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# Co-fermentation improves the functional properties and nutritional quality of infant complementary food products

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Food-to-food fortification and fermentation are effective strategies to enhance the product functionality and nutrient density of infant complementary foods. However, their effectiveness hinges on a deep understanding of ingredient combinations. Our research focused on the physicochemical and techno-functional aspects of sorghum–baobab blends, comparing two processes: ‘co-ferment-cook’ and ‘ferment-cook-fortify’. The results show that both techniques improved the water absorption capacity by 17–20% and the water solubility index increased by over 100% while maintaining a comparable nutritional composition and energy density. The calculated energy density (2048.8–2345 kJ day<sup>-1</sup>) was sufficient for both blends for infants 6–11 months old with an average breast milk intake. Viscosity, another crucial factor for complementary feeding, improved significantly ( $P < 0.05$ ) after co-ferment-cook compared to ferment-cook-fortify reaching a value suitable for children older than 18 months. Starch digestibility increased with co-ferment-cook, while protein digestion increased with fortified non-fermented foods. In conclusion, our findings emphasize that combining fermentation and fortification processing steps is optimal for balancing the nutritional and techno-functional properties of sorghum porridges for infant complementary foods. Processing parameters must be optimized to reach the viscosity suitable for complementary feeding at the assigned soluble solid contents for the age group 6–24 months.

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## 1. Introduction

Child malnutrition in sub-Saharan Africa (SSA) is exacerbated by climate change, manifested by recurrent droughts that threaten the food and nutritional security of vulnerable population groups.<sup>1,2</sup> Climate-resilient crops (CRCs), especially small grains such as sorghum, contain high amounts of essential amino acids, fibre and micronutrients and have been used as an alternative to mainstream cereal crops to overcome the double-edged impact of droughts and malnutrition.<sup>3</sup> Sorghum is culturally significant in many African societies, where it is often incorporated into traditional diets and culinary practices, including complementary foods.<sup>4</sup> The goal of complementary feeding during weaning is to consume nutritionally complete and balanced foods that adapt to the culture of the family and the country.<sup>5</sup>

Sorghum’s nutritional value is comparable to other staple cereals such as wheat, rice, and corn.<sup>6</sup> Its protein content

ranges from 11 to 13%.<sup>6</sup> The most representative amino acids are glutamic acid and non-polar amino acids (proline, leucine, and alanine), but there is a deficiency of lysine, tryptophan, methionine, cysteine, isoleucine, valine, and threonine.<sup>7,8</sup> Some scholars have classified sorghum as a low nutritional value food because it contains anti-nutrients such as phytic acid.<sup>9</sup> The chelating properties of phytic acid cause mineral ion deficiencies in human nutrition.<sup>10</sup> In addition to that, phytic acid hinders enzymatic activities for protein degradation in the small intestine and stomach.<sup>11</sup> For this reason, reducing the phytic acid content is crucial to improving the nutritional characteristics of sorghum or foods obtained from this cereal, especially if they are intended for weaning children.

Infants have limited oromotor abilities, making them unable to chew and swallow thick and viscous foods. Therefore, porridge is usually cooked and diluted with water, decreasing its nutrient and energy density. Consequently, infants must consume large quantities of porridge to have adequate nutrient intake, but their reduced gastric capacity limits the amount they can consume.<sup>12</sup> For an easy-to-swallow, semi-liquid porridge considered suitable for infants, a viscosity limit of 3 Pa s has been suggested, commonly measured with

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an assumed oral shear rate of 50 per s and temperatures of 30–40 °C.<sup>12</sup>

Cost-effective strategies such as spontaneous fermentation have been useful in overcoming these challenges. Spontaneous fermentation is based on uncontrolled fermentation conditions in the presence of a consortium of bacteria, fungi and/or yeasts indigenous to the raw materials used as the inoculum.<sup>13</sup> According to recent work,<sup>14</sup> spontaneous fermentation and food-to-food fortification are two strategies that could improve sorghum porridge viscosity for consumption by >18 months old infants. However, the viscosity of the fermented sorghum porridge is not significantly improved compared to that of unfermented porridge. Yet, fermentation improves the nutritional profile as it decreases the phytic acid content and increases the digestibility of starch, although it does not have the same effect on the digestibility of proteins,<sup>14</sup> which are insufficient to meet infant growth and development needs.

To meet the requirements of complementary food, cereal-based foods may be enriched with food ingredients that are high in limiting nutrients. A potential ingredient for sorghum-based porridges is fruit pulp or juice from indigenous, climate-resistant fruit trees.<sup>15,16</sup> Baobab is a promising ingredient used to fortify sorghum porridge to improve its nutritional value and sensory properties.<sup>17</sup> Nevertheless, despite the reported improvement in the nutritional quality of fortified fermented cereal-based foods, infants' nutrient needs are still unmet due to the porridges' high viscosities that limit adequate intake.<sup>4</sup>

Another strategy, co-fermentation, has emerged as a promising method for producing foods with high energy density and appropriate viscosity specifically designed for household use in sub-Saharan Africa.<sup>14</sup> Notwithstanding the unfolding benefits of co-fermentation, the cumulative complexity of final product quality in this category of foods still needs to be fully explored, starting with the viscosity and energy density. It is critical to establish the underlying principles of how spontaneous co-fermentation changes the techno-functional properties of the raw materials to which they are applied. Therefore, this research studied the effect of spontaneous fermentation on nutritional and techno-functional properties using sorghum and baobab as ingredients. To achieve this aim, we compared co-ferment-cook and ferment-cook-fortify as processing technologies to analyse physicochemical, functional and anti-nutritional properties, viscosity and starch/protein digestion. These results will provide insights into the impact of fermentation production steps on the nutritional and functional quality of the processed raw materials.

