was  $500 \pm 70$  ng/g DM, and in this study, we  
181 obtained an average content of  $512 \pm 57$  ng/g dm ( $n = 38$ ). A blank sample (that contained all  
182 the reagents, but no food sample) was also analyzed in each series of samples, and the folate  
183 value obtained for the blank sample was deducted from the value obtained for the samples, to  
184 take into account the folate brought by the enzymes.

185

#### 186 **2.2.4. Determination of bioaccessible folate content and folate bioaccessibility**

187 Folate bioaccessibility was assessed using the static *in vitro* digestion model described by  
188 Minekus et al. (2014), with minor modifications. This method includes oral (simulated  
189 salivary fluid, SSF, pH 7), gastric (simulated gastric fluid, SGF, pH 3), and small intestinal  
190 (simulated intestinal fluid, SIF, pH 7) phases (cf. Figure 4 in supplementary material). As the  
191 folate content of pancreatin was high, purified enzymes (i.e., trypsin, chymotrypsin, and  
192 pancreatic  $\alpha$ -amylase) were used. Prior to *in vitro* digestion, the enzyme activity was checked  
193 to guarantee the method efficiency, as described by Minekus et al. (2014). After quantifying  
194 the folate content of several bile extracts, the one with the lowest folate content (77 ng/g) was  
195 chosen, in order not to skew the bioaccessible folate content due to high endogenous content  
196 in the bile extracts.

197 In a 50 ml Falcon tube, 5 g of roughly ground sample was mixed with 4 ml of SSF containing  
198  $\alpha$ -amylase, 25  $\mu$ l of  $\text{CaCl}_2$  solution and 975  $\mu$ l of Milli-Q water to reach a final ratio of

199 food/reagents of 50 : 50 (W/ V). The mixture was incubated at 37°C in a shaking water bath  
200 for 2 min. Then, 8 ml of SGF with pepsin and 5 µl of CaCl<sub>2</sub> solution were added, and the pH  
201 was adjusted to 3 with 6 M HCl. After adjusting the volume to 20 ml with Milli-Q water, the  
202 mixture was incubated in a shaking water bath at 37°C for 2 hours. To stimulate intestinal  
203 digestion, 10 ml of SIF containing bile extract, 6 ml of SIF containing pancreatic α-amylase, 1  
204 ml trypsin solution, 1 ml chymotrypsin solution, and 40 µl of CaCl<sub>2</sub> solution were added.  
205 After adjusting the pH to 7 with 2M NaOH, samples were incubated in a shaking water bath  
206 at 37°C for 2 hours. Finally, samples were centrifuged at 8000 g at 4°C for 30 min, and  
207 supernatants (i.e., the digesta) were stored at -20°C for bioaccessible folate analysis. All  
208 samples were digested in duplicates.

209 The bioaccessible folate content was determined in 4 ml of digesta (i.e., supernatants of the  
210 digested samples) that contained the bioaccessible folate, extracted from the food matrix  
211 during the *in vitro* digestion. The folate analysis was carried out as described above, except  
212 that only the conjugase (γ-glutamylhydrolase) was added to convert the bioaccessible  
213 polyglutamyl folate into the mono and diglutamyl folate forms that were quantified using the  
214 microbiological assay.

215 Bioaccessible folate (in µg/100g fresh weight, FW) corresponds to the folate fraction that is  
216 released from the food matrix, and potentially available for absorption through the intestinal  
217 barrier. Folate bioaccessibility (in %) was calculated using the following formula:

218 
$$\text{Folate bioaccessibility (\%)} = \text{Bioaccessible folate/Total folate} * 100.$$

219

#### 220 **2.2.5. Estimation of CBFF contribution to the folate intake of infants**

221 To estimate the daily folate intake (DFI, in µg/day) from the seven CBFF, known data on the  
222 daily frequency (DF) of consumption and portion sizes (PS, in g) of each CBFF for young  
223 children aged 6 to 35 months (Hama, 2012) were used. Based on these data and on the total  
224 folate content (TFC in µg/100g FW) of the seven CBFF, their DFI was calculated as follows:

225  $DFI = (TFC/100) * PS * DF$

226 For breastfed children, the folate intake from breast milk was also taken into account, based  
227 on a folate content of 85 µg/l in breast milk (Allen, 2012), as well as the average daily amount  
228 of breast milk consumed by children in developing countries, as described by the World  
229 Health Organization (WHO) (1998).

230 Then, the respective contribution of CBFF and breastmilk to the recommended folate intake  
231 (RNI) were calculated, taking into account the specific RNI for each age group (FAO/WHO,  
232 2004). The DFI needed from other food products (DFI-OFPP) than breast milk to reach 100%  
233 of the RNI was also calculated. Finally, based on the DFI-OFPP, the daily CBFF intake needed  
234 to reach 100% of the RNI, if only breast milk and CBFF were consumed, was calculated.

235

#### 236 **2.2.6. Statistical analysis**

237 Statistical analyses were performed using Statgraphics Centurion 18 (FranceStat, Neuilly,  
238 France). The Spearman's rank correlation was used to determine the strength of the  
239 relationships between variables. One-way analysis of variance (ANOVA) and Newman-Keuls  
240 *post-hoc* tests were used to identify significant differences between samples. Differences were  
241 considered statistically significant with *p*-values <0.05.

242 Three-level nested ANOVA was also carried out to test the inter-producer and the inter-day  
243 (within producers) variabilities. These variables are nested, meaning that each value of these  
244 nominal variables (i.e., the subgroups 'producer' and 'day') are found in combination with  
245 only one value of the higher-level nominal variable (i.e., the groups 'product' or 'producer').

246 The linear model for three-way unbalanced nested arrangement is given by:

247  $y_{ijkl} = \mu + \alpha_i + \beta_{j(i)} + \gamma_{k(ij)} + e_{ijkl}$

248 where:

249  $y_{ijkl}$  is the  $l^{\text{th}}$  observation within the  $k^{\text{th}}$  level of factor 'day' within the  $j^{\text{th}}$  level of factor  
250 'producer' within the  $i^{\text{th}}$  level of factor 'product';  $\mu$  represents the overall mean;  $\alpha_i$  is the fixed

251 effect due to the  $i^{\text{th}}$  level of factor ‘product’ (i.e., either the type of raw material used, or the  
252 type of CBFF produced), with  $i = 1$  to 3 (for raw materials) or  $i = 1$  to 7 (for CBFF);  $\beta_{j(i)}$  is the  
253 random effect due to the  $j^{\text{th}}$  level of factor ‘producer’ nested within the  $i^{\text{th}}$  level of factor  
254 ‘product’, with  $j = 1$  to 20;  $\gamma_{k(ij)}$  is the random effect due to the  $k^{\text{th}}$  level of factor ‘day’ nested  
255 within the  $j^{\text{th}}$  level of factor ‘producer’ nested within the  $i^{\text{th}}$  level of factor ‘product’, with  $k =$   
256 1 to 60; and  $e_{ijkl}$  is the residual error of the observation  $y_{ijkl}$ .

257 Variance components were calculated and expressed as percentages.

258

### 259 **3. Results**

#### 260 **3.1. Description of food products and processes**

261 The processes used to produce the seven CBFF from different raw materials (corn, sorghum,  
262 and pearl millet) are summarized in Figure 2. More details on the description of process steps  
263 and food products are provided in the supplementary materials.

264 *Akassa*, *doncounou*, and *kaffa* are thick fermented and cooked gelatinized doughs that are  
265 consumed as staple foods by all age categories, and generally eaten with a sauce. These three  
266 CBFF are produced using similar processes. The main difference between *akassa* and  
267 *doncounou* is that *doncounou* is made from non-dehulled corn (whole-grain), whereas *akassa*  
268 is made from debranned and degermed corn. *Kaffa* is made from sorghum. *Akassa*,  
269 *doncounou*, and *kaffa* processes included grain soaking, washing, draining, wet milling,  
270 sieving, paste fermentation, hydrothermal cooking, and wrapping of gelatinized dough balls in  
271 plastic bags. *Doncounou* processing also included a steam-cooking step.

