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

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

1. Introduction

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

Cereals are the main crops in Africa (45% of arable land), and make an important contribution to the human diet in this continent, due to the high consumption of cereal-based products (Galati et al., 2014). They cannot be eaten raw, and are processed before consumption. Fermentation is widely used in many West African countries to transform cereals (sorghum, maize and millet) into edible products (Guyot, 2012). Traditional cereal-based fermented foods (CBFF), such as porridges or gelatinized doughs, are frequently consumed by young children in West Africa (Greffeuille et al., 2010; Hama, 2012). Fermentation is one of the oldest ways to preserve food products, and to enhance their sanitary and nutritional quality through the production of numerous nutritional and anti-microbial substances by fermenting microorganism (Nout, 2009). Fermentation is also considered as a good way to increase the folate content of food products, because yeast and many lactic acid bacteria (LAB) can synthesize folate during

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{figure1.png}
\caption{Folate content of CBFF and other food products.}
\end{figure}

\textbf{Abbreviations:} ANOVA, analysis of variance; AOAC, Association of Official Analytical Chemists; CBFF, cereal-based fermented food; CFC, cereal-based fermented food folate contents; DF, daily frequency; DFI, daily folate intake; DFI-OFP, daily folate intake needed from other food products; DM, dry matter; FAO, Food and Agriculture Organization of the United Nations; FW, fresh weight; LAB, lactic acid bacteria; PS, portion size; RNI, recommended nutrient intake; SGF, simulated gastric fluid; SIF, simulated intestinal fluid; SSF, simulated salivary fluid; TPU, traditional production unit; WAFCT, West African food composition table; WHO, World Health Organization

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fermentation (Moslehi-Jenabian et al., 2010; Saubade et al., 2017a). Previous works have reported the isolation of folate-producing strains from different cereals (Capozzi et al., 2012; Salvucci et al., 2016). Moreover, studies on CBFF that are commonly consumed in industrialized countries, such as rye, wheat and oat sourdoughs, have shown that fermentation can improve their folate content (Kariluoto et al., 2004, 2006a, Laiño et al., 2013; Jägerstad et al., 2005).

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

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

Therefore, the aim of this study was to provide information on the influence of processing methods of African CBFF on their total folate content and folate bioaccessibility. It also allowed evaluating the potential contribution to the recommended folate intake of young children of these CBFF that are frequently consumed in Burkina Faso and other West African countries (i.e., porridges, fritters, gelatinized doughs, dumplings).

2. Material and methods

2.1. Materials

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

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

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

For five other CBFF (akassa, doncounou, massa, fura, and ben-kida), samples of raw materials (i.e., grains of pearl millet, or corn) and final products (fermented foods, as eaten by consumers) were collected in three different TPUs for each food type. Moreover, in each TPU, samples were collected on three different days. The process steps were not followed in details, but were listed by recording the producers’ declarations, including estimations of the fermentation and cooking times. For kaffa, the least commonly eaten CBFF, samples of raw material (sorghum) and final product were collected on three different days from only one TPU.

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

2.1.3. Chemicals and enzymes

Porcine pancreatic α-amylase (E-PANAA, #150402, 110.8 U/mg) was purchased from Megazyme (Bray, Ireland). Chicken pancreas γ-glutamylhydrolase (P2002) was obtained from R-Biopharm. Protease from Streptomyces griseus (P5147, 3.5 units/mg), Aspergillus oryzae alpha-amylase (A9857, #SLBM0836 V, 50.4 U/mg), pepsin from porcine gastric mucosa (P7000, #BCBF9832 V, 536 U/mg), bovine pancreatic trypsin (T0303, #SLBR6593 V, 160.6 U/mg) and chymotrypsin (C4129, #SLBG2821 V, 40 U/mg), and bovine and ovine bile extracts (B8381, #SLBH1720 V, 881.3 g/mol) were purchased from Sigma-Aldrich (St Louis, MI, USA). All the enzymes were stored at −20 °C. All other used chemicals were of analytical grade.