## 2. Materials and methods

### 2.1 Sample preparation

Hulled, finely milled sorghum meal and baobab fruit pulp were purchased from a local market in Zimbabwe. The dry baobab pulp powder was removed from the seeds by blending and passed through a sieve (frame diameter 200 mm, mesh

size 900 µm) (Retsch, Haan, Germany). Sorghum porridge (SP), fermented sorghum porridge (FSP), baobab pulp-fortified sorghum porridge BFoSP, co-fermented baobab pulp-fortified sorghum porridge FoCFSP, and baobab pulp-fortified fermented sorghum porridge FoFSP were produced as shown in Fig. 1. Specifically, sorghum flour was mixed with tepid water in a 1:3 ratio (*i.e.*, 67 g and 200 g, respectively) to obtain a slurry that was cooked (continuous agitation, 65 °C, 15 min) as determined from previous studies and described.<sup>14</sup> During this process, 100 ml of water was added to obtain a sorghum porridge (SP). For fermentation, the slurry was allowed to spontaneously ferment at 25 °C for 48 h before cooking to produce fermented sorghum porridge (FSP). BFoSP and FoCFSP were first produced as described for SP and FSP, respectively. Thereafter, they were allowed to spontaneously ferment by the endogenous bacteria in the sorghum, baobab and environment. For FoCFSP, sorghum flour was replaced by sorghum and baobab pulp powder mixture (*i.e.* 56.67 g of sorghum + 10.0 g of baobab pulp). For BFoSP, baobab pulp powder (10.0 g) was added to FSP cooled to 50 °C.

Depending on the particular analysis, samples were either freeze-dried with liquid nitrogen to obtain a dry slurry/porridge powder or used as wet slurry.

### 2.2 Characterization of sorghum samples

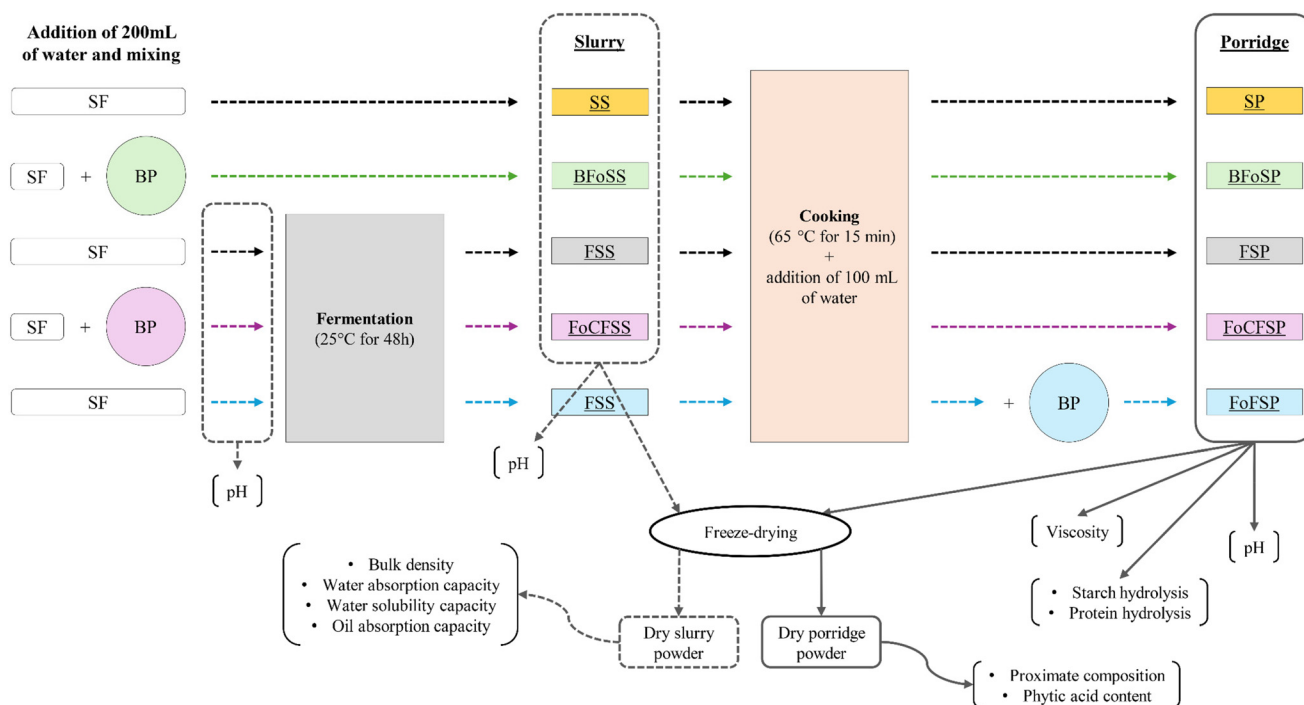
**2.2.1 Functional properties.** The dry slurry was milled to a powder and kept at 4 °C before analysis of functional properties. Bulk density was determined as described by Simonyan *et al.*,<sup>18</sup> water absorption (WAC) and water solubility index (WSI) as described by Kaur and Singh,<sup>19</sup> and oil absorption capacity (OAC) as described by Kaur and Singh.<sup>20</sup>

**2.2.2 Phytic acid content.** The phytic acid content of dry porridge powder was measured using a Megazyme kit (phytic acid (phytate)/total phosphorus, Megazyme Inc., Bray, Ireland). The extraction process started with 1 g of freeze-dried sample, followed by an enzymatic dephosphorylation reaction and a colourimetric determination of phosphorus.