272 *Massa* are fritters (small and thick pancakes fried in oil) made from pearl millet batter, often  
273 prepared as street food and eaten as snacks. They are consumed alone or crumbled in  
274 porridge. Processing included grain washing and sun drying, before dry milling in a  
275 community mill, mixing with water and kneading of the dough, fermentation during settling,

276 and frying. Frying led to oil adsorption in the fritters, with average oil contents of  $15.5 \pm 4.6$ ,  
277  $6.7 \pm 2.8$ , and  $17.1 \pm 2.4$  g/100g FW for *massa* produced in TPU 1, 2 and 3, respectively.  
278 *Fura* is a semi-solid dumpling meal made from pearl millet. It can be consumed in different  
279 ways. *Fura* balls can be made into porridge by crumbling and mixing them with porridge,  
280 fermented milk, or water with sugar. This can be used as food, refreshing drink, or weaning  
281 food for infants. *Fura* processing included grain dehulling before washing and sun drying (for  
282 30 min for producers P2 and P3, and up to 10 h for P1). After dry milling, sieving was an  
283 optional step. Two fermentation steps were carried out: one before and one after cooking.  
284 *Ben-saalga* and *ben-kida* are fermented porridges made from pearl millet, very often used as  
285 complementary food for infants and young children. Their processes were similar and  
286 included the following steps: soaking and washing of the grains, wet-milling, addition of  
287 water and sieving, fermentation (during settling), and cooking. For the preparation of *ben-*  
288 *kida*, previously prepared lumps (made from pearl-millet flour) were added to the boiling  
289 mixture to produce porridge with a granulated texture.

290

### 291 **3.2. Dry matter content of raw materials and fermented foods**

292 Measurement of the raw material DM content (Table 1) did not highlight any significant  
293 difference among cereal grains, whatever the producer or the day of production. Conversely,  
294 the DM content of the different CBFF types (Table 1) varied significantly. DM content was  
295 very low in porridges (*ben-kida* and *ben-saalga*) (5.7 to 8.3 g/100g). The DM contents of the  
296 three gelatinized doughs (*akassa*, *doncounou*, *kaffa*) varied greatly (from 11.1 to 30.2  
297 g/100g). The highest DM content (56.7-63.6 g/100g) was observed in fritters (*massa*). The  
298 DM content of *doncounou* and *massa* were significantly different among TPUs. A nested  
299 analysis of variance showed that for *doncounou*, this was due to inter-producer variability  
300 (88.6% of the variance) rather than inter-day variability (11.0% of the variance). Conversely,

301 for *massa*, 89.4% of variance was explained by inter-day variability, and only 10.5% by inter-  
302 producer variability.

303 The *massa* DM content (Table 1) included the lipid content brought by oil absorption during  
304 frying. The defatted DM content was also calculated, and was  $48.1 \pm 3.8$ ,  $50.0 \pm 4.4$  and  $45.1$   
305  $\pm 5.7$  g/100g for *massa* produced in TPU 1, 2 and 3, respectively.

306

### 307 **3.3. Folate content of raw materials and fermented foods**

308 Analysis of the folate content of raw materials and fermented foods (Table 1) showed  
309 significant differences among cereal types. The folate content of raw corn and sorghum ranged  
310 from 13.8 to 21.2  $\mu\text{g}/100\text{g}$  FW, whereas it was much higher in pearl millet (54.4 to 73.4  
311  $\mu\text{g}/100\text{g}$  FW). The corn grain color did not influence the folate content, because no significant  
312 difference was observed between white and yellow corn ( $15.7 \pm 3.0$   $\mu\text{g}/100\text{g}$  FW and  $17.5 \pm$   
313  $2.9$   $\mu\text{g}/100\text{g}$  FW, respectively). The folate content variability in pearl millet grains was mainly  
314 due to inter-producer variability (32.5% of variance) and inter-day variability (26.1% of  
315 variance).

316 Significant differences in total folate content were observed in the seven CBFF. A three-level  
317 nested analysis of variance showed that 84.0% of variance was due to inter-food variability,  
318 13.0% to inter-producer variability, and 1.9% to inter-day variability. Folate content was very  
319 low in porridges (*ben-kida* and *ben-saalga*: 1.8 to 2.9  $\mu\text{g}/100\text{g}$  FW), and also in gelatinized  
320 doughs (*akassa*, *doncounou*, and *kaffa*: 1.8 to 5.2  $\mu\text{g}/100\text{g}$  FW). The highest folate content was  
321 observed in dumplings (*fura*): 16.2 to 31.3  $\mu\text{g}/100\text{g}$  FW. Folate content in *massa* and *fura*  
322 significantly varied from one producer to another. A nested ANOVA showed that for *massa*  
323 this variability was due to both inter-producer and inter-day variability (52.5% and 37.7% of  
324 the variance, respectively), whereas for *fura* it was mostly explained by inter-producer  
325 variability (87.8%) rather than inter-day variability (6.4%). Indeed, the folate content of *fura*

326 produced in TPU 1 (the one that used sun drying instead of draining) was significantly lower  
327 than in the other TPUs.

328

### 329 **3.4. Influence of processing on CBFF folate content**

330 The first step of *akassa* processing (i.e., corn grain debranning and degerming) resulted in  
331 major folate losses because debranned corn contained only  $49.6 \pm 14.0\%$  of the folate detected  
332 in the initial grains (Table 6, in supplementary material). The folate content ranged from 13.8  
333 to  $24.3 \mu\text{g}/100\text{g DM}$  in whole-grain corn, and from 5.0 to  $12.8 \mu\text{g}/100\text{g DM}$  in debranned corn.  
334 Folate loss ranged from 23% to 66%, but no significant difference among TPUs was observed.  
335 Table 2 summarizes the folate loss or gain in the final food products compared with the raw  
336 materials. To avoid the influence of the various water amounts added during processing, the  
337 folate content of the different CBFF was expressed in  $\mu\text{g}/100\text{g DM}$ , and compared with that  
338 of cereal grains. The high standard deviations observed for some *massa* and *fura* samples  
339 were probably due to their variable dry matter contents. The highest folate loss was observed  
340 in fritters (*massa*; reduction between 59% and 76%), followed by porridges (reduction  
341 between 44% and 59% for all *ben-kida* and *ben-saalga* samples, regardless of the producer).  
342 Conversely, folate content in *kaffa* was increased by 66%. Folate content was greatly reduced  
343 (by 53%) in the dumplings made by the first *fura* producer, whereas no folate loss was  
344 observed in the *fura* made by the other producers. Likewise, folate content was not  
345 significantly different between raw material and CBFF in most *akassa* and *doncounou*  
346 samples, except for one *doncounou* producer (folate loss of 22%) and one *akassa* producer  
347 (folate gain of 78%).

348

### 349 **3.5. Impact of the different *ben-saalga* processing steps on folate content**

350 The changes in folate content distribution in the samples from the four TPUs (expressed as  
351 percentages of the pearl millet folate content, on a dry matter basis) (Figure 3A) indicated that  
352 with the exception of producer P2, the soaking step resulted in a significant decrease in folate  
353 content (20% to 49% loss, compared with raw pearl millet). This was partly due to folate  
354 diffusion in the soaking water (5% to 15%). The folate content in soaked grains varied from  
355  $24.2 \pm 4.1 \mu\text{g}/100\text{g FW}$  (for P4) to  $38.5 \pm 10.5 \mu\text{g}/100\text{g FW}$  (for P2). Folate loss in soaking  
356 water was higher for producer P2, compared with the other producers, probably due to the  
357 longer soaking time (from 24 to 48 h soaking for producer P2, compared with 10 h, 24 h, and  
358 22 h for producers P1, P3 and P4, respectively). After the soaking step, only two producers  
359 (P1 and P2) applied a washing step, which did not significantly affect folate content.  
360 Conversely, wet-milling resulted in a significant folate loss, compared with soaked grains,  
361 except for producer P4. Indeed, the paste of milled soaked grains contained only 41% to 61%  
362 of the folate initially present in pearl millet grains (Figure 3A). The sieving step also led to  
363 folate loss due to fractionation (draff removal). Indeed, all sieved batter samples contained  
364 significantly less folate than the pastes of milled grains (20% to 37% of the initial pearl millet  
365 folate content). The folate content varied from  $1.0 \pm 0.3$  to  $4.1 \pm 1.4 \mu\text{g}/100\text{g FW}$  in the  
366 sieved batter, and from  $8.3 \pm 2.2$  to  $11.7 \pm 3.2 \mu\text{g}/100\text{g FW}$  in draff. The sum of the amount of  
367 folate present in the sieved batter and in draff samples was significantly lower than the total  
368 amount of folate in milled grains (except for producer P3). This shows that folate losses also  
369 occurred due to other causes. The sieved batter was then left to rest at ambient temperature  
370 (decantation and fermentation) for 10 to 13 h (producers P1, P3 and P4) and for 24 h  
371 (producer P2). The folate content in the decanted paste (21% to 28% of the initial pearl millet  
372 folate) and in supernatant (6% to 18% of the initial pearl millet folate) samples was  
373 comparable among producers (Figure 3B). The sum of the folate present in the decanted paste  
374 and total supernatant (Decanted paste + TS) samples was significantly higher than the folate  
375 fraction in the sieved batter samples for producers P1 and P2. To prepare the final products,

376 producers used only part of the supernatant (used supernatant, US) to dilute the decanted paste  
377 and cook the dish (Decanted paste + US) to obtain *ben-saalga*. Then, *ben-saalga* was cooked  
378 for 5 to 10 min (producers P1 and P4) and for 15 to 20 min (producers P2 and P3). The  
379 cooking step did not significantly affect folate distribution. Nevertheless, for all producers,  
380 the cooked *ben-saalga* contained only 21% to 35% of the folate initially present in raw pearl  
381 millet grains.