2.2. Methods

2.2.1. Ben-saalga mass balance assessment

The traditional processing of pearl millet into ben-saalga has been described before (Tou et al., 2006). To follow the folate losses and gains during ben-saalga processing, and to monitor folate distribution in pearl millet fractions, a mass balance assessment was carried out at each TPU, as described by Saubade et al. (2018). Briefly, the weight of all utensils and sampled products (samples taken for folate analysis, raw materials, intermediate products, by-products, and final products) was recorded at
each step of the process. The dry matter (DM) content of all samples was measured, then each sample weight and DM content were combined with the folate content to calculate the amount of folate (in μg) in the original pearl millet grains, and in the intermediate and final products. The folate content distribution in the intermediate and final products was expressed as the percentage of the initial folate quantity in pearl millet grains.

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

The DM content of all samples was measured by drying the samples in an air-circulating oven at 105°C in open dishes until constant weight (overnight, ~16 h). Samples were then cooled in a desiccator and weighted, and the DM content was expressed as g DM/100 g sample (ICC Standard methods, 2003).

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

#### 2.2.3. Determination of total folate content

Total folate content was determined in triplicate using the microbiological assay described by Karluto and Piironen (2009) and adapted by Saubade et al. (2018). Briefly, thawed samples were diluted in extraction buffer and heated at 100°C for 10 min. After adjusting the pH to 7.2, a tri-enzymatic extraction with α-amylase (20 mg/ml in 1% sodium ascorbate), chicken pancreas γ-glutamylhydrolase (100 mg/ml in Milli-Q water) and protease (3 mg/ml in 1% sodium ascorbate) was used to release folate from the food matrix. After appropriate dilutions of the supernatants, folate concentration was determined in microtiter plates with Lactobacillus rhamnosus ATCC 7469 as the growth indicator organism, folic acid (Sigma-Aldrich F7876) as the calibration standard, and Folic Acid Casei Medium (Difco, Sparks, MD, USA) as the assay medium. The method performance was confirmed by analyzing a certified reference material (BCR 121 Wholemeal flour, IRMM, Geel, Belgium) for each incubation. The certified folate value for this reference material was 500 ± 70 ng/g DM, and in this study, we obtained an average content of 512 ± 57 ng/g dm (n = 38). A blank sample (that contained all the reagents, but no food sample) was also analyzed in each series of samples, and the folate value obtained for the blank sample was deducted from the value obtained for the samples, to take into account the folate brought by the enzymes.

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

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

In a 50 ml Falcon tube, 5 g of roughly ground sample was mixed with 4 ml of SSF containing α-amylase, 25 μl of CaCl₂ solution and 975 μl of Milli-Q water to reach a final ratio of food/ reagents of 50 : 50 (W/V). The mixture was incubated at 37°C in a shaking water bath for 2 min. Then, 8 ml of SGF with pepsin and 5 μl of CaCl₂ solution were added, and the pH was adjusted to 3 with 6 M HCl. After adjusting the volume to 20 ml with Milli-Q water, the mixture was incubated in a shaking water bath at 37°C for 2 h. To stimulate intestinal digestion, 10 ml of SIF containing bile extract, 6 ml of SIF containing pancreatic α-amylase, 1 ml trypsin solution, 1 ml chymotrypsin solution, and 40 μl of CaCl₂ solution were added. After adjusting the pH to 7 with 2 M NaOH, samples were incubated in a shaking water bath at 37°C for 2 h. Finally, samples were centrifuged at 8000 g at 4°C for 30 min, and supernatants (i.e., the digesta) were stored at ~20°C for bioaccessible folate analysis. All samples were digested in duplicates.

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

Bioaccessible folate (in μg/100 g fresh weight, FW) corresponds to the folate fraction that is released from the food matrix during the in vitro digestion. To estimate the daily folate intake (DFI, in μg/day) from the seven CBFF, known data on the daily frequency (DF) of consumption and portion sizes (PS, in g) of each CBFF for young children aged 6 to 35 months (Hama, 2012) were used. Based on these data and on the total folate content (TFC in μg/100 g FW) of the seven CBFF, their DFI was calculated as follows:

$$DFI = \frac{(TFC/100) \times PS \times DF}{100}$$

For breastfed children, the folate intake from breast milk was also taken into account, based on a folate content of 85 μg/1 l in breast milk (Allen, 2012), as well as the average daily amount of breast milk consumed by children in developing countries, as described by the World Health Organization (WHO) (1998).

