LWT - Food Science and Technology 198 (2024) 115951

Contents lists available at ScienceDirect

LWT

journal homepage: www.elsevier.com/locate/lwt

Effect of pre-treatment on physicochemical, microstructural and pasting properties of pearl millet and cowpea

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ARTICLE INFO

Keywords:
Pre-treatment
Physicochemical
Microstructure
Pearl millet
Cowpea

ABSTRACT

The effects of soaking, germination and fermentation on physicochemical, microstructural and pasting properties of improved and drought-tolerant Mozambican varieties of pearl millet and cowpea were investigated.

Total starch content in both germinated pearl millet (GPM, 67.2 % of DM) and germinated cowpea (GCP, 47.4 % of DM) was found to be affected by germination, which consequently affected the amylose content of GPM and GCP. Morphological and pasting properties of pearl millet starch granules were altered by germination, with numerous holes and broken starch granules developing, leading to a drastic reduction in final viscosity (4 mPa.s). Cowpea starch granule structure was not markedly affected by pre-treatment, but peak (310 mPa.s) and final viscosity (196 mPa.s) were decreased by germination. Cowpea flour had smaller particle size distribution than pearl millet, but no significant differences in the flour were observed after pre-treatment (soaking, germination, fermentation). Therefore, these simple, low-cost and culturally acceptable treatments can be used to alter technical functionality and improve the nutritional benefits of flour, e.g. different pre-treatments of pearl millet and cowpea could be used to develop food products with high energy density and acceptable sensory profile such as porridge for undernourished children in low and middle-income countries.

1. Introduction

Pearl millet and cowpea are well-known seeds cultivated in many countries worldwide and contribute significantly to African food security. In Mozambique, these seeds play an essential role in the diet due to their high nutrient content and potential value as an affordable source of protein. The protein in pearl millet has a balanced amino acid profile and a high methionine content, which makes it an excellent complement to legumes. Overall, pearl millet seeds are nutritionally comparable or even superior to cereals such as wheat and rice (Rai, Gowda, Reddy, & Sehgal, 2008). Cowpea is a plant protein source that could be used to enrich infant cereal food in low and middle-income countries in Africa or Asia. Its high lysine content makes it an excellent complement to cereal seeds to enhance protein quality in the human diet. Cowpea is therefore one of the most important legumes in Africa (Prinyawiwatkul, McWatters, Beuchat, Phillips, & Uebersak, 1996). In recent years, pearl millet and cowpea have attracted attention due to their capacity to grow in harsh climate conditions and potential nutritional benefits. Therefore, they are an excellent choice for incorporation into children's diets to alleviate protein-energy malnutrition in climate-vulnerable countries such as Mozambique (Goudar et al., 2023; Kapravelou, Martínez, Martino, Porres, & Fernández-Fígares, 2020; Almeida-Dominguez, Serna-Saldivar, Gomez, & Rooney, 1993).

The use of simple, affordable and culturally acceptable pre-treatments, such as soaking, germination and fermentation, are common at a household level in many low-middle-income countries like Mozambique before milling to soften the seeds and to enhance their nutritional value. These pre-treatments reduce the levels of anti-nutritional factors and improve digestibility and sensory characteristics (Nkhata, Ayua, Kamau, & Shingiro, 2018; Oladiran & Emmambux, 2022). Pearl millet and cowpea seeds are usually soaked for different food preparations (Henshaw, McWatters, Oguntunde, & Phillips, 1996; Ocheme & Chinma, 2008). This soaking process activates endogenous enzymes, such as alpha- and beta-amylase, and some water-soluble components leach out into the soak water. Germination pre-treatment increases the activity of hydrolytic enzymes, which break down

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https://doi.org/10.1016/j.lwt.2024.115951
Received 20 October 2023; Received in revised form 4 March 2024; Accepted 6 March 2024
Available online 13 March 2024

starch, protein and cell-wall polysaccharides. Germination of seeds effectively reduces the viscosity of thick porridges without dilution with water, and thus maintains high energy density (Alexander, 1983; Mosha & Svanberg, 1990; Ocheme & Chinma, 2008). In fermentation pre-treatment, microorganisms and enzymes produced by the natural microflora alter the composition of seed components, thus improving the nutritional value and altering the microstructure, leading to improved digestibility and texture (Nout & Ngoddy, 1997). The study is of particular interest because there is currently a lack of detailed information on the effects of these pre-treatments on the physicochemical, microstructural and pasting properties of Mozambican varieties of pearl millet and cowpea. In Mozambique, the changara variety of pearl millet and the variety 10 of cowpea are among the highly recommended, improved and locally available varieties due to their adaptability to volatile climatic conditions like drought. These varieties are high-yielding which contributes to food and nutritional security (Intsormil, 2012; Huynh et al., 2013). A better understanding of the chemical and physical modifications made to these seeds by pre-treatments such as soaking, germination and fermentation is therefore essential to successfully develop food products with high nutritional value, better texture and sensory acceptability for undernourished children in Mozambique.

This initial study aimed to determine the effects of seed pre-treatment (soaking, germination and fermentation) on physicochemical, microstructural and pasting parameters, proximate composition, starch and amylose content in pearl millet and cowpea. The overall goal was to gain better insights into the physicochemical attributes of different flour samples, produced by simple processing steps, intended for use in developing nutritional porridge products for children in Mozambique.

2. Materials and methods

2.1. Sample preparation and pre-treatments

Pearl millet (*Pennisetum glaucum*) variety Changara and cowpea (*Vigna unguiculata L.*) variety 10 were acquired from the Mozambican Agricultural Research Institute in Montepuez, Cabo Delgado. The samples were divided into two batches (800 g each), and sorted and cleaned, with damaged grains, stones and all other extraneous material removed and discarded. Each batch was then divided into four 200-g portions for four treatments (washing, soaking, germination and fermentation).

In the washing pre-treatment, the seeds were washed quickly for 10 s in tap water to remove dust, followed by drying in an oven with fan (Memmert, Germany) at 40 °C for 24 h. In the soaking pre-treatment, the seeds were steeped in tap water at room temperature (21 ± 2 °C) for 10 min and then dried. In the germination pre-treatment, the seeds were soaked in tap water at room temperature for 24 h, in a 1:3 (w/w) ratio for pearl millet and 1:4 (w/w) for cowpea, and then spread on germinator trays at 30 ± 1 °C in an oven without fan. Seeds were sprayed with water twice a day and removed from the oven after 48 h of germination. The sprouted seeds were washed for 10 s in tap water and dried in an oven with fan, after which dried rootlets were removed by rubbing between the hands. In the fermentation pre-treatment, the seeds were soaked in tap water at room temperature (21 ± 2 °C), in a 1:3 (w/w) ratio for pearl millet and 1:4 (w/w) for cowpea, and allowed to ferment by the action of endogenous microflora at 30 ± 1 °C for 72 h in an oven without fan. The seeds were then washed for 10 s in tap water, dried in an oven with fan.