382

### 383 **3.6. Bioaccessible folate and folate bioaccessibility**

384 Significant differences in bioaccessible folate content (expressed as  $\mu\text{g}/100\text{g}$  fresh weight)  
385 were observed among CBFF types (Table 3). A three-level nested analysis showed that 80.5%  
386 of total variance was due to inter-food variability, whereas inter-producer variability and  
387 inter-day variability accounted for 7.1% and 10.2% of variance, respectively. Bioaccessible  
388 folate was very low (0.4 to 3.2  $\mu\text{g}/100\text{g}$  FW) in porridges (*ben-kida*, *ben-saalga*) and most of  
389 the gelatinized doughs (*akassa*, *doncounou*, and *kaffa*). The highest bioaccessible folate  
390 content (4.5 to 8.6  $\mu\text{g}/100\text{g}$  FW) was observed in fritters (*massa*) and dumplings (*fura*);  
391 however, the bioaccessible folate content significantly varied among producers. Nested  
392 analyses of variance showed that for *fura*, 62.5% of the variance was due to inter-producer  
393 variability and 18.2% of the variance to inter-day variability. Conversely, for *massa*, 79.6% of  
394 the variance was due to inter-producer variability and only 16.4% to inter-day variability.

395 Folate bioaccessibility values also varied among the seven studied CBFF. The inter-food  
396 variability was the main contributor (73.8%) to the total variance, whereas the inter-producer  
397 and inter-day variability only accounted for 7.9% and 9.9% of variance, respectively. Folate  
398 bioaccessibility values were low (between 23% and 30%) for *akassa*, *kaffa* and *fura*, and  
399 average (between 30% and 57%) for the two porridges (*ben-kida*, *ben-saalga*). The highest  
400 values (up to 81%) were observed in *massa*. Folate bioaccessibility was much higher for  
401 *doncounou* (57-67%) than for *akassa* (24-29%), two gelatinized doughs made of corn.

402 The bioaccessible folate content was significantly correlated only with the DM content and the  
403 total folate content of the fermented foods ( $r = 0.80$  and  $r = 0.87$ , respectively).

404

### 405 **3.7. CBFF contribution to the recommended folate intake of infants**

406 The total folate contents were used to calculate the potential contribution of the seven CBFF  
407 to the recommended folate intake of children aged 6-35 months (Table 4). The estimated daily  
408 folate intakes from CBFF were 0.8 to 6.4  $\mu\text{g}/\text{day}$ , 2.7 to 15.2  $\mu\text{g}/\text{day}$ , and 7.9 to 34.6  $\mu\text{g}/\text{day}$ ,  
409 for children aged 6-11 months, 12-23 months, and 24-35 months, respectively. When taking  
410 into account the contribution of breast milk to the recommended folate intake for these three  
411 age categories, 30%, 69% and 100% of the folate intake should come from sources other than  
412 milk. However, the potential contribution of the seven studied CBFF to the recommended  
413 DFI was low, from 1% to 8%, 2% to 10%, and 5% to 23% for children aged 6-11 months, 12-  
414 23 months, and 24-35 months, respectively. When the bioaccessible folate contents were used  
415 to calculate the potential contribution of the seven CBFF to the recommended folate intake of  
416 young children, their potential contribution to the recommended DFI was even lower (from 0  
417 to 8%), (Table 5 in supplementary material).

418 Taking into account the folate intake from breast milk, the daily folate intake that should  
419 come from other food sources (DFI-OFP) was 23.9, 103.3, and 150.0  $\mu\text{g}/\text{day}$  for children  
420 aged 6-11 months, 12-23 months, and 24-35 months, respectively (Table 4). If the DFI-OFP  
421 were to come only from cereal-based fermented foods, the amount of the various CBFF that  
422 should be consumed to reach 100% of the recommended folate intakes would range from 93-  
423 1073 g/day for children aged 6-11 months to 584-6735 g/day for children aged 24-35 months,  
424 which would not be realistic.

425

#### 426 **4. Discussion**

427 CBFF are staple foods in many African countries (Nout, 2009). To the best of our knowledge,  
428 the influence of traditional processing on the folate content of CBFF commonly eaten in  
429 Africa had not been studied yet, except for *ben-saalga* (Saubade et al., 2018). Moreover,  
430 folate bioaccessibility in traditional African CBFF has never been investigated. The present  
431 study provides new information on CBFF frequently consumed in Burkina Faso. Nevertheless,  
432 our findings are also relevant for many other West African countries. Indeed, the analyzed food  
433 products are also frequently produced and consumed in Benin and Togo (*akassa, doncounou,*  
434 *massa, kaffa, ben-saalga* commonly called *koko* in Benin), Ghana (*fura, doncounou, kaffa*),  
435 Ivory Coast (*akassa, massa, ben-saalga* and *ben-kida* commonly called *baca* or *coco baca*),  
436 Niger (*kaffa, doncounou*), and Nigeria (*fura, massa* commonly called *wômi*) (Ayo et al.,  
437 2008; Greffeuille et al., 2010; Inyang & Zakari, 2008; Owusu-Kwarteng et al., 2012; Soro-  
438 Yao et al., 2013).

439 Considerable variations were observed in the folate content of the raw materials. Pearl millet  
440 grains displayed the highest folate content, and corn and sorghum the lowest folate content.  
441 The folate content of corn and sorghum was close to that of the West African Food  
442 Composition Table (WAFCT), whereas that of pearl millet was much higher than the one  
443 reported in the WAFCT (29-31  $\mu\text{g}/100\text{g}$  FW) and also in a recent study (Saubade et al.,  
444 2017b; Stadlmayr et al., 2012). This large variation in folate contents may be due to  
445 differences in grain cultivars, growing conditions, and storage conditions, as previously  
446 reported (Edelmann et al., 2013; Kariluoto et al., 2010).

447 Moreover, folate content was low in all studied CBFF, irrespective of the food type, producer  
448 and starting raw material. *Fura* exhibited the highest folate content (16 to 31  $\mu\text{g}/100\text{g}$  FW),  
449 and *ben-saalga, ben-kida* and *akassa* the lowest (1.8 to 3.1  $\mu\text{g}/100\text{g}$  FW). The difference in  
450 folate content between raw material and final food products indicates that part of the initial  
451 folate content was lost during food processing to obtain fritters (*massa*) and porridges (*ben-*

452 *saalga* and *ben-kida*). Conversely, for dumplings (*fura*), the folate loss during food processing  
453 depended on the producer (folate loss observed only for one producer). This suggests that  
454 folate was not degraded during *fura* processing and that the added water only ‘diluted’ the  
455 vitamins initially present in the grains. Alternatively, some loss might have occurred but  
456 folate was produced at one stage or another to counterbalance this loss, for example during  
457 the fermentation steps. Owusu-Kwarteng et al. (2012) described the diversity of LAB species  
458 involved in the traditional processing of *fura*, and some of these LAB might be able to  
459 produce folate (Saubade et al., 2017a).

460 Analysis of the CBFF process steps suggests that some procedures may particularly affect  
461 folate content. For instance, debranning performed during *akassa* processing particularly  
462 affected folate content (50% folate loss during this step, in average). This explains the  
463 significantly lower folate content in this dish compared with *doncounou*, another gelatinized  
464 dough produced from non-debranned corn grains. Indeed, vitamin B9 is mainly located in the  
465 outer layers and germ of cereal grains, and is largely lost during debranning/degerming  
466 (Liukkonen et al., 2003; Pomeranz, 1988). During grain soaking, folate could have been lost  
467 through leaching in soaking water (Witthöft et al., 1999). The longer soaking time (24-48 h)  
468 used by producer P2 during *ben-saalga* processing, compared with the other producers (10-24  
469 h soaking), might explain the higher folate content in the P2 soaking water sample. Folates  
470 are sensitive to heat, light, and oxidation. Sun drying, which is performed in the TPU yards,  
471 also could have affected folate content through the combined action of atmospheric oxygen  
472 and light (Preedy, 2013; Strandler et al., 2015). This could explain the significantly lower  
473 folate content of the *fura* dumplings produced in the TPU that included a very long sun drying  
474 step. Moreover, the CBFF under study were cooked (porridges), fried (fritters), or boiled in  
475 water (dumplings, gelatinized doughs). Cooking can lead to thermal and oxidative  
476 degradation of folates (Delchier et al., 2016; Witthöft et al., 1999). Specifically, frying can  
477 generate significant folate losses in various food products (Aramouni et al., 1986; Arcot et al.,

478 2002a; Hefni and Witthöft, 2014). Boiling in water (i.e., hydrothermal cooking) results in  
479 significant losses due to folate leaching from the food matrix into the cooking water (Delchier  
480 et al., 2016; Hefni and Witthöft, 2014).