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

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

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

$$DFI = \frac{(TFC/100) \times PS \times DF}{100}$$

For breastfed children, the folate intake from breast milk was also taken into account, based on a folate content of 85 μg/1 l in breast milk (Allen, 2012), as well as the average daily amount of breast milk consumed by children in developing countries, as described by the World Health Organization (WHO) (1998).

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

#### 2.2.6. Statistical analysis

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

Three-level nested ANOVA was also carried out to test the inter-producer and the inter-day (within producers) variabilities. These variables are nested, meaning that each value of these nominal variables (i.e., the subgroups ‘producer’ and ‘day’) are found in combination with only one value of the higher-level nominal variable (i.e., the groups ‘product’ or ‘producer’). The linear model for three-way unbalanced nested arrangement is given by:

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

where:
3. Results

3.1. Description of food products and processes

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

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

Massa are fritters (small and thick pancakes fried in oil) made from pearl millet batter, often prepared as street food and eaten as snacks. They are consumed alone or crumbled in porridge. Processing included grain washing and sun drying, before dry milling in a community mill, mixing with water and kneading of the dough, fermentation during settling, and frying. Frying led to oil absorption in the fritters, with average oil contents of 15.5 ± 4.6, 6.7 ± 2.8, and 17.1 ± 2.4 g/100 g FW for massa produced in TPU 1, 2 and 3, respectively.

Fura is a semi-solid dumpling meal made from pearl millet. It can be consumed in different ways. Fura balls can be made into porridge by crumbling and mixing them with porridge, fermented milk, or water with sugar. This can be used as food, refreshing drink, or weaning food for infants. Fura processing included grain dehulling before washing and sun drying (for 30 min for producers P2 and P3, and up to 10 h for P1). After dry milling, sieving was an optional step. Two fermentation steps were carried out: one before and one after cooking.

Ben-saala and ben-kida are fermented porridges made from pearl millet, very often used as complementary food for infants and young children. Their processes were similar and included the following steps: soaking and washing of the grains, wet-milling, addition of water and sieving, fermentation (during settling), and cooking. For the preparation of ben-kida, previously prepared lumps (made from pearl-millet flour) were added to the boiling mixture to produce porridge with a granulated texture.

3.2. Dry matter content of raw materials and fermented foods

Measurement of the raw material DM content (Table 1) did not highlight any significant difference among cereal grains, whatever the producer or the day of production. Conversely, the DM content of the different CBFF types (Table 1) varied significantly. DM content was very low in porridges (ben-kida and ben-saala) (5.7–8.3 g/100 g). The DM contents of the three gelatinized doughs (akassa, doncouonou, kaffa) varied greatly (from 11.1–30.2 g/100 g). The highest DM content (56.7–63.6 g/100 g) was observed in fritters (massa). The DM content of doncouonou and massa were significantly different among TPUs. A nested analysis of variance showed that for doncouonou, this was due to inter-producer variability (88.6% of the variance) rather than inter-day variability (11.0% of the variance). Conversely, for massa, 89.4% of variance was explained by inter-day variability, and only 10.5% by inter-producer variability.

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

3.3. Folate content of raw materials and fermented foods

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

Significant differences in total folate content were observed in the seven CBFF. A three-level nested analysis of variance showed that 84.0% of variance was due to inter-food variability, 13.0% to inter-producer variability, and 1.9% to inter-day variability. Folate content was very low in porridges (ben-kida and ben-saala: 1.8 to 2.9 µg/100 g FW), and also in gelatinized doughs (akassa, doncouonou, and kaffa: 1.8 to 5.2 µg/100 g FW). The highest folate content was observed in dumplings (fura): 16.2 to 31.3 µg/100 g FW. Folate content in massa and fura significantly varied from one producer to another. A nested ANOVA showed that for massa this variability was due to both inter-producer and inter-day variability (52.5% and 37.7% of the variance, respectively), whereas for fura it was mostly explained by inter-producer variability (87.8%) rather than inter-day variability (6.4%). Indeed, the folate content of fura produced in TPU 1 (the one that used sun drying instead of draining) was significantly lower than in the other TPUs.