The seeds were pre-milled using a Tecator machine (Cemotec, Sweden) to break them into small pieces. The washed samples were further milled with the entire hull. In the soaking, germination and fermentation pre-treatments, the seeds were partially dehulled following the Mozambican tradition, which consists of spreading the pre-milled seeds on a tray and removing the hulls using compressed air, and then milling the remaining seed components. All samples were milled using a laboratory cyclone sample mill (Retsch, Haan, Germany) to pass through a sieve with mesh 0.5 mm.

These pre-treatment methods were inspired by methods described by Adebiyi, Obadina, Mulaba-Bafubiandi, Adebo, and Kayitesi (2016), Griffith and Castell-Perez (1998), Ibrahim, Habiba, Shatta, and Embaby (2002) and Ocheme and Chinma (2008), with minor modifications relating to duration of drying for the materials and length for fermentation for cowpea, in order to standardise the treatments and make them easily replicable in practice in Mozambican communities.

The milled samples were stored in polyethylene zipper plastic bag at room temperature (21 ± 2 °C) before analysis. Samples from the two original batches of each seed type were used to prepare replicates for all treatments. Each treatment was analysed at least in duplicate and results are reported as mean values of repeats.

2.2. Proximate composition analysis

Dry matter (DM) content was determined by drying for 16 h at 105 °C according to AACC method 44-15A (2000). Total dietary fibre was quantified according to the Uppsala method (Theander, Aman, Westerlund, Andersson, & Petersson, 1995), further modified for separate analysis of extractable and unextractable dietary fibre components (Andersson, Merker, Nilsson, Sørensen, & Åman, 1999). Crude protein content was measured by the Kjeldahl method, according to the Nordic Committee on Food Analysis (1976), using a 2520 digestor, Kjeltec 8400 analyser unit and 8460 sampler unit (all from Foss, Denmark). Protein content was calculated from nitrogen content (N x 6.25). Fat content was determined according to EU Commission Directive 152/2009 EC (2009) using a Hydrotec 8000 and Soxtec 8000 extraction unit (both from Foss, Denmark). Ash was determined by incineration in an oven at 600 °C for 3 h.

2.3. Total starch and amylose content

The starch content in flours was determined by selective hydrolysis with thermostable α-amylase and amyloglucosidase (Åman, Westerlund & Theander, 1994). The amount of glucose released was quantified using kit K-GLUC (GOPOD: glucose oxidase/peroxidase) from Megazyme (Bray, Ireland). Amylose was measured using a colorimetric method based on iodine complex formation stabilised with trichloroacetic acid (Chrastil, 1987). The amylose content in flour samples was determined based on a standard curve developed using waxy barley and barley starch (Lyckeby, Kristianstad, Sweden) with varying amylose level (0%, 16%, 32% and 48%). The absorbance value was read against water at 620 nm. The results are reported as mean percentage of total starch content.

2.4. Morphological properties

Pearl millet and cowpea flour were analysed using scanning electron microscopy (SEM) (Hitachi TM-1000, Tokyo, Japan) with 2500X magnification (30 μm scale). Samples were mounted on aluminium stubs and spray-coated with a thin film of carbon using a carbon coater (Cressington Scientific Instruments, Sputter coater-108 auto, Watford, UK). After coating, the samples were transferred to the SEM specimen chamber and subjected to an electron beam under vacuum.

2.5. Particle size distribution

Particle size distribution of the flours was determined by sieve fractionation using an AS 200 shaker (Retsch, Haan, Germany). Each sample (50 g) was sifted with a set of five graded sieves (425, 250, 150, 75 and 50 μm) and a collection pan. Fractionation was carried out at 1.5 mm amplitude for 10 min with 10 s sieving intervals to ensure complete fractionation.

2.6. Pasting properties

Pasting properties were determined by a rapid visco analyser (RVA) (Newport Scientific, Australia), using a 3 g sample of flour dispersed in 25 mL deionised water in an aluminium canister. The samples were heated from 50 °C to maximum temperature 95 °C and held at 95 °C for 2.5 min, before cooling to 50 °C, using the general standard method (Std1). Pasting temperature, peak viscosity, peak time, breakdown, strength, setback and final viscosity were recorded.

2.7. Statistical analysis

All analyses were carried out on the two replicate batches used for the pre-treatments. Statistical analyses of data were performed using the statistical software Minitab version 19.2. Analysis of variance (ANOVA) was performed using the general linear model procedure, with pre-treatment, seed types and their interaction as factors. Tukey's comparison test was used to identify significant differences between group means, with significance level set at 95% confidence level.

Multivariate analysis of the data obtained in the study was applied separately for pearl millet and cowpea, to assess the effect of the pre-treatments. Principal component analysis (PCA) score and loadings plots were used to visualise differences between batches, and differences and relationships between variables. These analyses were performed using the software SIMCA 17 (Sartorius Stedim Data Analytics AB).

3. Results and discussion

3.1. Proximate composition

Cowpea samples contained nearly twice as much crude protein (23.6–27.9% of DM) as pearl millet samples (12.1–12.9% of DM) (Table 1). The protein in cowpea is rich in lysine, but low in methionine, so combining cowpea with pearl millet, which is rich in methionine, can result in a porridge with high protein quality suitable for undernourished children (Prinyawiwatkul et al., 1996). Germinated cowpea had a significantly higher protein content than soaked cowpea (Table 1), which is in agreement with previous findings (Ghavidel & Prakash, 2007). During germination, carbohydrates stored in the endosperm are consumed by the plant embryo to provide energy for cellular processes, which leads to DM losses due to carbon dioxide and water incorporation in growing shoots and roots. This process may have contributed to the observed increase in protein content in cowpea. In contrast, Jirapa, Normah, Zamaliah, Asmah, and Mohamad (2001) found no significant differences in protein content in cowpea after germination, although their germination treatment had a significant effect on in vitro protein digestibility. Pre-treatment by fermentation significantly increased the protein content in cowpea (Table 1), again possibly as a result of loss of dry matter (mainly carbohydrates), which fermentative microorganisms consume as a source of energy. In contrast, Giami (1993) observed only a non-significant increase in crude protein content of cowpea after 72 h of fermentation.