### 2.3 Biochemical analysis

**2.3.1 Proximate composition of porridges.** The moisture content of dry porridge powder was analysed using the AOAC method 925.09.<sup>21</sup> Macronutrient content was measured for freeze-dried porridge powders. The pH of porridges was measured with a pH meter (pH1002 VWR pHEnominal) for all samples before and after cooking. Protein content ( $N \times 5.71$ ) was determined by the Dumas combustion method using an analyzer (EA 112 NC, Thermo Fisher Scientific Inc., Waltman, USA) following the manufacturer's protocol. Cellulose and D-methionine were used to prepare the control and calibration curves, respectively. Lipid contents were determined by the Soxhlet method with petroleum ether as the extraction solvent. Total dietary fibre was measured using a commercial Megazyme kit (K-TDFR, Megazyme Int, Wicklow, Ireland) whereby ash and protein residues were corrected for the corresponding soluble dietary fibre (SDF) and insoluble dietary fibre (IDF) values. Total dietary fibre (TDF) was calcu-





**Fig. 1** Scheme of the sample preparation and summary of the analyses carried out. SF; sorghum flour; BP; baobab pulp; SS; sorghum slurry; FSS; fermented sorghum slurry; BFoSS; sorghum slurry fortified with baobab pulp; FoCFSS; co-fermented sorghum slurry fortified with baobab pulp; SP; sorghum porridge; FSP; fermented sorghum porridge; BFoSP; sorghum porridge fortified with baobab pulp; FoCFSP; co-fermented sorghum porridge fortified with baobab pulp; FoFSP; sorghum porridge fortified with baobab pulp added after cooking.

lated as the sum of SDF and IDF. The carbohydrate content (g per 100 g) was calculated by difference. Energy values were then computed using Atwater's conversion factors from Bazaz *et al.*<sup>22</sup>

$$\text{Energy (kJ per 100 g)} = 4.18[(4 \times \text{carbohydrate \%}) + (4 \times \text{protein \%}) + (9 \times \text{fat \%})]$$

## 2.4 Viscosity

The viscosity of the porridges was measured using a GmbH viscometer (Anton Paar, GmbH, Austria) equipped with a 50 mm measuring plate and a 1 × 0.5, Groove probe (A-8054 Graz, Anton Paar, GmbH, Austria), subjecting the sample to increasing shear rates. The test, shear rate range, plate and probe settings were chosen following the method of Makame *et al.*<sup>12</sup> Porridge viscosity at a 50 s<sup>-1</sup> shear rate was taken to represent in-mouth handling of the bolus by infants,<sup>23</sup> and as porridge texture suitable for infant dysphagia management.<sup>24</sup>

## 2.5 Simulated *in vitro* gastrointestinal digestion

*In vitro* digestion was carried out on 5 g of freshly produced porridge. Simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were used for *in vitro* digestion according to Minekus *et al.*<sup>25</sup> Aliquots were taken at different gastric and intestinal digestion times, *i.e.*, SGP at 0 and 2 h, and SIP at 15, 30, 60 and 120 min. Enzyme inactivation was performed by adding absolute ethanol for starch digestion, then resting for

30 min at room temperature before centrifugation at 10 000g for 10 min. Enzyme inactivation for protein digestion was performed by adding 25 μL pefabloc® (0.1 M). Samples were left for 30 min at room temperature after which 0.83 mL 5% TCA was added, followed by centrifugation at 10 000g for 10 min. Supernatants were collected and stored at -20° C for further analysis. As a blank, 5 ml MilliQ water was digested.

**2.5.1 Starch hydrolysis.** An aliquot of 0.1 ml of the supernatant obtained after the addition of ethanol and centrifugation was mixed with an amyloglucosidase solution (27.17 U ml<sup>-1</sup>) in 0.1 M sodium acetate buffer (pH 4.8) and incubated at 37 °C for 1 h. The amount of glucose was then quantified using a Megazyme D-glucose assay kit (GOPOD FORMAT, K-GLUC, Megazyme Inc., Bray, Ireland). To obtain the corresponding amount of starch, the glucose content was multiplied by a factor of 0.9 and the results were expressed as g of hydrolysed starch per 100 g of dry starch.<sup>26</sup>

**2.5.2 Protein hydrolysis.** The concentration and quantification of free amino groups (NH<sub>2</sub>) in TCA samples was determined using the *ortho*-phthalaldehyde (OPA) method.<sup>27</sup>

## 2.6 Statistical analysis

One-way analysis of variance (ANOVA) and *post-hoc* Tukey's (HSD) test were performed using Excel Stat software for comparing the mean differences of samples at a significance level of *p* < 0.05.



### 3. Results and discussion

#### 3.1 Effect of co-fermentation on functional properties

**3.1.1 Water and oil absorption capacity.** The WAC significantly decreased for FSS, BFoSS, and FoCFSS by 19%, 21% and 17%, respectively, compared to the untreated sorghum samples (Table 1). No statistically significant difference between FSS and FoCFSS was found ( $P < 0.05$ ). The difference is marginal when considering single treatments (FSS or BFoSS) and their combination (FoCFSS). This implies that the WAC of sorghum is reduced by fermentation regardless of blending with baobab, giving a powder with less water absorption. The trend aligns with Alka *et al.*<sup>28</sup> who reported reduced WAC during the spontaneous fermentation of selected cereals. The addition of baobab fruit pulp flour further lowered the WAC level, which is in line with previous findings.<sup>29</sup> WAC denotes the amount of water available for starch gelatinization<sup>14</sup> and translates to water absorbed and retained. The lower WAC of FSS and FoCFSS may be related to the modification of starch structures during fermentation.<sup>30</sup> A lower WAC implies a lower water uptake by the slurry and, therefore, lowers bulking, which benefits infant feeding since reduced water absorption is ideal for preparing thinner gruels with a high caloric density per unit volume.<sup>31</sup>