3.4. Influence of processing on CBFF folate content

The first step of akassa processing (i.e., corn grain debranning and degerming) resulted in major folate losses because debranned corn contained only 49.6 ± 14.0% of the folate detected in the initial grains (Table 6, in supplementary material). The folate content ranged from 13.8 to 24.3 µg/100 g DM in whole-grain corn, and from 5.0 to 12.8 µg/100 g DM in debranned corn. Folate loss ranged from 23% to 66%, but no significant difference among TPUs was observed.

Table 2 summarizes the folate loss or gain in the final food products compared with the raw materials. To avoid the influence of the various water amounts added during processing, the folate content of the different CBFF was expressed in µg/100 g DM, and compared with that of cereal grains. The high standard deviations observed for some massa and fura samples were probably due to their variable dry matter contents. The highest folate loss was observed in fritters (massa; reduction between 59% and 76%), followed by porridges (reduction between 44% and 59% for all ben-kida and ben-saala samples, regardless of the producer). Conversely, folate content in kaffa was increased by 66%. Folate content was greatly reduced (by 53%) in the dumplings made by the first fura producer, whereas no folate loss was observed in the fura made by the other producers. Likewise, folate content was not significantly different between raw material and CBFF in most akassa and doncouonou samples, except for one doncouonou producer (folate loss of 22%) and one akassa producer (folate gain of 78%).

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batter), the defatted dry matter was used for the calculation, to take into account loss or gain during the processing of cereal grains into fermented foods.

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/100 g FW of food “as eaten” (i.e., non-defatted samples).

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

<table>
<thead>
<tr>
<th>Food products (and respective raw materials)</th>
<th>Producer</th>
<th>Raw matter content (g/100 g FW)</th>
<th>Fermented food content (μg/100 g FW)</th>
<th>Total folate content (μg/100 g FW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akassa (gelatinized dough, corn)</td>
<td>P1</td>
<td>89.7 ± 0.5</td>
<td>11.4 ± 1.1</td>
<td>13.8 ± 1.3</td>
</tr>
<tr>
<td>(sieved batter)</td>
<td>P2</td>
<td>89.0 ± 0.1</td>
<td>11.1 ± 0.2</td>
<td>16.8 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>89.6 ± 0.4</td>
<td>13.0 ± 1.1</td>
<td>21.2 ± 1.4</td>
</tr>
<tr>
<td>Doncounou (gelatinized dough, corn)</td>
<td>P1</td>
<td>88.4 ± 0.7</td>
<td>24.1 ± 0.9</td>
<td>31.5 ± 0.8</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>88.8 ± 0.6</td>
<td>26.6 ± 1.6</td>
<td>44.8 ± 0.6</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>90.4 ± 2.4</td>
<td>30.2 ± 0.1</td>
<td>36.4 ± 0.6</td>
</tr>
<tr>
<td>Kaffa (gelatinized dough, sorghum)</td>
<td>P1</td>
<td>88.9 ± 0.4</td>
<td>17.4 ± 3.1</td>
<td>26.3 ± 2.6</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>89.1 ± 0.7</td>
<td>63.8 ± 8.1</td>
<td>72.6 ± 4.5</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>90.8 ± 0.8</td>
<td>56.7 ± 3.8</td>
<td>72.6 ± 10.9</td>
</tr>
<tr>
<td>Massa* (batter fritters, pearl-millet)</td>
<td>P1</td>
<td>90.0 ± 0.6</td>
<td>8.3 ± 0.9</td>
<td>6.6 ± 0.4</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>90.5 ± 0.7</td>
<td>6.1 ± 0.7</td>
<td>6.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>90.4 ± 0.5</td>
<td>7.9 ± 0.7</td>
<td>6.9 ± 0.6</td>
</tr>
<tr>
<td>Ben-saalga (dumplings, pearl-millet)</td>
<td>P1</td>
<td>89.0 ± 0.7</td>
<td>5.8 ± 0.4</td>
<td>6.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>P2</td>
<td>90.0 ± 0.3</td>
<td>6.6 ± 1.9</td>
<td>7.0 ± 1.3</td>
</tr>
<tr>
<td></td>
<td>P3</td>
<td>89.3 ± 0.9</td>
<td>5.7 ± 0.6</td>
<td>6.6 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>P4</td>
<td>89.2 ± 0.1</td>
<td>6.6 ± 0.1</td>
<td>7.3 ± 0.2</td>
</tr>
</tbody>
</table>