The crude protein content in pearl millet samples did not show any significant variation between the different pre-treatments (Table 1). Thus, germination and fermentation of pearl millet did not increase the protein content, whereas some previous studies have found that these pre-treatments can improve protein digestibility due to breakdown of complex proteins into more soluble peptides (Annor, Tyl, Marcone, Ragaee, & Marti, 2017; Elkhalifa & Bernhardt, 2010). Adebiyi, Obadina, Adebo, and Kayitesi (2017) and Sade (2009) observed an apparent increase in the protein content of germinated and fermented pearl millet seed.

In the present study, the total fat content was significantly higher in pearl millet samples (5.9–6.6% of DM) than in cowpea samples (2.3–2.5% of DM) (Table 1). However, pre-treatment by soaking, germination or fermentation did not affect the fat content in either pearl

Table 1
Total dietary fibre, crude protein, crude fat, ash and starch content (% of dry matter (DM)) and amylose content (% of starch) in pearl millet and cowpea subjected to different pre-treatments[1].

Pre-treatment	Total dietary fibre [2]	Crude protein (N x 6.25)	Crude fat	Ash	Total starch	Amylose (% of starch)
Pearl millet						
Washed	7.0 ± 1.1^{bc}	12.8 ± 0.1^{d}	6.0 ± 0.4^{a}	1.3 ± 0.1^{c}	71.0 ± 0.5^{a}	32.1 ± 1.4^{ab}
Soaked	6.0 ± 0.3^{c}	12.7 ± 0.02^{d}	6.1 ± 0.2^{a}	1.3 ± 0.0^{c}	69.9 ± 0.2^{ab}	33.1 ± 0.6^{ab}
Germinated	7.7 ± 0.3^{bc}	12.1 ± 0.2^{d}	5.9 ± 0.2^{a}	1.0 ± 0.1^{cd}	67.2 ± 0.7^{b}	31.5 ± 0.4^{b}
Fermented	6.8 ± 0.4^{bc}	12.9 ± 0.3^{d}	6.6 ± 0.3^{a}	0.9 ± 0.0^{d}	70.6 ± 0.1^{a}	33.6 ± 1.0^{ab}
Cowpea						
Washed	13.5 ± 1.1^{a}	23.6 ± 0.0^{c}	2.4 ± 0.4^{b}	3.4 ± 0.0^{a}	44.6 ± 0.6^{e}	33.6 ± 0.7^{ab}
Soaked	7.9 ± 0.4^{bc}	24.7 ± 0.2^{c}	2.3 ± 0.2^{b}	3.5 ± 0.0^{a}	48.1 ± 0.1^{d}	36.2 ± 0.0^{ab}
Germinated	8.3 ± 0.0^{bc}	27.9 ± 0.6^{a}	2.5 ± 0.3^{b}	3.4 ± 0.2^{a}	47.4 ± 1.8^{de}	32.5 ± 3.1^{ab}
Fermented	8.4 ± 0.2^{b}	26.3 ± 0.4^{b}	2.3 ± 0.1^{b}	2.9 ± 0.1^{b}	51.9 ± 0.2^{c}	36.9 ± 0.8^{a}

Values within columns followed by different letters are significantly different at $p < 0.05$ in Tukey's pair-wise comparison of pre-treatments and seed types.
[1)] Mean value ± standard deviation of two replicates on % of dry weight basis, except for amylose.
[2)] Sum of extractable and unextractable fractions, except oligosaccharides (DP 3–9).

millet or cowpea, which is in agreement with previous findings by Sade (2009) for germinated and fermented pearl millet. For cowpea, however, Jirapa et al. (2001) observed a significant decrease in total fat content after 48 h of germination. The total fat content in pearl millet is much higher than that in major cereal crops like rice, wheat and maize, improving the energy value (Rai et al., 2008).

Ash content in pearl millet samples (0.9–1.3% of DM) was significantly lower than that in cowpea samples (2.9–3.5% of DM) (Table 1). In both cases, fermentation of samples significantly decreased the ash content compared with soaked samples, possibly due to leaching of soluble inorganic salts during fermentation. Similar results have been reported by Adebiyi et al. (2017) and Sade (2009) for fermented pearl millet, and by Onweluzo and Nwabugwu (2009) for fermented pigeon pea flour. In the present study, there was a slight decrease in ash content of germinated samples of pearl millet and cowpea compared with soaked samples, but the differences were not statistically significant.

Total dietary fibre content in pearl millet and cowpea was calculated as the sum of extractable and unextractable dietary fibre. Pre-treatment and seed type both had statistically significant effects on total dietary fibre (TDF) content, with cowpea samples having significantly higher TDF levels (7.9–13.5% of DM) than pearl millet (6.0–7.7% of DM) (Table 1). Washed cowpea seeds had a significantly higher TDF content than soaked, germinated or fermented cowpea. This might be due to presence of the hulls, since washed cowpea was milled as whole grains while seeds used for other pre-treatment were dehulled, indicating that dehulling significantly affected the TDF content. No significant differences were observed in TDF content in pearl millet. Similarly, Griffith and Castell-Perez (1998) found that grain decortication significantly reduced the TDF content of cowpea, but not pearl millet.

In comparison with the soaking pre-treatment, germination and fermentation tended to increase the TDF content in both cowpea and pearl millet (Table 1). Veena, Urooj, and Puttaraj (1995) and Benítez et al. (2013) observed similar changes after germination and fermentation of cowpea, while Sharma, Saxena, and Riar (2016) observed similar changes after germination of barnyard millet. This increase in TDF content may be because of enzymatic breakdown of cell-wall polysaccharides during seed germination or fermentation.

3.2. Total starch content and amylose

Starch is an essential component of cereals and legumes used in the human diet. Total starch content was significantly higher in pearl millet (67.2–71.0% of DM) than in cowpea (44.6–51.9 % of DM) (Table 1). Total starch content in both germinated pearl millet and cowpea was slightly lower than in the soaked samples, possibly due to breakdown of starch granules by α-amylase, the principal enzyme activated during germination. It is known that the germination process is responsible for activating enzymatic activity in sprouted seeds, resulting in breakdown of carbohydrates into simpler forms. Similarly, in previous studies, germination of seeds has generally been found to decrease starch content (Elkhalifa & Bernhardt, 2010; Griffith & Castell-Perez, 1998; Veena, Urooj, Puttaraj, 1995; Yang et al., 2021).