481 The present work also shows that the folate content of the gelatinized doughs produced in two  
482 TPUs (*kaffa*, and the *akassa* made by producer P1) was significantly higher than that of their  
483 respective raw materials (sorghum and corn), suggesting folate production during the  
484 fermentation steps. Indeed, it was previously reported that fermentation positively influences  
485 folate content (Nout, 2009), due to the ability of yeast and some LAB to produce folate in  
486 fermenting foods (Moslehi-Jenabian et al., 2010; Saubade et al., 2017a). However, despite  
487 this folate content increase, the final folate content in these ready-to-eat foods was still very  
488 low, due to their low DM content.

489 Saubade et al. (2018) carried out a mass balance assessment during the processing of pearl  
490 millet grains into *ben-saalga*, but in controlled laboratory conditions. In the present study,  
491 although the final *ben-saalga* folate content was comparable to the 1.5 to 2.5 µg/100g FW  
492 reported by Saubade et al. (2018), the influence of the different processing steps was not  
493 similar. This highlights the difference between analyses in controlled time/temperature  
494 conditions and in the yard of TPUs. Specifically, Saubade et al. (2018) reported folate  
495 production (+26% to +31%) during pearl millet soaking, whereas in the TPUs folate was lost  
496 during the soaking step (-19% to -49%). In the TPU yard, grains are left in water at ambient  
497 temperature, exposed to sunlight and atmospheric oxygen, until the end of the soaking step (Tou  
498 et al., 2006). This can lead to folate oxidization or photo-degradation (Delchier et al., 2016;  
499 Preedy, 2013). Moreover, the soaking time was variable (10 to 24h) among TPUs, and might  
500 not have been sufficient to promote germination, which leads to folate synthesis (Kariluoto et  
501 al., 2006b). Saubade et al. (2018) found that the fermentation step did not have any significant  
502 effect on folate content. Conversely, in the present study, a significant gain of folate was

503 observed during the fermentation step in two TPUs, suggesting folate production by  
504 microorganisms present in the batter. Spontaneous fermentation can lead to variable microbial  
505 composition (Saubade et al., 2017a) due to the endogenous bacteria present in the cereals, food  
506 utensils and manual handling. This could explain the folate content variability in *ben-saalga*  
507 from the different TPUs. In *ben-saalga*, LAB are the predominant microorganisms during  
508 fermentation (Saubade et al., 2017b; Tou et al., 2006). LAB isolated from *ben-saalga* display a  
509 good genetic potential for folate synthesis, and some specific strains can produce folate during  
510 fermentation (Greppi et al., 2017). A recent study also showed that *ben-saalga* folate content  
511 could be increased by using back-slopping or starter cultures combined with amylolytic LAB  
512 strains (Bationo et al., 2019b).

513 Folate bioaccessibility in CBFF was very variable (from 23% to 81%) depending on the food  
514 product. Bioaccessibility could have been influenced by i) the total folate content in non-  
515 digested foods; ii) the food product structure that governs the digestion patterns of the  
516 carbohydrate fractions in cereal foods (Lovegrove et al., 2017; Mishra & Monro, 2012) and  
517 might influence the release of folate from food matrices; and iii) the stability of folate vitamers  
518 during the digestion process. Ringling & Rychlik (2017) also observed a noticeable variability of  
519 folate bioaccessibility in various food matrices. They found that this variability was due to  
520 differences in the food product composition and in folate vitamer distribution within the food  
521 products. Specifically, they observed differences in vitamer stability during *in vitro* digestion  
522 that affected their relative bioaccessibility. For example, tetrahydrofolate was completely lost  
523 during the digestion simulations. In the current study, the seven CBFF could be classified in  
524 groups that behaved differently during digestion and presented distinct folate bioaccessibility  
525 profiles:

- 526 • Porridges were characterized by very low bioaccessible folate (< 1.5 µg/100g FW), due to  
527 the low total folate content and moderate bioaccessibility ratios (30% to 57%). In these  
528 semi-liquid foods, most folate was already in a solubilized form and should have been

529 bioaccessible. However, porridges had little structural resilience in digestion conditions,  
530 and folate was not protected by the food matrix during the gastric phase. As many folate  
531 vitamers are sensitive to low pH, the observed moderate bioaccessibility ratios could be  
532 due to loss of labile folate in acidic gastric conditions (Ringling et al., 2017; Seyoum &  
533 Selhub, 1998; Strandler et al., 2015).

534 • Gelatinized doughs made from debranned grains (*akassa*, *kaffa*) displayed low bioaccessible  
535 folate (0.4-1.6  $\mu\text{g}/100\text{g}$  FW) and low bioaccessibility (23-30%). Folate bioaccessibility was  
536 similarly low in dumplings (*fura*), but bioaccessible folate was much higher (4-9  $\mu\text{g}/100\text{g}$   
537 FW) due to a higher initial total folate content. In these three structurally weak foods, the  
538 starchy matrix hydrolysis started during the oral phase of digestion, possibly leading to the  
539 degradation of non-protected folate during the gastric phase.

540 • The two CBFF with the highest folate bioaccessibility ratio were both made from whole-  
541 grain cereals, and had either a dense semi-solid structure (*doncounou*, 57-67%  
542 bioaccessibility) or a solid structure (*massa*, 67-81% bioaccessibility). These dense  
543 matrices provided secondary structure and generated particles that were resistant to  
544 digestion (Mishra & Monro, 2012), and persisted long enough to protect folate from the  
545 acidic gastric conditions. Moreover, it is known that micronutrient encapsulation in the  
546 plant cell walls modulates the rate and extent of nutrient release during digestion (Parada &  
547 Aguilera, 2007). Therefore, the presence of bran particles in *massa* and *doncounou* may  
548 have protected folate during the gastric phase, and allowed a higher proportion of folate to  
549 reach the intestinal phase (neutral pH) in a non-degraded form.

550 In many West African countries, traditional millet-, sorghum- and maize-based fermented  
551 dishes are frequently consumed by young children (Greffeuille et al., 2010). Porridges are  
552 mainly consumed as foods to complement breast milk. Then, when children can eat family  
553 dishes, porridges are progressively replaced by thicker starchy staple foods (such as  
554 gelatinized doughs) that are eaten with various sauces. Snacks, such as fritters or dumplings,

555 are given more regularly (Hama, 2012). Our study shows that, whatever the age group, the  
556 contribution of the studied CBFF to the recommended folate intake was far from sufficient to  
557 cover the folate requirement of young children. By taking into account the food intake  
558 reported by Hama (2012), 6-11-month-, 12-23-month- and 24-35-month-old children would  
559 get respectively 1-8%, 2-10% and 5-23% of the recommended folate intake through CBFF  
560 consumption. Moreover, the potential contribution of the seven studied CBFF to the  
561 recommended DFI was limited: up to 23% when considering the total folate contents, and  
562 only up to 8% when considering the bioaccessible folate contents. This suggests that many  
563 children might be at risk of folate deficiency, especially non-breastfed children, if their diet  
564 were mainly based on CBFF. However, in Burkina Faso and other West African countries,  
565 most of these CBFF are consumed with other food products with higher folate contents, such  
566 as sauces made from green leafy vegetables and legumes (e.g., groundnut or soybean)  
567 (Greffeuille et al., 2010).

568

## 569 **5. Conclusion**

570 This work generated new information on the influence of traditional processing on the folate  
571 content and the first data on folate bioaccessibility in seven CBFF commonly consumed in  
572 West Africa that can be useful to complete the existing food composition tables. The total  
573 folate and bioaccessible folate contents of the seven studied products (gelatinized doughs,  
574 porridges, dumplings, fritters) were rather low, suggesting that populations whose diet is  
575 mainly based on these CBFF may be at risk of folate deficiency. The folate content of most of  
576 the food products was strongly and negatively affected by the laborious processing. Some  
577 processing steps could be adapted to limit such losses (controlled volumes, controlled soaking  
578 and fermentation times, and limitation of direct exposure to sunlight). Another way to  
579 increase the folate content in CBFF could be to promote folate production during the  
580 fermentation step, by using folate-producing bacterial strains. Finally, the observed

581 differences in folate bioaccessibility ratios among the studied food products suggest a strong  
582 effect of the food matrix that should be investigated more precisely. The influence of  
583 processing on folate bioaccessibility in other folate-rich foods frequently consumed by young  
584 children together with CBFF (*i.e.*, green leafy vegetables, legumes) should also be analyzed.