Results are the mean ± 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). For massa samples, the results are given in g or μg/100 g 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.

<table>
<thead>
<tr>
<th>Food products (and raw materials)</th>
<th>Producer</th>
<th>Folate content (μg/100 g DM)</th>
<th>Folate loss or gain (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Akassa (gelatinized dough, corn)</td>
<td>P1</td>
<td>15.4 ± 1.5</td>
<td>78%*</td>
</tr>
<tr>
<td>(sieved batter)</td>
<td>P2</td>
<td>18.7 ± 1.4</td>
<td>11%</td>
</tr>
<tr>
<td>(gelatinized dough, corn)</td>
<td>P3</td>
<td>23.6 ± 1.5</td>
<td>13%</td>
</tr>
<tr>
<td>Doncounou (gelatinized dough, corn)</td>
<td>P1</td>
<td>17.8 ± 2.8</td>
<td>10%</td>
</tr>
<tr>
<td>(gelatinized dough, corn)</td>
<td>P2</td>
<td>16.7 ± 2.2</td>
<td>7%</td>
</tr>
<tr>
<td>(gelatinized dough, corn)</td>
<td>P3</td>
<td>15.2 ± 1.9</td>
<td>22%*</td>
</tr>
<tr>
<td>Kaffa (gelatinized dough, sorghum)</td>
<td>P1</td>
<td>18.3 ± 2.9</td>
<td>66%*</td>
</tr>
<tr>
<td>Massa* (batter fritters, pearl-millet)</td>
<td>P2</td>
<td>63.9 ± 3.9</td>
<td>76%*</td>
</tr>
<tr>
<td>(gelatinized dough, corn)</td>
<td>P3</td>
<td>70.4 ± 4.4</td>
<td>59%*</td>
</tr>
<tr>
<td>(sieved batter)</td>
<td>P2</td>
<td>81.7 ± 4.3</td>
<td>53%*</td>
</tr>
<tr>
<td>(sieved batter)</td>
<td>P3</td>
<td>72.9 ± 4.1</td>
<td>2%</td>
</tr>
<tr>
<td>(gelatinized dough, corn)</td>
<td>P3</td>
<td>71.9 ± 1.2</td>
<td>3%</td>
</tr>
<tr>
<td>Ben-saalga (dumplings, pearl-millet)</td>
<td>P1</td>
<td>60.5 ± 4.4</td>
<td>49%*</td>
</tr>
<tr>
<td>(dumplings, pearl-millet)</td>
<td>P2</td>
<td>72.1 ± 7.6</td>
<td>44%*</td>
</tr>
<tr>
<td>(gelatinized dough, corn)</td>
<td>P3</td>
<td>65.7 ± 7.8</td>
<td>51%*</td>
</tr>
<tr>
<td>Ben-saalga (dumplings, pearl-millet)</td>
<td>P4</td>
<td>70.3 ± 7.4</td>
<td>54%*</td>
</tr>
</tbody>
</table>

Results are the mean ± 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). For massa samples, the results are given in g or μg/100 g FW of food “as eaten” (i.e., non-defatted samples).