Results show a decreased OAC when compared to unfermented/unfortified samples. However, there was no significant difference between the FoCFSS, FSS and BFoSS samples ( $P < 0.05$ ). OAC is the binding of fat by the non-polar side chain of proteins.<sup>32</sup> Thus, OAC is high when protein content is high, which agrees with the results for FSS. However, fortification significantly lowered the OAC (Table 1), due to the lower protein content of the fortified samples (Table 2). Since the oil absorption rate is high in matrices with high protein content, the assumption is that the fortified samples have fewer hydrophobic interaction sites than SS. According to another study,<sup>33</sup> fermentation causes a slight decrease in OAC. Moreover, Onyeneke<sup>34</sup> reported that a higher OAC would be beneficial for flavour retention and mouth feel, both characteristics that are critical for infant feeding. A low OAC could be advantageous for viscosity because unabsorbed oil helps separate the par-

**Table 2** Viscosity of porridges at 40 °C (50 and 87 shear rates ( $s^{-1}$ ) and pH (before and after cooking)

Porridge type	Apparent viscosity (Pa s)		pH	
	Shear rate (50 $s^{-1}$ )	Shear rate (87 $s^{-1}$ )	Before cooking	After cooking
SP	4.06 ± 0.20 <sup>b</sup>	2.58 ± 0.11 <sup>c</sup>	6.33 ± 0.03 <sup>a</sup>	6.23 ± 0.02 <sup>a</sup>
FSP	12.26 ± 2.15 <sup>a</sup>	7.36 ± 1.20 <sup>ab</sup>	3.84 ± 0.07 <sup>b</sup>	3.93 ± 0.09 <sup>b</sup>
BFoSP	4.65 ± 0.41 <sup>b</sup>	3.07 ± 0.24 <sup>c</sup>	3.78 ± 0.02 <sup>bc</sup>	3.92 ± 0.03 <sup>b</sup>
FoCFSP	7.81 ± 3.32 <sup>ab</sup>	4.90 ± 1.98 <sup>bc</sup>	3.66 ± 0.02 <sup>d</sup>	3.81 ± 0.08 <sup>b</sup>
FoFSP	12.17 ± 2.23 <sup>a</sup>	7.75 ± 1.26 <sup>a</sup>	3.73 ± 0.09 <sup>cd</sup>	3.43 ± 0.13 <sup>c</sup>

The viscosity was measured at shear rates (50  $s^{-1}$  and 87  $s^{-1}$ ) that are compatible with infant chewing and swallowing. The optimal viscosity is: 3 Pa s for children under 10 months; 10 Pa s for children over 18 months. pH measurements were carried out before and after cooking to see the influence of cooking on pH. Different superscripts in the same column denote a statistically significant difference ( $P < 0.05$ ). SP: sorghum porridge; FSP: fermented sorghum porridge; BFoSP: sorghum porridge fortified with baobab pulp; FoCFSP: co-fermented sorghum porridge fortified with baobab pulp; FoFSP: sorghum porridge fortified with baobab pulp added after cooking.

ticles, reducing their interaction and allowing them to move more independently, thus reducing friction and resistance within the mixture. However, this depends on several factors, such as the proportion of monomeric or polymeric proteins, type and concentration of oil, amylose/amylopectin ratio, and starch content, which are primary contributors to final viscosity<sup>35–37</sup> therefore warranting further investigation for these ingredients.

**3.1.2 Water solubility index and bulk density.** An extensive range was observed (16.26–3.14  $g\ g^{-1}\ DW$ ) for WSI in the order of BP > FoCFSS > BFoSS > SS > FSS. Fermentation significantly reduced the WSI, as shown by FSS. FSS and BFoSS/FoCFSS differed significantly, without a significant difference between BFoSS and FoCFSS ( $P < 0.05$ ). Since BP has the highest WSI, it seems to have caused the shift for the fermented-fortified samples to the higher values. WSI is related to the presence of soluble molecules and is used as an indicator for starch degradation, where a high WSI relates to a high extent of dextrinisation and gelatinization.<sup>38</sup> A lower WSI means less starch degradation, resulting in less soluble food molecules.<sup>39</sup> Thus, the higher WSI for BFoSS and FoCFSS shows that adding baobab before cooking causes starch degradation, probably by some baobab endogenous enzymes or yeasts/molds,<sup>40–42</sup> making it more readily available for digestion. This is correlated with a high digestibility of food, which is ideal for infant nutrition.

Regarding bulk density, a significant difference was observed between BFoSS and FoCFSS, where FoCFSS had the highest BD in the order FoCFSS > FSS > SS > BFoSS. The bulk density increased by 15% with co-fermentation. The bulk density of fermented sorghum flour aligned with that found by Ojha *et al.*<sup>9</sup> but is inconsistent with the findings of Ea *et al.*,<sup>43</sup> where BD decreased with sorghum and sorghum blend fermentation. Bulk density (BD), or packing density, represents the weight of powder per unit volume.<sup>39</sup> Thus, a higher BD translates to more flour at a constant volume,<sup>44</sup> which limits

**Table 1** Functional properties of blended flour samples

Sample	WAC ( $g\ g^{-1}$ )	WSI ( $g\ g^{-1}$ )	OAC ( $g\ g^{-1}$ )	Bulk density ( $g\ ml^{-1}$ )
BP	4.41 ± 0.27 <sup>b</sup>	16.26 ± 1.32 <sup>a</sup>	1.99 ± 0.09 <sup>a</sup>	0.47 ± 0.03 <sup>c</sup>
SS	5.51 ± 0.26 <sup>a</sup>	4.44 ± 0.59 <sup>c</sup>	1.01 ± 0.05 <sup>b</sup>	0.79 ± 0.02 <sup>ab</sup>
FSS	4.45 ± 0.16 <sup>b</sup>	3.14 ± 0.17 <sup>c</sup>	0.91 ± 0.12 <sup>bc</sup>	0.80 ± 0.03 <sup>ab</sup>
BFoSS	4.37 ± 0.28 <sup>b</sup>	9.21 ± 0.75 <sup>b</sup>	0.85 ± 0.06 <sup>c</sup>	0.72 ± 0.07 <sup>b</sup>
FoCFSS	4.57 ± 0.09 <sup>b</sup>	9.46 ± 1.16 <sup>b</sup>	0.84 ± 0.02 <sup>c</sup>	0.84 ± 0.04 <sup>a</sup>

Different superscripts in the same column denote a statistically significant difference ( $P < 0.05$ ). WAC: water absorption capacity; WSI: water solubility index; OAC: oil absorption capacity; BD: bulk density. SS: sorghum slurry; FSS: fermented sorghum slurry; BFoSS: sorghum slurry fortified with baobab pulp; FoCFSS: co-fermented sorghum slurry fortified with baobab pulp.



nutrient and calorie intake. A relatively lower bulk density is beneficial for infant feeding preparations<sup>31</sup> since it helps to reduce porridge thickness and viscosity, which is important for swallowing.