585

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594

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787 **Figure captions**

788

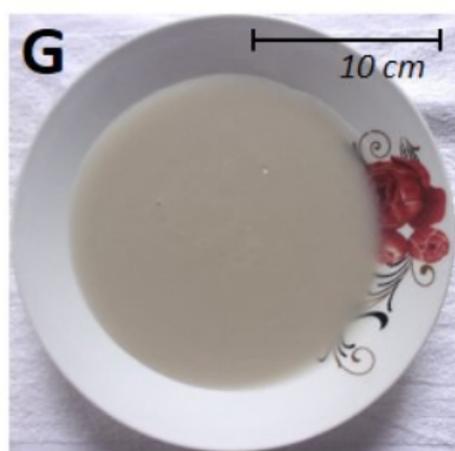
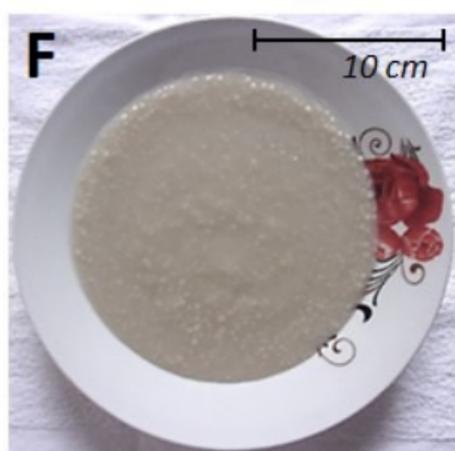
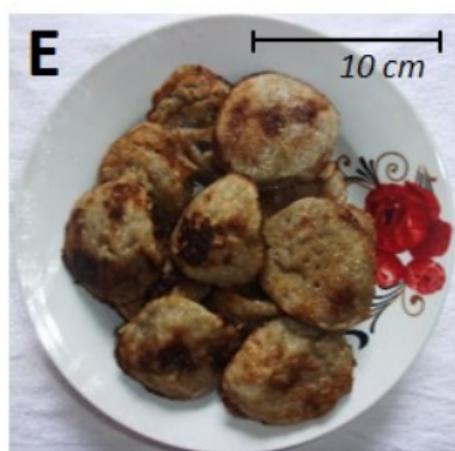
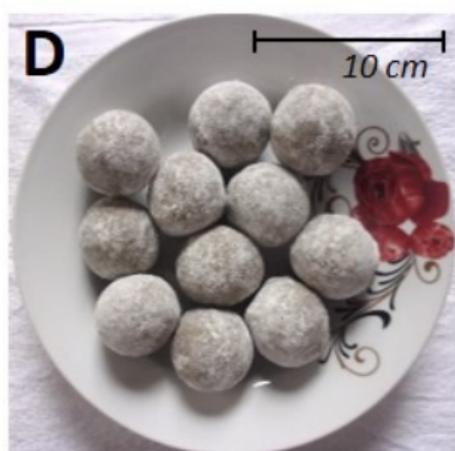
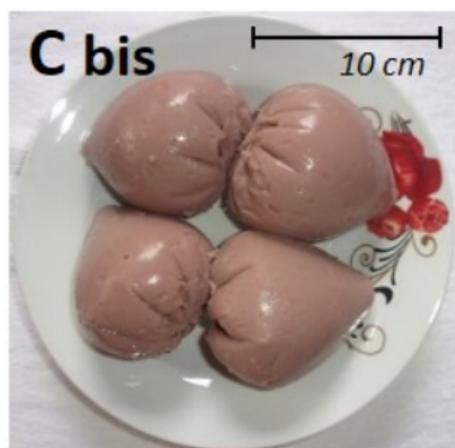
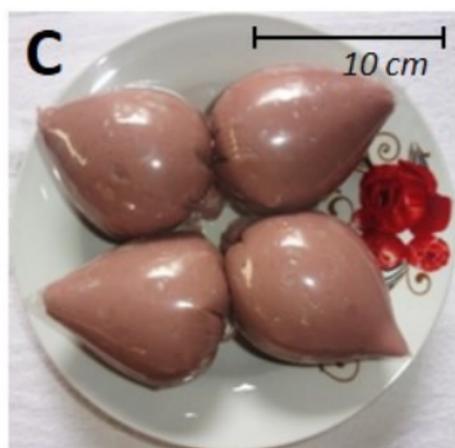
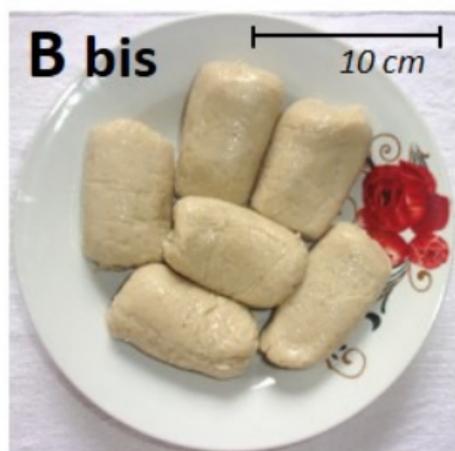
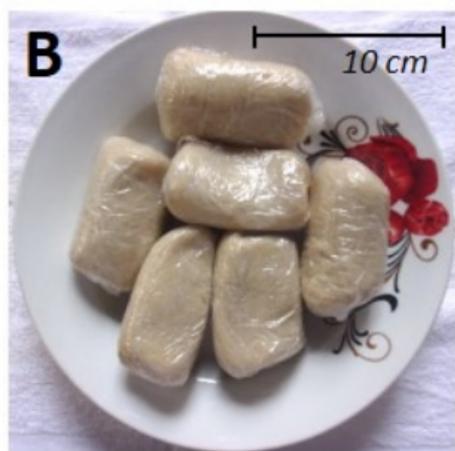
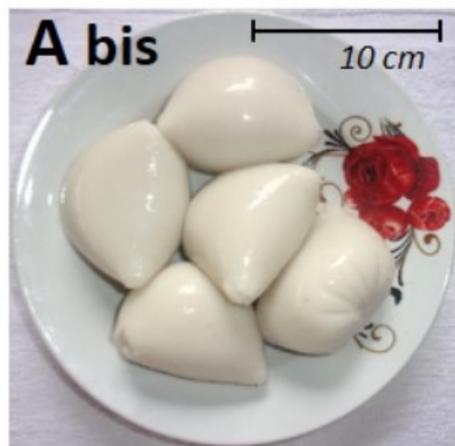
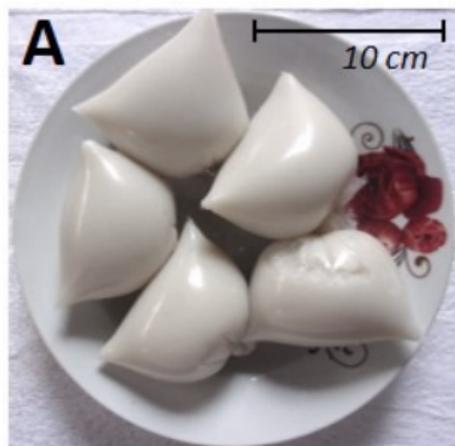
789 **Figure 1.** Photos of the seven studied cereal-based fermented foods. A, B and C: *akassa*,  
790 *doncounou* and *kaffa* as sold (*i.e.*, wrapped in plastic bags); A-bis, B-bis and C-bis:  
791 *akassa*, *doncounou* and *kaffa* as eaten; D: *fura*; E: *massa*; F: *ben-kida*; G: *ben-*  
792 *saalga*.