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

The changes in folate content distribution in the samples from the four TPU’s (expressed as percentages of the pearl millet folate content, on a dry matter basis) (Fig. 3A) indicated that with the exception of producer P2, the soaking step resulted in a significant decrease in folate content (20% to 49% loss, compared with raw pearl millet). This was partly due to folate diffusion in the soaking water (5% to 15%). The folate content in soaked grains varied from 24.2 ± 4.1 μg/100 g FW (for P4) to 38.5 ± 10.5 μg/100 g FW (for P2). Folate loss in soaking water was higher for producer P2, compared with the other producers, probably due to the longer soaking time (from 24 to 48 h soaking for producer P2, compared with 10 h, 24 h, and 22 h for producers P1, P3 and P4, respectively). After the soaking step, only two producers (P1 and P2) applied a washing step, which did not significantly affect folate content. Conversely, wet-milling resulted in a significant folate loss, compared with soaked grains, except for producer P4. Indeed, the paste of milled soaked grains contained only 41% to 61% of the folate initially present in pearl millet grains (Fig. 3A). The sieving step also led to folate loss due to fractionation (draf fry) Indeed, all sieved batter samples contained significantly less folate than the pastes of milled grains (20% to 37% of the initial pearl millet folate content). The folate content varied from 1.0 ± 0.3 to 4.1 ± 1.4 μg/100 g FW in the sieved batter, and from 8.3 ± 2.2 to 11.7 ± 3.2 μg/100 g FW in draft. The sum of the folate content in the sieved batter and in draft samples was significantly lower than the total amount of folate in milled grains (except for producer P3). This shows that folate losses also occurred due to other causes. The sieved batter was then left to rest at ambient temperature (decanteration and fermentation) for 10 to 13 h (producers P1, P3 and P4) and for 24 h (producer P2). The folate content in the decanted paste (21% to 28% of the initial pearl millet folate) and in supernatant (6% to 18% of the initial pearl millet folate) samples was comparable among producers (Fig. 3B). The sum of the folate present in the decanted paste and total supernatant (Decanted paste + TS) samples was significantly higher than the folate fraction in the sieved batter samples for producers P1 and P2. To prepare the final products, producers used only part of the supernatant (used supernatant, US) to dilute the decanted paste and cook the dish (Decanted paste + US) to obtain ben-saalga. Then, ben-saalga was cooked for 5 to 10 min (producers P1 and P4) and for 15 to 20 min (producers P2 and P3). The cooking step did not significantly affect folate distribution. Nevertheless, for all producers, the cooked ben-saalga contained only 21% to 35% of the folate initially present in raw pearl millet grains.

### 3.6. Bioaccessible folate and folate bioaccessibility

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

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

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

3.7. CBFF contribution to the recommended folate intake of infants

The total folate contents were used to calculate the potential contribution of the seven CBFF to the recommended folate intake of children aged 6–35 months (Table 4). The estimated daily folate intakes from CBFF were 0.8 to 6.4 μg/day, 2.7 to 15.2 μg/day, and 7.9 to 34.6 μg/day, for children aged 6–11 months, 12–23 months, and 24–35 months, respectively. When taking into account the contribution of
breast milk and CBFF were consumed.

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

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 categories, 30%, 69% and 100% of the folate intake should come from sources other than milk. However, the potential contribution of the seven studied CBFF to the recommended DFI was low, from 1% to 8%, 2% to 10%, and 5% to 23% for children aged 6–11 months, 12–23 months, and 24–35 months, respectively. When the bioaccessible folate contents were used to calculate the potential contribution of the seven CBFF to the recommended folate intake of young children, their potential contribution to the recommended DFI was even lower (from 0 to 8%), (Table 5 in supplementary material).

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

4. Discussion

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

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

Moreover, folate content was low in all studied CBFF, irrespective of the food type, producer and starting raw material. *Fura* exhibited the highest folate content (16–31 μg/100 g FW), and *ben-saalga*, *ben-kida* and *akassa* the lowest (1.8–3.1 μg/100 g FW). The difference in folate content between raw material and final food products indicates that part of the initial folate content was lost during food processing to obtain fritters (*massa*) and porridges (*ben-saalga* and *ben-kida*). Conversely, for dumplings (*fura*), the folate loss during food processing depended on the producer (folate loss observed only for one producer). This suggests that folate was not degraded during *fura* processing and that the added water only ‘diluted’ the vitamins initially present in the grains. Alternatively, some loss might have occurred but folate was produced at one stage or another to counterbalance this loss, for example during the fermentation steps. Owusu-Kwarteng et al. (2012) described the diversity of LAB species involved in the traditional processing of *fura*, and some of these LAB might be able to produce folate (Saubade et al., 2017a).