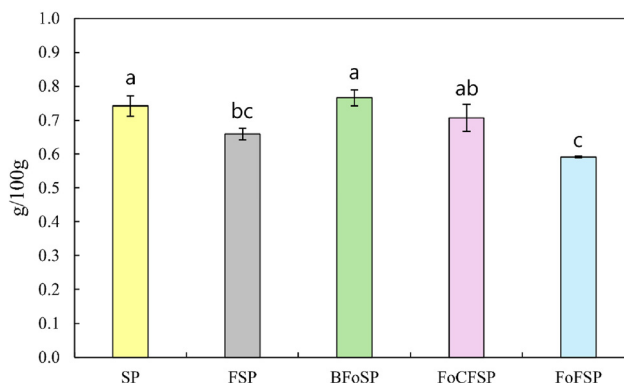
**3.1.3 pH and viscosity.** All samples, except for SP, had a pH < 4.6. The fermented-fortified porridges' pH ranged from 3.43 to 3.93 (Table 2). No significant change in pH was found before or after cooking the porridge except for FoCFSP ( $P < 0.05$ ). This is caused by the addition of baobab before cooking; baobab is acidic by nature due to its high amounts of ascorbic acid and citric acid. The low pH after cooking is attributed to the production of lactic acid by microorganisms during fermentation, causing the transformation of food constituents into organic acids. The low pH values are beneficial from food safety and sensory property points of view because not even the harmful *Clostridium botulinum* spores survive this level of acidity.<sup>45</sup>

Porridge viscosities at a consumption temperature of 40 °C at shear rates compatible with chewing and swallowing<sup>23</sup> are reported in Table 2. The results show no significant difference between the viscosities of BFoSP and FoCFSP. The lower viscosity is related to the reduced WAC (Table 1) and low pH after cooking. The gelatinization of the starch molecules depends on several factors, such as pH and salt, sugar, and protein concentrations. Sugars compete with starch for water, while hydrophobic fats and protein coat starch, decreasing water absorption and resulting in less granular swelling and reduced paste viscosity.<sup>32</sup> The acidic nature of baobab, in combination with heat, may have caused starch hydrolysis, resulting in the gelatinized starch paste being thinner for BFoSP and FoCFSP. Thus, the acid effect is minimized by rapid heating or adding baobab after heat treatment, as indicated by the high viscosity of FoFSP.

The viscosity of SP and BFoSP was found to be suitable for 6–11-month-old infants, while fermented porridges had viscosities that were slightly above the suggested limit.<sup>46</sup> There is currently no consensus on which range of shear rates constitutes the most representative conditions concerning chewing and swallowing processes, although a shear rate value of 50–90  $s^{-1}$  is considered a reasonable order of magnitude for in-mouth handling of the bolus,<sup>23</sup> suitable also for dysphagic infants.<sup>24</sup> However, the increase in shear rate caused a rapid decrease in apparent viscosity for all samples as observed in the study of Zhang *et al.*<sup>47</sup> for the curdlan and myofibrillar blends. As reported by Trèche,<sup>48</sup> 1 and 3 Pa s correspond approximately to the limits for drinkable (<1 Pa s), spoonable (>1 Pa s and <3 Pa s), and thick (>3 Pa s) gruel, with the indication that food suitable for children aged 6–11 months (in most contexts, particularly in Africa) should have a viscosity below 4.5 Pa s.

### 3.2 Effect of co-fermentation on the content of phytic acid

No significant difference was found in phytic acid contents in SP and BFoSP (Fig. 2). However, a significantly lower phytic acid content was observed for FoFSP (*i.e.*, ferment-cook-fortify), which confirms that adding baobab pulp after ferment-



**Fig. 2** Phytic acid contents of blended porridges. Different superscripts denote a statistically significant difference ( $P < 0.05$ ). SP: sorghum porridge; FSP: fermented sorghum porridge; BFoSP: sorghum porridge fortified with baobab pulp; FoCFSP: co-fermented sorghum porridge fortified with baobab pulp; FoFSP: sorghum porridge fortified with baobab pulp added after cooking.

tation and cooking causes a significant phytic acid reduction ( $P < 0.05$ ). Fermentation decreases the phytic acid content of sorghum porridge (FSP < SP) and baobab-fortified sorghum porridge (FoCFSP < BFoSP), as previously reported.<sup>29,49</sup> The reduction in phytic acid was probably because of the increased microbial phytase activity in fermented products or by interactions between phytic acid and other components, such as fibre, thus leading to reduced recovery.<sup>50</sup> Additionally, baobab reportedly has high content of, namely ascorbic acid and citric acid.<sup>51,52</sup> Acidic conditions (pH 2.5–4.5) trigger the endogenous phytase enzyme.<sup>53,54</sup> Furthermore, soaking increases water activity in the matrix during spontaneous fermentation, which activates phytase. As reported by Vidal-Valverde *et al.*,<sup>55</sup> a 37% reduction in phytic acid was observed in lentils soaked in acidic conditions. Phytic acid creates insoluble complexes with essential micronutrients such as Fe, Mn, Ca, and Zn, making them unavailable for absorption into the bloodstream. Phytase hydrolyses the hexa form of phytic acid into phosphate esters (IP5-IP), inferior forms of phytic acid with a lower affinity for metal ions like Fe and Zn.<sup>56</sup> Reducing phytic acid is necessary for low-protein foods, especially for children who suffer from health problems such as stunting and cannot get the complete intake of micronutrients from other sources. Further studies are required to assess the mineral absorption from the products of these treatments.