Total starch content in fermented pearl millet (70.6% of DM) showed no statistically significant differences compared with soaked pearl millet (69.9% of DM) (Table 1). In contrast, Khetarpaul and Chauhan (1990) observed a decrease in starch content in pearl millet during fermentation in a study where pure cultures were used for fermentation. On the other hand, total starch content in fermented cowpea (51.9% of DM) was significantly higher than in soaked cowpea (48.1% of DM) (Table 1). The higher protein content in fermented cowpea might have protected the starch granules surface for enzymatic action due to reduced starch swelling. A study by Zhu, Liu, Sang, Gu, and Shi (2010), reported after the protein matrix is disrupted, the starch granules become less rigid and susceptible to enzymatic attack. However, Veena et al. (1995) found no significant differences in total starch content in cowpea after fermentation.

Amylose comprised 33.1% of the total starch content in soaked pearl millet and 36.2% of total starch in soaked cowpea (Table 1). A study by Badi, Hoseney, and Finney (1976) reported much lower amylose content in pearl millet starch (17% of total starch). The amylose content in pearl millet in the present study ranged from 32.1 to 33.6 %, which is a high level compared with some cereal starches (Emmambux & Taylor, 2013). Different varieties, seed origins, crop growing conditions and estimation methods may explain these differences. A study by Faki, Desikachar, Paramahans, and Tharanathan (1983) and Wani et al. (2016) reported the amylose content in cowpea starches ranged from 25.8 to 33% of total starch, which is slightly lower than in the present study. Legume starch has a relatively high amylose/amylopectin ratio compared with cereals (Hoover & Sosulski, 1985).

The pre-treatments tested in this study showed no significant effects on amylose content of cowpea and pearl millet. However, the amylose content in germinated pearl millet and cowpea samples decreased slightly compared with soaked samples (Table 1). Yang et al. (2021) reported similar results for germinated proso millet. The slight decrease in amylose after germination may have been caused by an increase in α-amylase activity and enzyme hydrolysis of macromolecules such as amylose and amylopectin during germination, leading to production of small molecules such as dextrin, maltose and oligosaccharide (Li, Oh, Lee, Baik, & Chung, 2017). According to Frias, Fornal, Ring, and Vidal-Valverde (1998), the digestibility of starch may improve with decreasing amylose in germinated samples.

3.3. Scanning electron microscopy

The SEM images showed different morphological properties of pearl millet and cowpea starch granules (Figs. 1 and 2). Pearl millet starch granules ranged in diameter from 6 to 12 μm and were round in shape, irregular and polygonal with several faces. Cowpea starch granules ranged in diameter from 12 to 24 μm and were oval or spherical in shape. Similar findings have been reported by Badi et al. (1976) and Faki et al. (1983) for pearl millet and cowpea starch, respectively.

From the SEM images, it was evident that washed and soaked pearl millet starch granules had similar morphology, with intact starch granules visible in both cases. Thus short-duration soaking (10 min) did not affect the morphology of the starch granules. On the other hand, the germination pre-treatment clearly affected pearl millet starch granule morphology, resulting in numerous holes and broken starch granules. In addition, the layers became apparent after germination, due to attack by amylases (Fig. 1, panel GPM). Some previous studies have also observed pores in starch granule in cereal grains after 48 h of germination, as a result of enzymatic degradation (Adebiyi et al., 2016; Li, Oh, Lee, Baik & Chung, 2017). It has been suggested that amylases penetrate the granules during germination and hydrolyse from the hilum region towards the outside (Krishna & Thayumanavan, 1998). However, the SEM images of germinated cowpea showed no holes in the granules (Fig. 2, panel GCP), probably due to low enzyme activity and higher protein content protecting the starch granules from damage (Zhu et al., 2010). Similar findings have been reported previously for starch granules in cowpea (Faki et al., 1983). Hoover and Sosulski (1985) postulated that the degree of attack by α-amylase also depends on factors such as granule size, amylose/amylopectin ratio, degree of crystallinity and degree of polymerisation.

A slightly irregular, loose matrix was apparent in fermented pearl millet (Fig. 1, panel FPM), probably due to enzymatic attack, whereas this type of irregular, loose matrix feature was less visible in fermented cowpea (Fig. 2, panel FCP). During fermentation, water uptake by the seed makes the starch granules smoother and loosely embedded in the protein matrix, microstructural differences that were more clearly visible in pearl millet than in cowpea. According to Lineback and Ponpipom (1977), pearl millet has a very active α-amylase system.

3.4. Particle size distribution

Fig. 3 shows particle size distribution (PSD) retained in each sieve, with values expressed as percentage of the total. Pearl millet flour retained significantly larger particle sizes (≥150 μm mesh size) than cowpea. The most significant percentage of fine particles was observed in cowpea flour, with most particles retained in the sieve with <150 μm mesh size. Similarly, Griffith and Castell-Perez (1998) observed very fine particle size in cowpea flour, with high percentages in a <74 μm sieve and the collection pan.

Large particle size distribution influences the resistance of starch granules to expand and rupture. According to Kerr, Ward, McWatters, and Resurreccion (2000), milling conditions and particle size influence the functional properties of the flour, as do type and amount of material extracted by water. In the present study, there were differences in particle size distribution depending on seed type, seed hardness and pre-treatment. Pre-treatments such as soaking, germination and fermentation are often used in Mozambique to soften the seed and facilitate the milling process. Milling can be done manually or using a commercial roller mill, which gives different particle sizes. Griffith and Castell-Perez (1998) found that germination of pearl millet and cowpea influenced the particle size distribution, with germinated flours producing a higher percentage of particles that passed through the sieve 210 μm mesh size. In the present study, no significant differences were observed between pre-treatments in pearl millet and cowpea. However, germinated and fermented pearl millet flour produced a higher percentage of particles that passed through in the sieve 150 and 75 μm mesh size and cowpea flour produced a higher percentage of particles that passed through in the sieve 50 and < 50 μm mesh size (Fig. 3). As suggested by Griffith and Castell-Perez (1998), all samples were