Analysis of the CBFF process steps suggests that some procedures may particularly affect folate content. For instance, debranning performed during *akassa* processing particularly affected folate content (50% folate loss during this step, in average). This explains the significantly lower folate content in this dish compared with *doncouou*, another gelatinized dough produced from non-debranned corn grains. Indeed, vitamin B9 is mainly located in the outer layers and germ of cereal grains, and is largely lost during debranning/degemering (Liukkonen et al., 2003; Pomeranz, 1988). During grain soaking, folate could have been lost through leaching in soaking water (Witthöft et al., 1999). The longer soaking time (24–48 h) used by producer P2 during *ben-saalga* processing, compared with the other producers (10–24 h soaking), might explain the higher folate content in the P2 soaking water sample. Folate are sensitive to heat, light, and oxidation. Sun drying, which is performed in the TPU yards, also could have affected folate content through the combined action of atmospheric oxygen and light (Preedy, 2013; Strandler et al., 2015). This could explain the significantly lower folate content of the *fura* dumplings produced in the TPU that included a very long sun drying step. Moreover, the CBFF under study were cooked (porridges), fried (fritters), or boiled in water (dumplings, gelatinized doughs). Cooking can lead to thermal and oxidative degradation of folates (Delchier et al., 2016; Witthöft et al., 1999). Specifically, frying can generate significant folate losses in various food products (Aramouni et al., 1986; Arcot et al., 2002a; Hefni and Witthöft, 2014). Boiling in water (i.e., hydrothermal cooking) results in significant losses due to folate leaching from the food matrix into the cooking water (Delchier et al., 2016; Hefni and Witthöft, 2014).

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

Saubade et al. (2018) carried out a mass balance assessment during the processing of pearl millet grains into *ben-saalga*, but in controlled laboratory conditions. In the present study, although the final *ben-saalga* folate content was comparable to the 1.5 to 2.5 μg/100 g FW reported by Saubade et al. (2018), the influence of the different processing steps was not similar. This highlights the difference between analyses in controlled time/temperature conditions and in the yard of TPUs. Specifically, Saubade et al. (2018) reported folate production (+26% to +31%) during pearl millet soaking, whereas in the TPUs folate was lost during the soaking step (-19% to -49%). In the TPU yard, grains are left in water at ambient temperature, exposed to sunlight and atmospheric oxygen, until the end of the soaking step (Tou et al., 2006). This can lead to folate oxidation or photo-degradation (Delchier et al., 2016; Preedy, 2013). Moreover, the soaking time was variable (10 to 24 h) among TPUs, and might not have been sufficient to promote germination, which leads to folate synthesis (Kariluoto et al., 2006b). Saubade et al. (2018) found that the fermentation step did not have any significant effect on folate content. Conversely, in the present study, a significant gain of folate was observed during the fermentation step in two TPUs, suggesting folate production by microorganisms present in the batter. Spontaneous fermentation can lead to variable microbial composition (Saubade et al., 2017a) due to the endogenous bacteria present in the cereals, food utensils and manual handling. This could explain the folate content variability in *ben-saalga* from the different TPUs. In *ben-saalga*, LAB are the predominant microorganisms during fermentation (Saubade et al., 2017b; Tou et al., 2006). LAB isolated from *ben-saalga* display a good genetic potential for folate synthesis, and some specific strains can produce folate during fermentation (Greppi et al., 2017). A recent study also showed that *ben-saalga* folate content could be increased by using back-stopping or starter cultures combined with amylolytic LAB strains (Bationo et al., 2019b).