### 3.3 Biochemical properties of co-fermented and fortified sorghum porridges

Table 3 shows the proximate composition and estimated energy intakes for infants (6–8, 9–11 and 12–24 months old) of the co-fermented and fortified porridges.

**3.3.1 Moisture content.** The moisture content of the porridges ranged from 3.34 to 9.43 g per 100 g. BFoSP, FoCFSP and FoFSP had the lowest moisture contents and showed no significant differences. Fermented samples had a lower moisture content because fermentation at 25 °C for 48 h probably





Table 3 Proximate composition of porridge samples consisting of sorghum flour and combinations with baobab pulp

Porridge type	Moisture (g per 100 g)	Fibre (g per 100 g)	Ash (g per 100 g)	Protein (g per 100 g)	Fat (g per 100 g)	Carbohydrate (g per 100 g)	Energy (kJ per 100 g)	Energy intake (kJ day <sup>-1</sup> )		
								6–8 months	9–11 months	12–24 months
SP	9.43 ± 1.06 <sup>a</sup>	14.98 ± 3.76 <sup>a</sup>	1.50 ± 0.07 <sup>b</sup>	10.55 ± 0.45 <sup>ab</sup>	0.52 ± 0.10 <sup>b</sup>	78.52 ± 1.26 <sup>b</sup>	1508.81 ± 21.42 <sup>c</sup>	1878.84	2150.48	2603.22
FSP	6.84 ± 1.52 <sup>b</sup>	15.18 ± 11.41 <sup>a</sup>	1.62 ± 0.18 <sup>b</sup>	11.13 ± 0.71 <sup>a</sup>	2.25 ± 0.08 <sup>a</sup>	80.40 ± 1.70 <sup>b</sup>	1614.94 ± 29.27 <sup>b</sup>	2011.00	2301.75	2786.33
BFoSP	3.34 ± 0.17 <sup>c</sup>	19.36 ± 1.23 <sup>a</sup>	2.20 ± 0.11 <sup>a</sup>	9.45 ± 0.92 <sup>b</sup>	2.44 ± 0.99 <sup>a</sup>	85.01 ± 0.90 <sup>a</sup>	1671.16 ± 38.74 <sup>a</sup>	2081.01	2381.88	2883.33
FoCFSP	3.58 ± 0.26 <sup>c</sup>	33.28 ± 10.94 <sup>a</sup>	2.14 ± 0.18 <sup>a</sup>	9.42 ± 0.97 <sup>b</sup>	1.83 ± 0.41 <sup>a</sup>	84.86 ± 0.84 <sup>a</sup>	1645.30 ± 11.68 <sup>ab</sup>	2048.81	2345.02	2838.71
FoFSP	3.44 ± 0.19 <sup>c</sup>	20.95 ± 2.77 <sup>a</sup>	2.06 ± 0.27 <sup>a</sup>	9.48 ± 0.58 <sup>b</sup>	1.66 ± 0.07 <sup>a</sup>	85.02 ± 0.64 <sup>a</sup>	1642.40 ± 2.89 <sup>ab</sup>	2045.20	2340.89	2833.71
RDI Low BME							2310	2933	4301	
RDI Average BME							1490	2004	3230	

Different superscripts in the same column denote a statistically significant difference ( $P < 0.05$ ). SP: sorghum porridge; FSP: fermented sorghum porridge; BFoSP: sorghum porridge fortified with baobab pulp; FoCFSP: co-fermented sorghum porridge fortified with baobab pulp; FoFSP: sorghum porridge fortified with baobab pulp added after cooking; BME: breast milk energy, RDI: recommended daily intake. Estimated energy intakes were calculated using 16.67 g of solid contents per 100 g of porridge at 3 meals per day for functional gastric capacities of 249 g, 285 g and 345 g per meal for 6–8, 9–11 and 12–24 month old children respectively.<sup>65</sup>

led to the evaporation of part of the water. The addition of baobab powder (BFoSP, FoCFSP, and FoFSP) resulted in moisture content lowering because baobab pulp has a low water content of only 10%,<sup>57</sup> compared to sorghum flour which has a moisture content of ~12%.<sup>58</sup> The moisture content might affect the blends' water retention capacity. Specifically, high fat and fibre contents corresponded to low moisture contents, signifying the concentration of the macronutrient profile in the dry matter. However, protein did not follow this expectation, where the highest protein content was observed for a relatively high moisture content in FSP.

**3.3.2 Ash and fibre content.** The ash content ranged from 1.50 to 2.20 g per 100 g. The contents increased with fermentation and fortification and were not significantly different for FoCFSP, FoFSP and BFoSP. The fortified samples appear to have a higher fibre content, although no statistically significant differences existed among the different porridges. Fortified samples had higher ash levels since baobab is a rich source of minerals such as potassium, magnesium, and calcium.<sup>57</sup> The values are similar to those reported for fortified cereal weaning foods. Furthermore, fermentation *via* hydrolysis of bound minerals and loss of dry matter improves ash extractability and the activation of microbial enzymes may have reduced the myriad anti-nutritional factors, increasing mineral solubility.