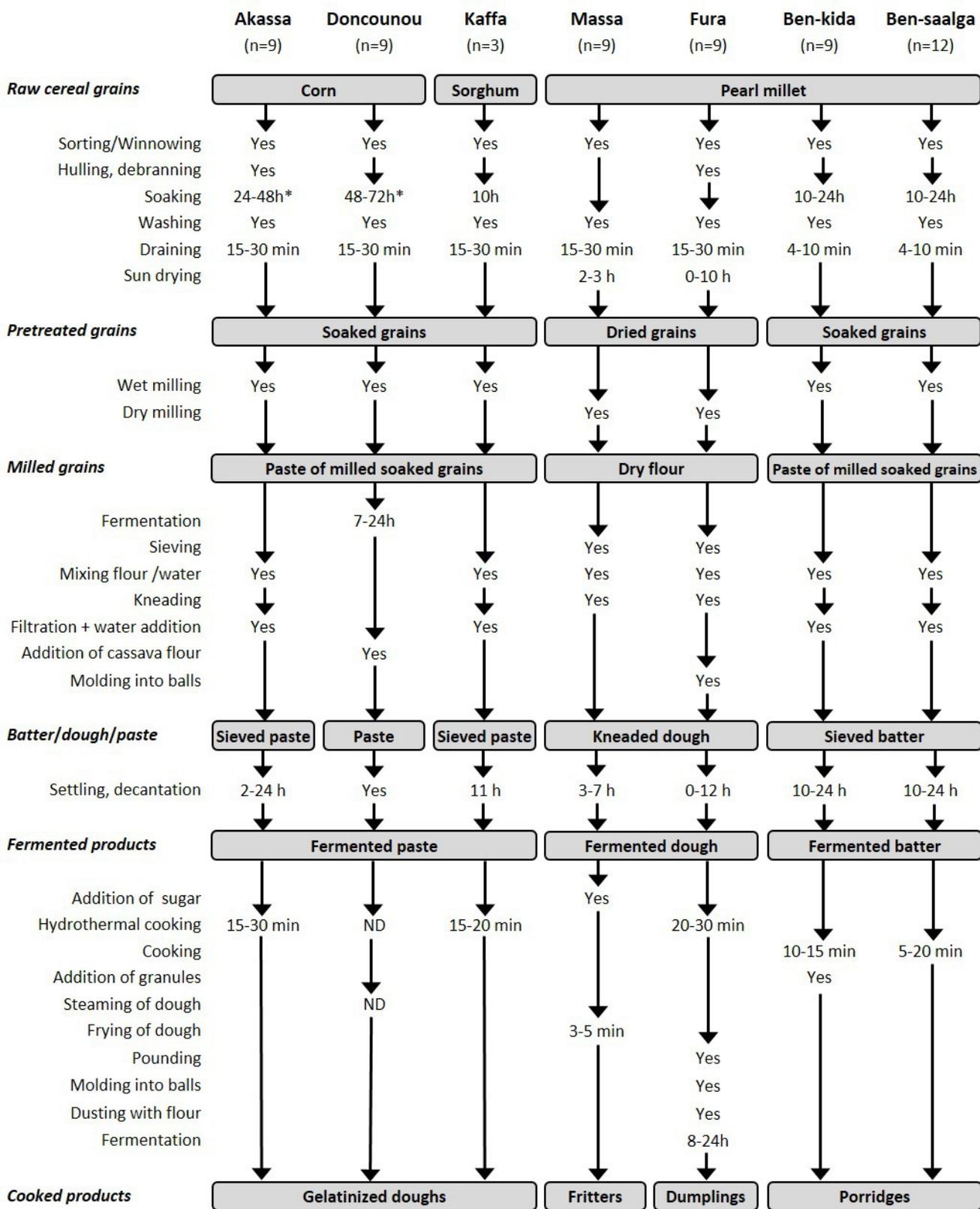
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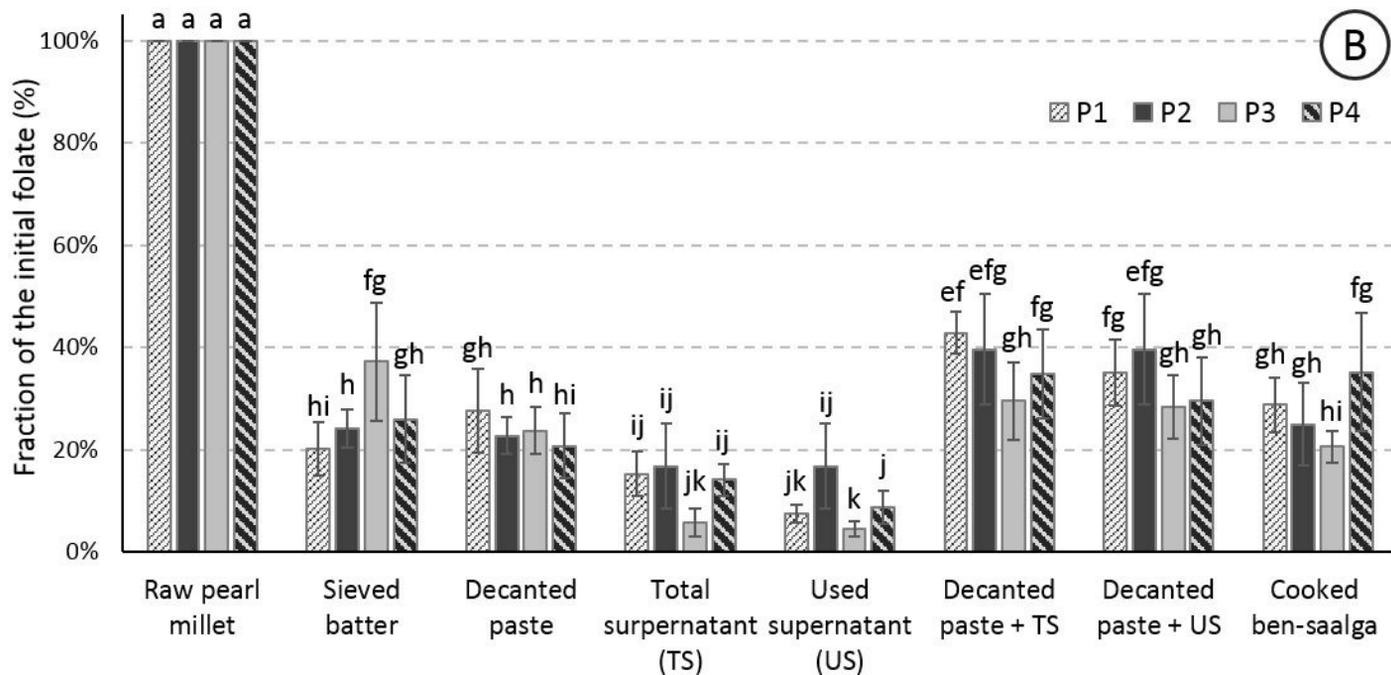
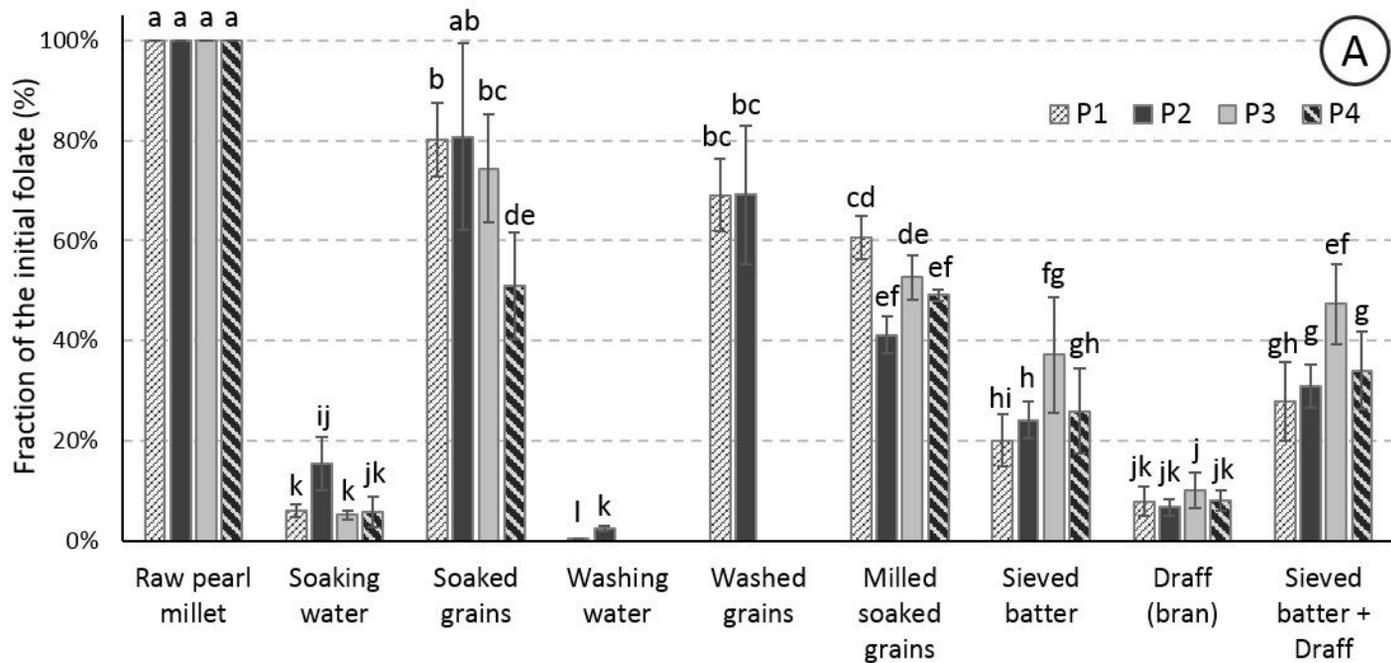
794 **Figure 2.** Processing of cereal-based fermented foods in traditional processing units. “Yes”  
795 indicates that the corresponding step was carried out. When available, the step  
796 duration was indicated. ND: duration not determined; n: number of samples. \*:  
797 Soaking water was renewed daily.