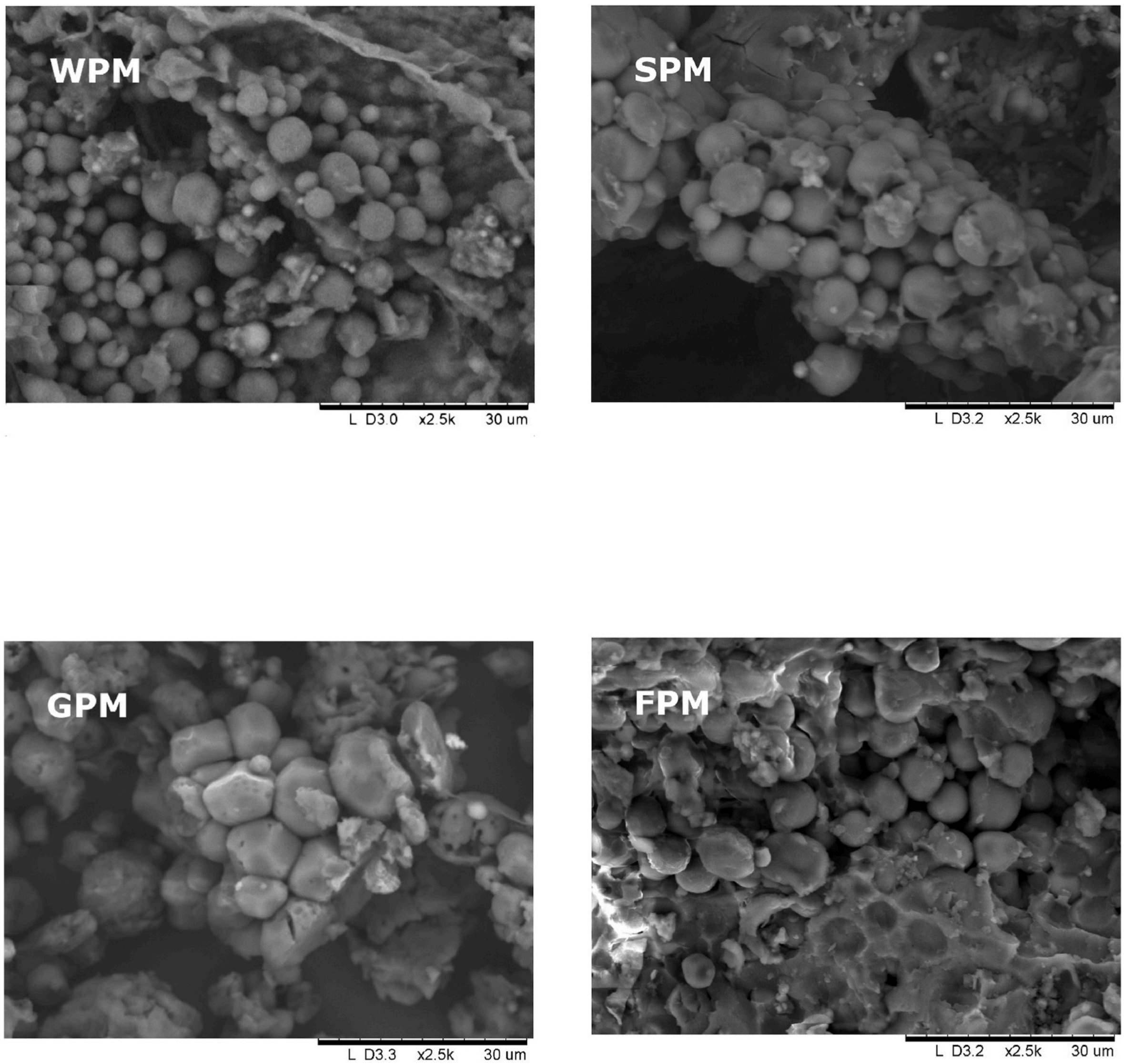

Fig. 1. SEM images of starch granules in pearl millet after different pre-treatments: WPM = washed pearl millet, SPM = soaked pearl millet, GPM = germinated pearl millet, FPM = fermented pearl millet.

classified as fine because they passed through the sieve 425 μm mesh size, which is reported to be a good particle size range to prepare complementary porridge with desirable viscosity values for undernourished children. Viscosity is an important characteristic in children's foods, as children and babies have less developed oral motor skills.

3.5. Pasting properties

The pasting properties of pearl millet and cowpea flours were assessed based on pasting graphs (Fig. 4), which provided information on the viscosity of the flour in water. The pasting characteristics of starches are affected by amylose and amylopectin content and by the arrangement of these in the starch granule. In pearl millet, the highest viscosity (2773 mPa.s) was observed in soaked samples, followed by washed samples (2488 mPa.s) (Fig. 4A). The highest peak viscosity (1387 mPa.s) in cowpea was observed in fermented samples (Fig. 4B). The high peak viscosity of the pearl millet samples indicated high swelling power of the starch granules and their resistance to shear. However, soaked cowpea showed no peak in viscosity, but rather a gradual rise over time. Hoover and Sosulski (1985) reported a similar pattern of peak viscosity for legume starches, which were characterised by absence of peak viscosity due to a gradual rise during a holding period at 95 °C. Peak viscosity reflects the ability of starch granules to swell freely before they start to break down. The absence of peak viscosity in soaked cowpea (Fig. 4B) might be because of presence of other molecules such as proteins and dietary fibre in large amounts, which might have affected the starch swelling process and the development of peak viscosity. For instance, higher protein and fibre content, fibre-starch and starch-protein interactions, and the structural arrangements of amylose and amylopectin molecular configurations influence the hydration rate of starch (Henshaw et al., 1996). The lowest peak viscosity was observed in the germinated pearl millet (53 mPa.s) and germinated cowpea (310 mPa.s) (Fig. 4), possibly because of enzymatic breakdown of the starch macromolecule to simple sugars during germination to support sprout growth. Breakdown of pearl millet and cowpea starch was observed when the samples were subjected to constant high temperature (95 °C) and stirring conditions (Fig. 4). However, the breakdown was more accentuated for pearl millet (Fig. 4A) than cowpea (Fig. 4B), indicating fragility of the swollen starch granules in pearl millet at shear and temperature. Interestingly, the results showed that germinated (55 mPa.s) and fermented (158 mPa.s) pearl millet underwent lower breakdown compared with the washed (300 mPa.s) and soaked (346 mPa.s) pearl millet. This is probably because the starch granules were already broken and could not swell to