Fermentation also did not influence fibre content, although fermenting microorganisms, which completely or partially can hydrolyse fibre by enzymatic degradation, can decrease the fibre content. The addition of baobab pulp was expected to result in a higher fibre content because the fibre content of baobab pulp is approximately 70–80% of its dry mass<sup>59</sup> whereas that of sorghum is 19%.<sup>60</sup>

**3.3.3 Protein and carbohydrate content.** The protein content ranged from 9.42% to 11.13%, with no significant difference for BFoSP, FoFSP, and FoCFSP. The differences in the carbohydrate content of the treated samples were marginal. FSP had a significantly higher protein content than FoFSP and FoCFSP, while the carbohydrate content of FoFSP and FoCFSP was about 5.3–5.6% higher than for FSP. Fortification decreased the protein content because baobab fruit has a low protein content.<sup>61</sup> Moreover, fermentation increases the number of microorganisms that utilise protein and amino acid molecules for their metabolism, thereby reducing protein content. Baobab also contains high levels of phenolics and tannins, whose antinutritional effects can be attributed to the formation of complexes with dietary protein rather than the inhibition of enzymes.<sup>62,63</sup> Protein content also depends on dominant microbes like yeasts or lactic acid bacteria. A reduction in protein content due to baobab fortification and fermentation has been reported in other studies.<sup>64</sup> Moreover, the protein increase for FSP could be due to non-nitrogen protein; thus, the findings require further investigation. The carbohydrate content was expected to be high for FoCFSP and FoFSP due to the high carbohydrate content of baobab. In FSP, the reduction of carbohydrates indicates the activation of  $\alpha$ -amylase and metabolic activity of microorgan-

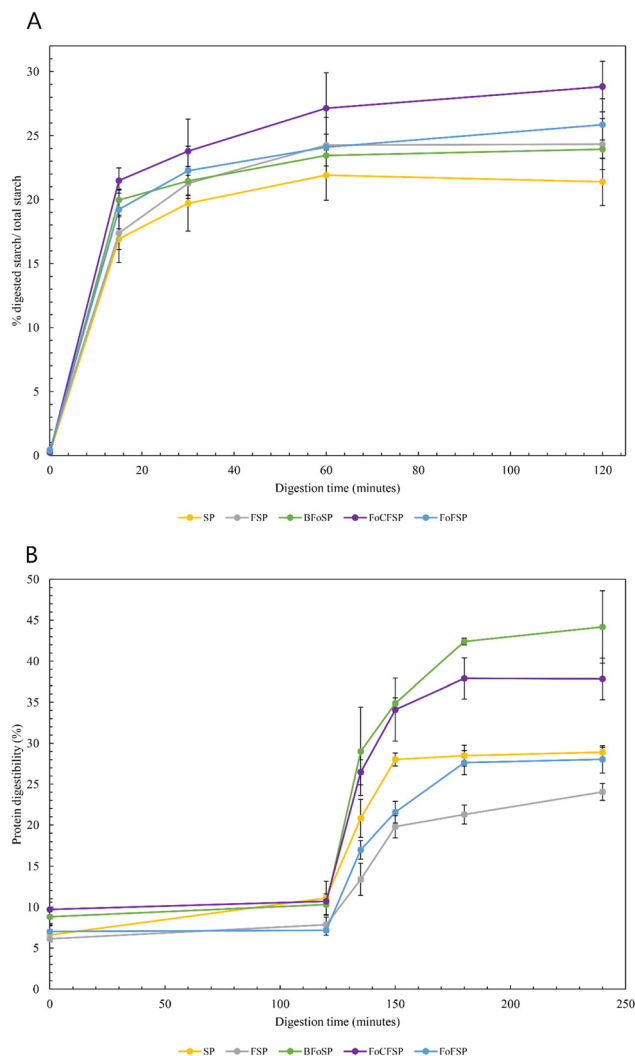
isms degrading carbohydrates to simple sugars. However, because of the combined effects and high dietary fibre, including pectins, the carbohydrate content in FoFSP and FoCFSP was higher.

**3.3.4 Fat content.** Fat content ranged from 0.52 to 2.44 g per 100 g, with SP having the lowest fat content. Fermented, fortified and fermented/fortified samples have a relatively higher fat content – up to 4 fold – compared with SP, especially FSP and BFoSP. No significant difference ( $p \leq 0.05$ ) was found in the fat content for FoFSP and FoCFSP. Co-fermentation increased the fat content compared to SP, even though baobab fruit pulp has a low fat content. This observation regarding fat content does not agree with the findings when baobab pulp was used as a starter for sorghum fermentation.<sup>29</sup> During fermentation, an increase in lipase activity and use of fat components by fermenting microorganisms may have occurred, resulting in the low fat content, but it seems that this correlation only exists for the FoCFSP and FoFSP (both fermented and fortified) samples compared to BFoSP (fortified but not fermented). As yet, the reason for the evident increase in fat in the FSP sample compared to SP is not clear. If, instead, FSP is compared with FoCFSP and FoFSP, it is possible to note that fortification decreases the fat content, probably because baobab fruit has a low fat content.<sup>61</sup>

**3.3.5 Energy content and estimated energy intake.** The energy content of BFoSP, FoCFSP and FoFSP was not significantly different. The energy content of these samples was higher by 8–10.8% compared to unfermented-unfortified samples. All fermented samples exhibited a higher energy content than the average whole-grain sorghum energy value. The higher energy content can be attributed to the blending of sorghum and baobab powder because the flours complemented one another, making an energy- and nutrient-dense formulation. We calculated the energy intake per day based on 16.67 g solid contents per 100 g porridge at 3 meals per day for functional gastric capacities of 249 g, 285 g and 345 g per meal for children aged 6–8, 9–11 and 12–24 months, respectively.<sup>65</sup> The data show that the estimates for the non-fermented sample are comparable to those reported by the National Research Council.<sup>66</sup> Furthermore, the estimated calculated energy intake for the fermented-fortified porridge samples was sufficient for infants 6–11 months old with an average breast milk intake.<sup>65</sup> The energy content of infant foods is vital for their nutritional status as it determines energy intake. However, consuming only fortified fermented porridge three times a day may not give all the required nutrients for healthy growth and development.