798

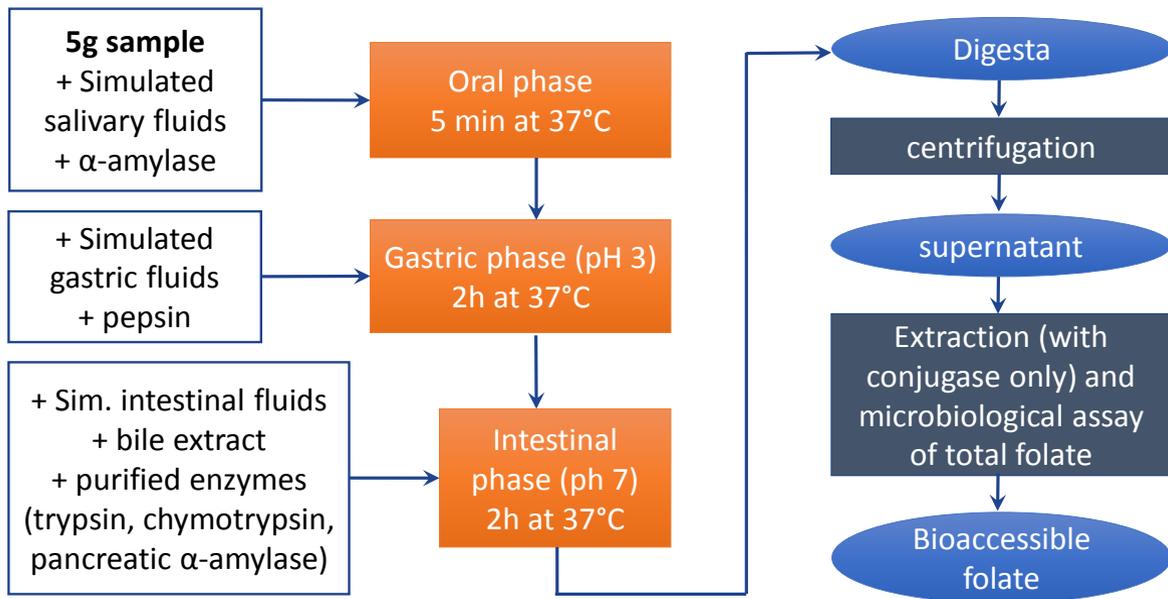
799 **Figure 3.** Changes in folate content distribution during the steps of the traditional processing  
800 of pearl millet into *ben-saalga*, in different production units (P1 to P4): percentage  
801 of the folate content of the original raw pearl millet grains, on a dry matter basis. A:  
802 from the raw material to the fractions after sieving; B: from the sieved batter to the  
803 final fermented and cooked product. Values are the mean of three samples collected  
804 on different days; bars indicate the standard deviations, and different letters indicate  
805 significant differences ( $p < 0.05$ ) in folate distribution among samples.







Bationo F., Humblot C., Songré Ouattara L.T., Hama Ba F., Le Merrer M., Chapron M., Kariluoto S., Hemery Y., 2019. Total folate in West-African cereal-based fermented foods: bioaccessibility and influence of processing. *Journal of Food Composition and Analysis*.



**Figure 4. Flow diagram of a simulated *in vitro* digestion method adapted from the method described by Minekus et al. (2014).**

**Table 1. Dry matter content and total folate content in raw materials and fermented foods**

Food products (and respective raw materials)	Producer	Dry matter content (g/100g FW)		Total folate content (µg/100g FW)	
		Raw materials	Fermented foods	Raw materials	Fermented foods
<i>Akassa</i> (gelatinized dough, corn)	P1	89.7 ± 0.5	11.4 ± 1.1 <sup>b</sup>	13.8 ± 1.3 <sup>j</sup>	3.1 ± 0.8 <sup>op</sup>
	P2	89.9 ± 0.1	11.1 ± 0.2 <sup>b</sup>	16.8 ± 1.3 <sup>jk</sup>	1.8 ± 0.3 <sup>o</sup>
	P3	89.6 ± 0.4	13.0 ± 1.1 <sup>b</sup>	21.2 ± 1.4 <sup>k</sup>	2.7 ± 0.4 <sup>o</sup>
<i>Doncounou</i> (gelatinized dough, corn)	P1	88.4 ± 0.7	24.1 ± 0.9 <sup>d</sup>	15.7 ± 2.4 <sup>j</sup>	4.7 ± 0.8 <sup>p</sup>
	P2	88.8 ± 0.6	26.6 ± 1.6 <sup>e</sup>	14.8 ± 2.0 <sup>j</sup>	4.8 ± 0.6 <sup>p</sup>
	P3	90.4 ± 2.4	30.2 ± 0.1 <sup>f</sup>	13.8 ± 1.7 <sup>j</sup>	3.6 ± 0.6 <sup>p</sup>
<i>Kaffa</i> (gelatinized dough, sorghum)	P1	88.9 ± 0.4	17.4 ± 3.1 <sup>c</sup>	16.3 ± 2.6 <sup>jk</sup>	5.2 ± 1.3 <sup>pq</sup>
<i>Massa*</i> (batter fritters, pearl-millet)	P1	89.4 ± 0.7	63.6 ± 8.1 <sup>i</sup>	57.2 ± 3.7 <sup>l</sup>	7.2 ± 1.3 <sup>q</sup>
	P2	89.8 ± 0.8	56.7 ± 3.8 <sup>h</sup>	72.6 ± 4.0 <sup>n</sup>	10.2 ± 1.0 <sup>qr</sup>
	P3	89.9 ± 0.4	62.2 ± 6.2 <sup>i</sup>	63.3 ± 3.9 <sup>lm</sup>	12.6 ± 3.2 <sup>rs</sup>
<i>Fura</i> (dumplings, pearl-millet)	P1	89.6 ± 0.3	42.5 ± 1.9 <sup>g</sup>	73.2 ± 3.9 <sup>n</sup>	16.2 ± 1.2 <sup>s</sup>
	P2	89.7 ± 0.8	42.2 ± 3.1 <sup>g</sup>	65.4 ± 3.7 <sup>mn</sup>	31.3 ± 2.5 <sup>t</sup>
	P3	89.1 ± 1.0	42.6 ± 1.2 <sup>g</sup>	64.0 ± 9.7 <sup>mn</sup>	29.6 ± 4.1 <sup>t</sup>
<i>Ben-kida</i> (porridge, pearl- millet)	P1	90.0 ± 0.6	8.3 ± 0.9 <sup>a</sup>	54.4 ± 4.2 <sup>l</sup>	2.5 ± 0.4 <sup>o</sup>
	P2	90.5 ± 0.7	6.1 ± 0.7 <sup>a</sup>	65.3 ± 7.0 <sup>mn</sup>	2.5 ± 0.3 <sup>o</sup>
	P3	90.4 ± 0.5	7.9 ± 0.7 <sup>a</sup>	59.3 ± 6.9 <sup>l</sup>	2.5 ± 0.8 <sup>o</sup>
<i>Ben-saalga</i> (porridge, pearl- millet)	P1	89.0 ± 0.7	5.8 ± 0.4 <sup>a</sup>	62.6 ± 6.3 <sup>lm</sup>	1.9 ± 0.3 <sup>o</sup>
	P2	90.0 ± 0.3	6.6 ± 1.9 <sup>a</sup>	70.3 ± 6.9 <sup>mn</sup>	2.9 ± 1.3 <sup>op</sup>
	P3	89.3 ± 0.9	5.7 ± 0.6 <sup>a</sup>	68.6 ± 7.4 <sup>mn</sup>	1.8 ± 0.2 <sup>o</sup>
	P4	89.2 ± 0.1	6.6 ± 0.1 <sup>a</sup>	73.4 ± 8.2 <sup>n</sup>	2.4 ± 1.0 <sup>o</sup>

Results are the mean ± standard deviations (for each producer, samples were collected on three different days, and analyzed in triplicate). ANOVA: In each column, values with no common letter are considered significantly different ( $p < 0.05$ ). No significant differences were observed for the dry matter content of raw materials. \*For *massa* samples, the results are given in g or µg /100g FW of food “as eaten” (*i.e.*, non-defatted samples).