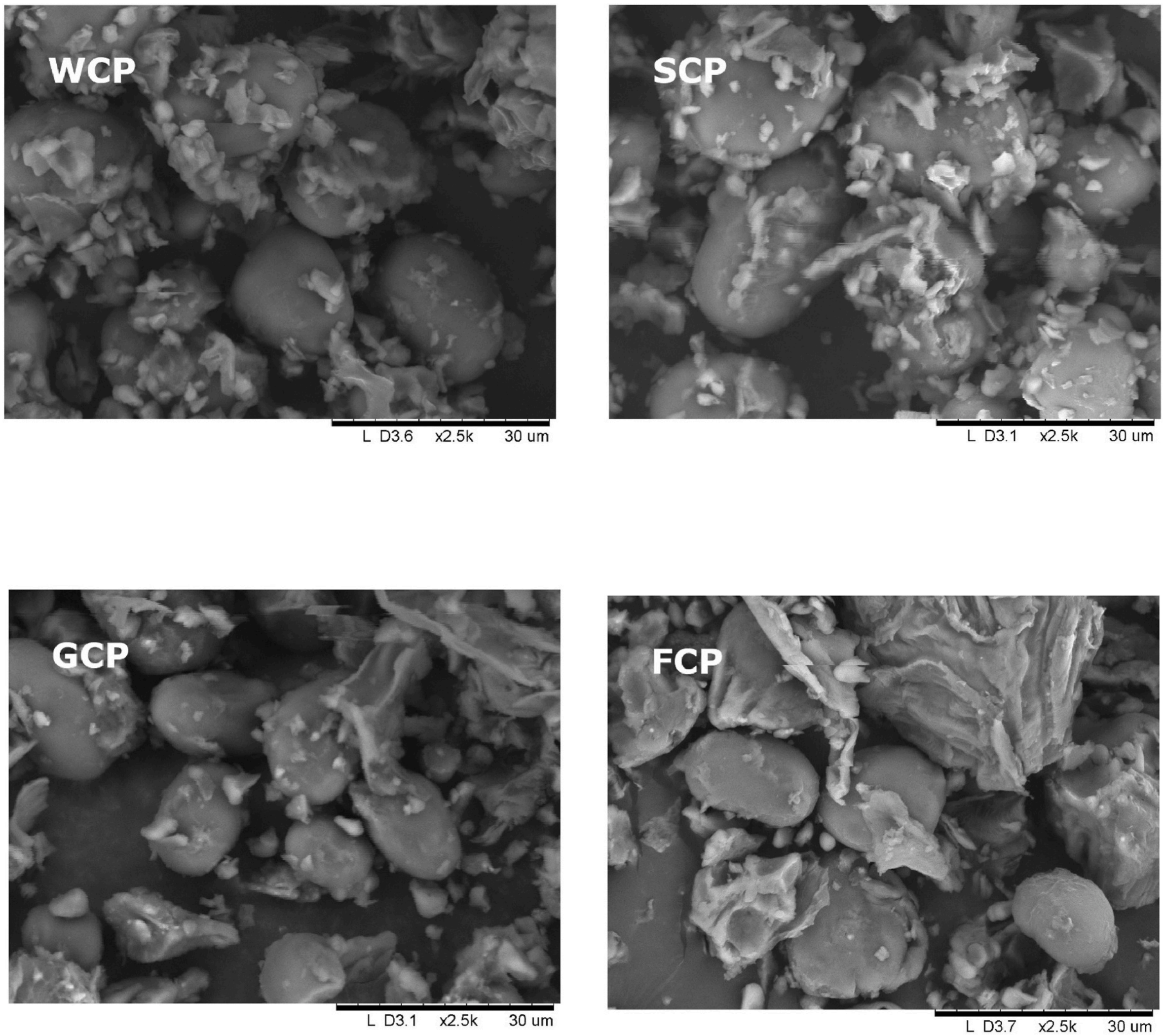

Fig. 2. SEM images of starch granules in cowpea after different pre-treatments: WCP = washed cowpea, SCP = soaked cowpea, GCP = germinated cowpea, FCP = fermented cowpea.

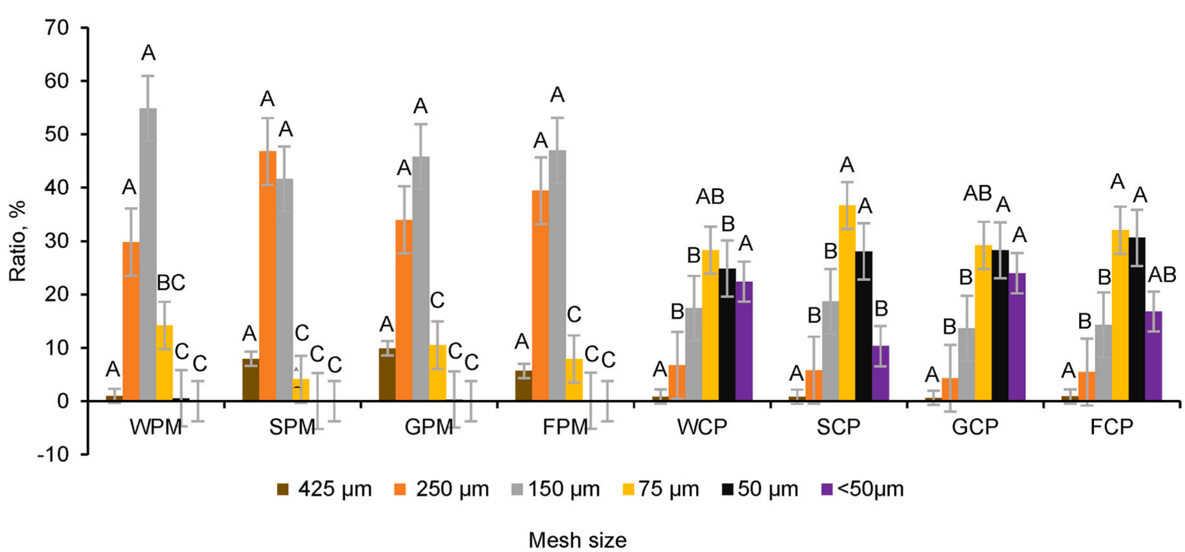

Fig. 3. Particle size distribution of pearl millet and cowpea flour: WPM = washed pearl millet, SPM = soaked pearl millet, GPM = germinated pearl millet, FPM = fermented pearl millet, WCP = washed cowpea, SCP = soaked cowpea, GCP = germinated cowpea, FCP = fermented cowpea. Error bars represent standard error. Bars marked with different letters are significantly different ($p < 0.05$) within each mesh size.