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

- Porridges were characterized by very low bioaccessible folate (< 1.5 μg/100 g FW), due to the low total folate content and moderate bioaccessibility ratios (30%–57%). In these semi-liquid foods, most folate was already in a solubilized form and should have been bioaccessible. However, porridges had little structural resilience in digestion conditions, and folate was not protected by the food matrix during the gastric phase. As many folate vitamers are sensitive to low pH, the observed moderate bioaccessibility ratios could be due to loss of labile folate in acidic gastric conditions (Ringling et al., 2017; Seyoum and Selhub, 1998; Strandler et al., 2015).
- Gelatinized doughs made from debranned grains (*akassa*, *kaffa*) displayed low bioaccessible folate (0.4–1.6 μg/100 g FW) and low bioaccessibility (23–30%). Folate bioaccessibility was similarly low in dumplings (*fura*), but bioaccessible folate was much higher (4–9 μg/100 g FW) due to a higher initial total folate content. In these three structurally weak foods, the starch matrix hydrolysis started during the oral phase of digestion, possibly leading to the degradation of non-protected folate during the gastric phase.
- The two CBFF with the highest folate bioaccessibility ratio were both made from whole-grain cereals, and had either a dense semi-solid structure (*doncouou*, 57–67% bioaccessibility) or a solid
structure (massa, 67–81% bioaccessibility). These dense matrices provided secondary structure and generated particles that were resistant to digestion (Mishra and Monro, 2012), and persisted long enough to protect folate from the acidic gastric conditions. Moreover, it is known that micronutrient encapsulation in the plant cell walls modulates the rate and extent of nutrient release during digestion (Parada and Aguilera, 2007). Therefore, the presence of bran particles in massa and doncouhou may have protected folate during the gastric phase, and allowed a higher proportion of folate to reach the intestinal phase (neutral pH) in a non-degraded form.

In many West African countries, traditional millet-, sorghum- and maize-based fermented dishes are frequently consumed by young children (Greffeuille et al., 2010). Porridges are mainly consumed as foods to complement breast milk. Then, when children can eat family foods, porridges are progressively replaced by thicker starchy staple foods (such as gelatinized doughs) that are eaten with various sauces. Snacks, such as fritters or dumplings, are given more regularly (Hama, 2012). Our study shows that, whatever the age group, the contribution of the studied CBFF to the recommended folate intake was far from sufficient to cover the folate requirement of young children. By taking into account the food intake reported by Hama (2012), 6–11-month, 12–23-month- and 24–35-month-old children would get respectively 1–8%, 2–10% and 5–23% of the recommended folate intake through CBFF consumption. Moreover, the potential contribution of the seven studied CBFF to the recommended DFI was limited: up to 23% when considering the total folate contents, and only up to 8% when considering the bioaccessible folate contents. This suggests that many children might be at risk of folate deficiency, especially non-breastfed children, if their diet were mainly based on CBFF. However, in Burkina Faso and other West African countries, most of these CBFF are consumed with other food products with higher folate contents, such as sauces made from green leafy vegetables and legumes (e.g., groundnut or soybean) (Greffeuille et al., 2010).

5. Conclusion

This work generated new information on the influence of traditional processing on the folate content and the first data on folate bioaccessibility in seven CBFF commonly consumed in West Africa that can be useful to complete the existing food composition tables. The total folate and bioaccessible folate contents of the seven studied products (gelatinized doughs, porridges, dumplings, fritters) were rather low, suggesting that populations whose diet is mainly based on these CBFF may be at risk of folate deficiency. The folate content of most of the food products was strongly and negatively affected by the laborious processing. Some processing steps could be adapted to limit such losses (controlled volumes, controlled soaking and fermentation times, and limitation of direct exposure to sunlight). Another way to increase the folate content in CBFF could be to promote folate production during the fermentation step, by using folate-producing bacterial strains. Finally, the observed differences in folate bioaccessibility ratios among the studied food products suggest a strong effect of the food matrix that should be investigated more precisely. The influence of processing on folate bioaccessibility in other folate-rich foods frequently consumed by young children together with CBFF (i.e., green leafy vegetables, legumes) should also be analyzed.

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Appendix A. Supplementary data

Supplementary material related to this article can be found in the online version, at doi:https://doi.org/10.1016/j.jfca.2019.103309.

References