**Table 2. Folate loss or gain during the processing of cereal grains into fermented foods**

Food products (and raw materials)	Producer	Folate content ( $\mu\text{g}/100\text{g DM}$ )		Folate loss or gain (%)
		Raw material	Processed food	
<i>Akassa</i> (gelatinized dough, corn)	P1	15.4 $\pm$ 1.5	27.5 $\pm$ 6.2	+78% *
	P2	18.7 $\pm$ 1.4	16.6 $\pm$ 3.0	-11%
	P3	23.6 $\pm$ 1.5	20.6 $\pm$ 4.1	-13%
<i>Doncounou</i> (gelatinized dough, corn)	P1	17.8 $\pm$ 2.8	19.6 $\pm$ 3.5	10%
	P2	16.7 $\pm$ 2.2	17.9 $\pm$ 1.8	7%
	P3	15.2 $\pm$ 1.9	11.8 $\pm$ 1.9	-22% *
<i>Kaffa</i> (gelatinized dough, sorghum)	P1	18.3 $\pm$ 2.9	30.4 $\pm$ 7.3	+66% *
<i>Massa</i> (batter fritters, pearl-millet) <sup>a</sup>	P1	63.9 $\pm$ 3.9	15.1 $\pm$ 3.3	-76% *
	P2	80.8 $\pm$ 4.5	20.5 $\pm$ 1.8	-75% *
	P3	70.4 $\pm$ 4.4	28.9 $\pm$ 9.6	-59% *
<i>Fura</i> (dumplings, pearl-millet)	P1	81.7 $\pm$ 4.3	38.1 $\pm$ 3.5	-53% *
	P2	72.9 $\pm$ 4.1	74.5 $\pm$ 6.9	2%
	P3	71.9 $\pm$ 11.2	69.7 $\pm$ 10.4	-3%
<i>Ben-kida</i> (porridge, pearl-millet)	P1	60.5 $\pm$ 4.4	30.8 $\pm$ 5.8	-49% *
	P2	72.1 $\pm$ 7.6	40.4 $\pm$ 4.1	-44% *
	P3	65.7 $\pm$ 7.8	32.0 $\pm$ 1.2	-51% *
<i>Ben-saalga</i> (porridge, pearl-millet)	P1	70.3 $\pm$ 7.4	32.3 $\pm$ 7.2	-54% *
	P2	78.1 $\pm$ 7.8	41.5 $\pm$ 9.5	-47% *
	P3	76.9 $\pm$ 8.1	31.8 $\pm$ 5.2	-59% *
	P4	82.3 $\pm$ 9.3	36.2 $\pm$ 1.5	-56% *

Results are the mean  $\pm$  standard deviations (samples collected on three different days for each producer, and analyzed in triplicate). \*: Significant difference of folate content between raw material and processed fermented food product (ANOVA,  $p < 0.05$ ). <sup>a</sup>: For *massa* (fritters made from pearl millet batter), the defatted dry matter was used for the calculation, to take into account the absorption of oil during frying.

**Table 3. Bioaccessible folate and folate bioaccessibility in fermented food products**

Food product	Producer	Bioaccessible folate ( $\mu\text{g}/100\text{g FW}$ )	Folate bioaccessibility (%)
<i>Akassa</i> (gelatinized dough, corn)	P1	0.9 $\pm$ 0.4 <sup>a</sup>	29.1 $\pm$ 6.8 <sup>i</sup>
	P2	0.4 $\pm$ 0.1 <sup>a</sup>	23.6 $\pm$ 5.4 <sup>i</sup>
	P3	0.7 $\pm$ 0.2 <sup>a</sup>	26.4 $\pm$ 6.2 <sup>i</sup>
<i>Doncounou</i> (gelatinized dough, corn)	P1	3.2 $\pm$ 0.8 <sup>c</sup>	66.9 $\pm$ 10.9 <sup>n</sup>
	P2	2.8 $\pm$ 0.5 <sup>c</sup>	57.4 $\pm$ 7.6 <sup>lm</sup>
	P3	2.2 $\pm$ 0.5 <sup>bc</sup>	62.4 $\pm$ 11.1 <sup>mn</sup>
<i>Kaffa</i> (gelatinized dough, sorghum)	P1	1.6 $\pm$ 0.4 <sup>ab</sup>	29.8 $\pm$ 4.5 <sup>i</sup>
<i>Massa</i> (batter fritters, pearl-millet)	P1	5.6 $\pm$ 0.5 <sup>e</sup>	77.6 $\pm$ 5.8 <sup>o</sup>
	P2	8.3 $\pm$ 1.3 <sup>fg</sup>	80.9 $\pm$ 7.7 <sup>o</sup>
	P3	8.6 $\pm$ 3.2 <sup>g</sup>	66.8 $\pm$ 13.3 <sup>n</sup>
<i>Fura</i> (dumplings, pearl-millet)	P1	4.5 $\pm$ 0.8 <sup>d</sup>	27.7 $\pm$ 4.9 <sup>i</sup>
	P2	7.4 $\pm$ 1.5 <sup>f</sup>	23.5 $\pm$ 4.9 <sup>i</sup>
	P3	8.2 $\pm$ 1.6 <sup>fg</sup>	27.8 $\pm$ 3.9 <sup>i</sup>
<i>Ben-kida</i> (porridge, pearl-millet)	P1	1.1 $\pm$ 0.2 <sup>ab</sup>	45.5 $\pm$ 10.7 <sup>jk</sup>
	P2	1.1 $\pm$ 0.2 <sup>ab</sup>	43.5 $\pm$ 9.0 <sup>j</sup>
	P3	1.4 $\pm$ 0.6 <sup>ab</sup>	55.7 $\pm$ 10.8 <sup>lm</sup>
<i>Ben-saalga</i> (porridge, pearl-millet)	P1	1.1 $\pm$ 0.3 <sup>ab</sup>	57.3 $\pm$ 7.5 <sup>lm</sup>
	P2	1.2 $\pm$ 0.4 <sup>ab</sup>	45.7 $\pm$ 8.1 <sup>jk</sup>
	P3	1.0 $\pm$ 0.2 <sup>a</sup>	52.9 $\pm$ 10.9 <sup>kl</sup>
	P4	0.6 $\pm$ 0.1 <sup>a</sup>	30.1 $\pm$ 8.8 <sup>i</sup>

Results are the mean  $\pm$  standard deviations (samples collected on three different days for each producer, and analyzed in quadruplicate). ANOVA: in each column, values without common letters are considered significantly different ( $p < 0.05$ ).

Folate bioaccessibility (%) = Bioaccessible folate / Total folate \* 100

**Table 4. Potential contribution of West-African cereal-based fermented foods to the recommended folate intakes for children aged 6-35 months**

	Daily CBFF folate intake (µg/day)	CBFF contribution to the RNI <sup>a</sup> (% of RNI)	Recommended folate intake (RNI) <sup>b</sup> (µg/day)	Daily breast milk intake <sup>c</sup> (ml/day)	Daily folate intake from breast milk <sup>d</sup> (µg/day)	Contribution of breast milk to RNI (% of RNI)	Folate intake from foods to reach 100% RNI <sup>e</sup> (µg/day)	CBFF intake needed to reach 100% RNI <sup>f</sup> (g/day)
<b>6-11 months (breastfed)</b>								
<i>Akassa</i>	3.2	4%						940
<i>Doncounou</i>	2.5	3%						549
<i>Kaffa</i>	4.6	6%						456
<i>Massa</i>	0.8	1%	80	660	56.1	70%	23.9	239
<i>Fura</i>	1.4	2%						93
<i>Ben-kida</i>	6.2	8%						961
<i>Ben-saalga</i>	6.4	8%						1073
<b>12-23 months (breastfed)</b>								
<i>Akassa</i>	10.8	7%						4066
<i>Doncounou</i>	8.2	5%						2372
<i>Kaffa</i>	15.2	10%						1973
<i>Massa</i>	2.7	2%	150	550	46.7	31%	103.3	1032
<i>Fura</i>	4.3	3%						402
<i>Benk-ida</i>	4.8	3%						4154
<i>Ben-saalga</i>	5.0	3%						4640
<b>24-35 months (non-breastfed)</b>								
<i>Akassa</i>	24.6	16%						5902
<i>Doncounou</i>	18.7	12%						3443
<i>Kaffa</i>	34.6	23%						2864
<i>Massa</i>	8.2	5%	150	0	0	0%	150.0	1499
<i>Fura</i>	14.0	9%						584
<i>Ben-kida</i>	7.9	5%						6029
<i>Ben-saalga</i>	8.2	5%						6735

<sup>a</sup> Contribution of CBFF to RNI calculated using the daily intakes and daily intake frequencies reported by Hama (2012) for various CBFF, and for the indicated age groups. <sup>b</sup> RNI values, as reported by FAO/WHO (2004). <sup>c</sup> Daily intake of breast milk as reported by (WHO, 1998). <sup>d</sup> Folate intake from breast milk based on a folate content of 85 µg/L in breast milk (Allen, 2012). <sup>e</sup> Folate intake needed from other food products than breast milk to reach the RNI. <sup>f</sup> Daily CBBF intake that would be needed if only breast milk and CBFF were consumed.