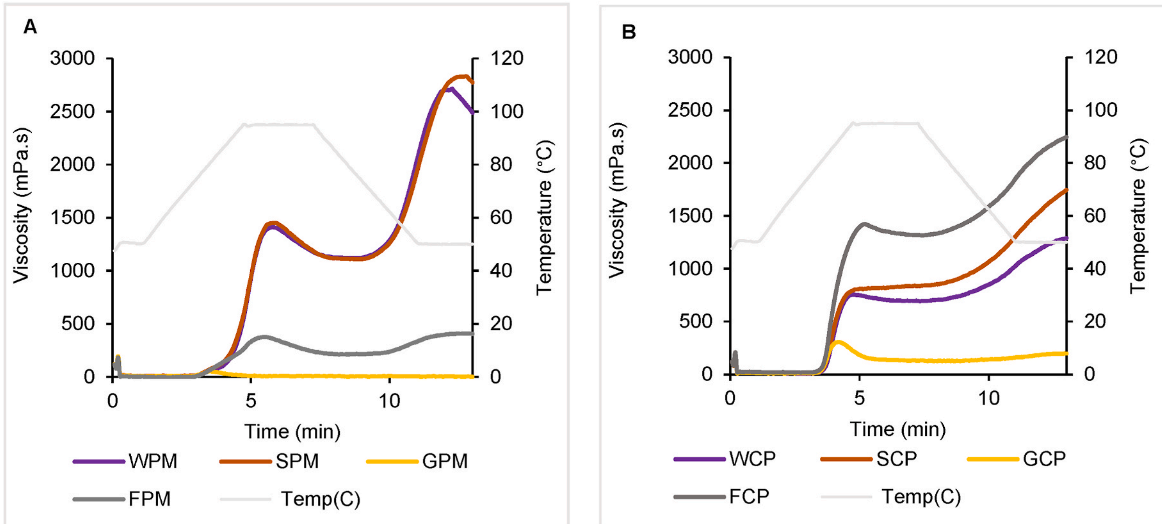

Fig. 4. Pasting properties of (A) pearl millet (WPM = washed pearl millet, SPM = soaked pearl millet, GPM = germinated pearl millet, FPM = fermented pearl mille) and (B) cowpea (WCP = washed cowpea, SCP = soaked cowpea, GCP = germinated cowpea, FCP = fermented cowpea).

the same extent as those in washed and soaked pearl millet. Yadav, Chhikara, Anand, Sharma, and Singh (2014) also reported shear-thinning behaviour of pearl millet samples. The behaviour of starch granules in cowpea was completely different, with very low breakdown of washed (65 mPa.s) and soaked (18 mPa.s) cowpea compared with germinated (187 mPa.s) and fermented (192 mPa.s) cowpea. Indicating that washed and soaked cowpea were more resistant to shear thinning than germinated and fermented cowpea. Germinated and fermented cowpea showed higher breakdown, probably because of the higher activity of amylolytic and proteolytic enzymes activated during the germination and fermentation process.

Setback indicates how starch behaves after heating, cooking and cooling, and it is an essential parameter in aggregation of starch molecules. The decrease in temperature in the flour paste during cooling allows more scope for hydrogen bonding and entanglement between the starch chains. Cooling of starch pastes also leads to renewed crystal formation of amylose-lipid complexes and recrystallisation of starch. Soaking pearl millet gave the highest setback of all pre-treatments (Fig. 4A). Germinated and fermented pearl millet had the lowest setback, probably due to breakdown of amylose by enzymes minimising the scope for entanglement between starch chains. For cowpea, the highest setback was observed in fermented samples (Fig. 4B). This was possibly because of disruption of the cell wall matrix by enzymatic action during fermentation, allowing the starch to swell freely, as observed by Zhu et al. (2010). This information must be considered in efforts to formulate complementary porridge with good appearance, paste stability and the right consistency for children under five years old.

Final viscosity in the pearl millet samples ranged widely, from 4 to 2773 mPa s (Fig. 4A), with soaked pearl millet showing higher final viscosity followed by washed, fermented and germinated pearl millet. Final viscosity in the cowpea samples also ranged widely, from 196 to 2117 mPa s (Fig. 4B), with fermented cowpea showing higher final viscosity followed by washed, soaked and germinated cowpea. This higher final viscosity in fermented cowpea might be because of the high protein content (Table 1), which possibly protected the starch granules from breakdown by enzymes during fermentation (Zhu et al., 2010). The germination process lowered the final viscosity of both pearl millet and cowpea samples, with the lowest values observed for germinated pearl millet. This difference reflects higher enzymatic attack on the starch granules in germinated pearl millet than in germinated cowpea, as also observed in the SEM images (Fig. 1, panel GPM, and Fig. 2, panel GCP). Previous studies have also found that germination decreases viscosity in cereal and legume flour due to breakdown of starch granules by α-amylase activated during germination (Griffith & Castell-Perez, 1998; Malleshi, Daodu & Chandrasekhar, 1989; Mosha & Svanberg, 1990).

Therefore, decreasing the viscosity by germinating seeds can be a good strategy when developing complementary porridge for undernourished children. Lower viscosity provides more flexibility in adjusting flour concentration and thus increasing energy density (Alexander, 1983; Mosha & Svanberg, 1990).

3.6. *Principal component analysis (PCA)*

Exploratory multivariate analysis was used to further distinguish the effects of pre-treatments on physicochemical and pasting properties of pearl millet and cowpea and to identify any differences between replicate batches (Fig. 5). In general, variables opposite to each other showed a negative relationship, while variables close to each other revealed a positive relationship. Based on the pearl millet PCA score and loading plot (Fig. 5A1 and 5A2), PC1 and PC2 accounted for 39.0% and 30.6% of the total variance, respectively. Any differences between the pre-treatments were mainly associated with higher pasting viscosity in washed and soaked pearl millet and higher total dietary fibre content in germinated pearl millet. Based on the score plots of pearl millet and cowpea (Fig. 5A1 and B1), there were differences between batch 1 and batch 2. These differences between batches were mainly seen for replicate germination and fermentation samples, and can be challenging to control due to complex biological and environmental factors varying to some extent. This is because these pre-treatments further modify the seeds during processing by increasing activity of enzymes, thereby changing the macromolecules present. PCA score and loading plots for cowpea (Fig. 5B1 and B2), PC1 and PC2 accounted for 40.9% and 30.2% of the total variance, respectively. They indicated that differences between pre-treatments were mainly related to higher total dietary fibre and ash content in washed cowpea, due to the presence of the hull, higher pasting properties in soaked and fermented cowpea, and germination contributing to increasing the protein content. Total dietary fibre content in both loading plots was inversely related to parameters such as starch, % amylose and some pasting parameters.