### 3.4 Digestibility

**3.4.1 Starch digestibility.** Starch *in vitro* digestibility was in the order FoCFSP > FoFSP > FSP > BFoSP > SP, as shown in Fig. 3A. Co-fermentation had the highest starch digestion. The significant difference between FoCFSP and the other samples is noteworthy. The increase in starch digestibility of the co-fermented samples can be related to an increased susceptibility to amylolytic activity after gelatinisation and solubilization,



**Fig. 3** Starch (A) and protein (B) hydrolysis as a function of time during gastric and intestinal *in vitro* digestion phase for protein and intestinal phase only for starch. Values are mean  $\pm$  SD of four independent samples. SP: sorghum porridge; FSP: fermented sorghum porridge; BFoSP: sorghum porridge fortified with baobab pulp; FoCFSP: co-fermented sorghum porridge fortified with baobab pulp; FoFSP: sorghum porridge fortified with baobab pulp added after cooking.

thus releasing starch from tissues.<sup>67</sup> The fermenting microflora enzymes have access to break down starch oligosaccharides into simple sugars. The expectation was that BFoSP would have a digestibility close to that of FoCFSP because of its higher WSI (Table 1) and also due to baobab addition before cooking, where the expectation was that the microbial enzymes of baobab would have catalytic activities that would subsequently increase digestibility. This was not the case, confirming the hypothesis that baobab enzymes are less significant in *in vitro* starch digestibility and that co-fermentation is more significant. Other studies confirm that plant amylases have a hydrolysis activity below 5% and scarcely hydrolyse raw starch.<sup>68</sup> Cooked sorghum flour has a lower starch digestibility than other grains, mainly because of the important role of the



protein in the flour paste in slowly digesting starch.<sup>69</sup> According to recent studies, the protein bodies could encapsulate the starch granules, which would then act as a protective layer, decreasing the digestibility of the starch.<sup>70</sup> Besides, baobab contains phenolic compounds, which alter functional properties when added to starch systems.<sup>71</sup> The additive effect of baobab organic acids and the phenolic compounds at lower temperatures in BFoSP was critical in reducing the starch digestibility. These findings are pivotal in determining the balance between *in vivo* glucose release and reducing postprandial hyperglycaemia in infant foods per age group for these fortifications. Finally, no correlation was found between phytic acid content and starch digestibility.

**3.4.2 Protein digestibility.** Protein *in vitro* digestibility was in the order of BFoSP > FoCFSP > SP > FoFSP > FSP (Fig. 3B). The protein digestibility of the samples ranged from 25% to 45%. As Fig. 3B shows, protein digestion in the stomach is very limited. This phenomenon is well known, especially for plant food products.<sup>70</sup> Furthermore, limited proteins due to the protease resistance of kafirins, which comprise 50–70% of the proteins in sorghum, result in lower digestibility than for other crops.<sup>72</sup> The trend in the results shows that adding baobab with co-fermentation or blending before cooking increased the *in vitro* protein digestion. Additionally, as reported in the literature,<sup>73</sup> the protease activity is higher at 60 °C than at 50 °C, and this would explain why, considering the fortified samples, BFoSP and FoCFSP have a higher digestibility than FoFSP. Moreover, considering that baobab contains some trypsin inhibitors<sup>74</sup> whose activity decreases after cooking,<sup>75</sup> the activity of these inhibitors is not modified significantly if fortification occurs after cooking (FoFSP), unlike in the other samples fortified before cooking (BFoSP and FoCFSP). Microbial activity during fermentation could have caused protein hydrolysis and increased availability of peptides and amino acids, showing increased FoCFSP digestibility. Further, the biosynthesis and modification of the structural configurations of peptides and amino acid nutrients can make them more accessible to enzymatic action during fermentation, a mechanism that warrants further investigation in the specific matrices.

## 4. Conclusion

Fermentation and fortification significantly improve the nutritional and functional qualities of infant complementary foods when using sorghum–baobab blends. More specifically, the co-fermentation of sorghum and baobab improves the functional properties of the porridge despite the absence of significant changes from a biochemical point of view. Furthermore, co-fermentation represents a fortification strategy that guarantees a product with higher digestibility of starch and proteins. Both co-ferment-cook and ferment-cook-fortify have improved the energy density, making them beneficial for complementary foods for infants 6–24 months old. Nutritionally, ferment-cook-fortify provided better results than co-fermentation. The

findings cement the applicability of these food processing methods and the blending of sorghum with baobab in infant complementary food production, depending on the specific general outcome purpose and age group. Therefore, when optimally processed and composited with other ingredients, sorghum can result in a nutrient-dense complementary food with the potential to counteract the problem of malnutrition. These findings contribute to ascertaining the role of processing practices and bring new information about their impact on the nutritional and functional quality of the end product. Recent economic, productive and social trends have led to the rediscovery of the potential of spontaneous fermentation in improving the unique quality of fermented products. The exploitation of indigenous bacteria and yeasts as a commercial option presents a strategy to restore traditional artisanal practices that have the potential for scaling up. Modern microbial biotechnologies should be sought to reconcile fermented food safety with instances of an enhanced contribution of microbes associated with spontaneous fermentation. In parallel, further studies on food processing and technology are needed to ensure adequate consistency, as infants reject food textures that cannot be swallowed and exacerbate malnutrition.

## Author contributions

Luigi Moriconi: formal analysis, investigation, data curation, and writing – review and editing. Elena Vittadini: funding acquisition, supervision, and writing – review and editing. Anita R. Linnemann: conceptualization, supervision, visualization, and writing – review and editing. Vincenzo Fogliano: funding acquisition, resources, supervision, and writing – review and editing. Ruth T. Ngadze: project administration, conceptualization, methodology, supervision, visualization, and writing – original draft, review and editing.

## Data availability

The data supporting this article have been included as part of the ESI.†

## Conflicts of interest

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

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