4. Conclusions

Soaking, germination, and fermentation can be low-cost processing methods to modify the physicochemical properties and alter the food microstructural arrangements of different macromolecules present in pearl millet and cowpea seeds. This study showed that these pre-

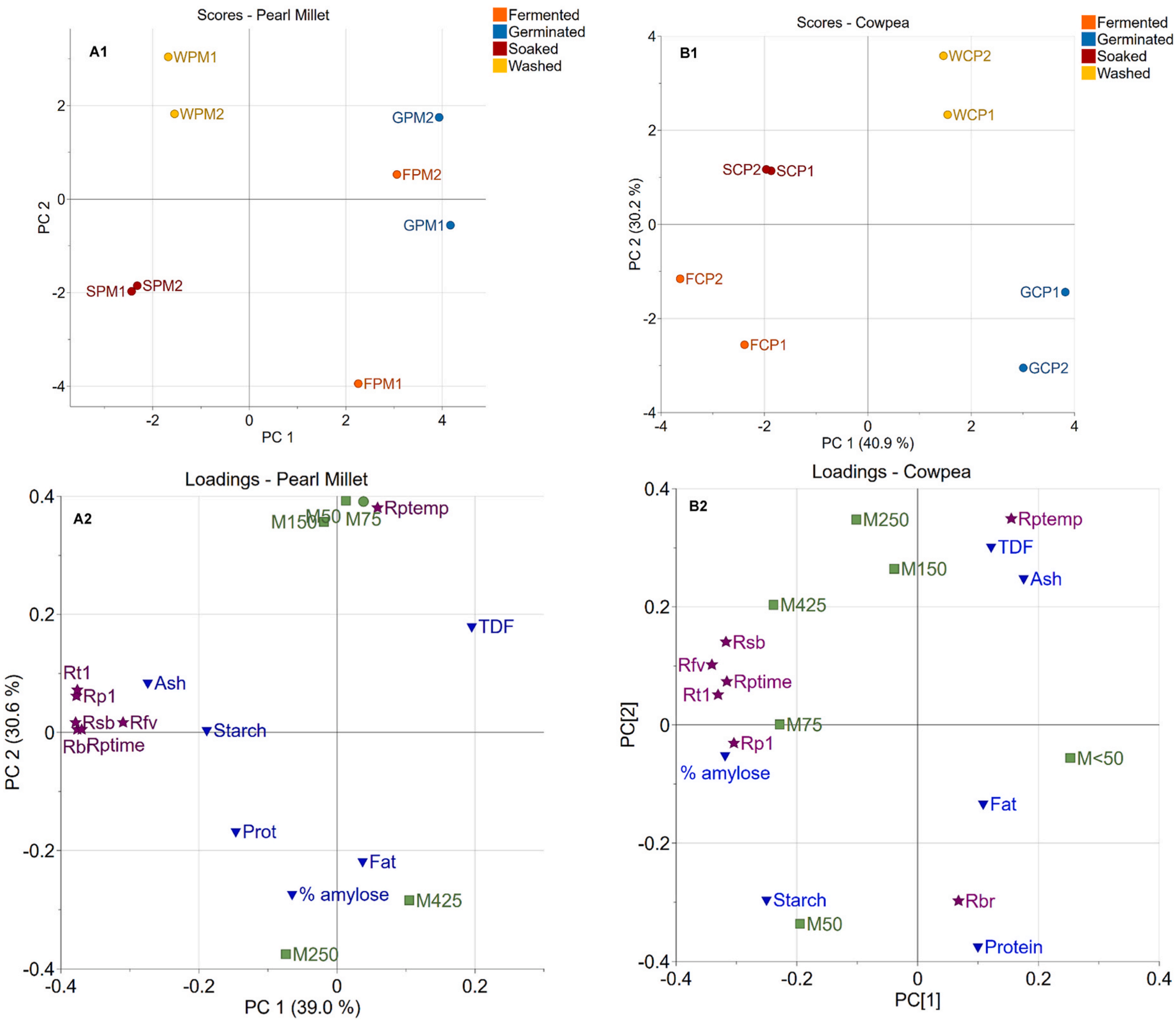

Fig. 5. Principal component analysis (PCA) plots for pearl millet and cowpea. (A1) Score plot for pearl millet (WPM = washed pearl millet, SPM = soaked pearl millet, GPM = germinated pearl millet, FPM = fermented pearl millet), 1 = batch 1, 2 = batch 2. (A2) Loading plot for pearl millet (TDF = total dietary fibre, M = mesh size, R = rapid visco analyser: p1 = peak 1, ptemp = pasting temperature, sb = setback, fv = final viscosity, br = breakdown, ptime = peak time, t1 = through 1. (B1) Score plot for cowpea (WCP = washed cowpea, SCP = soaked cowpea, GCP = germinated cowpea, FCP = fermented cowpea). (B2) Loading plot for cowpea.

treatments can also enhance the nutritional profile. Activation of enzymes during germination and fermentation was likely responsible for the changes in pasting and microstructural properties. Germination decreased total starch content in both pearl millet and cowpea, resulting in less entanglement between starch chains, as verified by the pasting properties. Numerous holes and broken starch granules in pearl millet were observed, drastically reducing the final viscosity of the pearl millet flour, while in cowpea germination did not affect the microstructure but led to reduced final viscosity. This important new knowledge on the effect of pre-treatments on the physicochemical and microstructural properties of Mozambican varieties of pearl millet and cowpea flour milled from pre-treated seeds can be applied to develop complementary porridge for malnourished children.

CRediT authorship contribution statement

Sunera Nurmomade: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Santanu Basu:** Writing – review & editing, Validation, Supervision, Data curation, Conceptualization. **Irene de Carvalho:** Writing – review & editing, Supervision, Conceptualization. **Maria Eduardo:** Writing – review & editing, Supervision, Conceptualization. **Roger Andersson:** Writing – review & editing, Validation, Supervision, Methodology, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

Acknowledgements

This work was supported by grants from the Swedish International Development Cooperation Agency (Sida), subprogram 1.2.1 (Sida Decision No: 2017/11862).

We are grateful to Gulaim Seisenbaeva at the Department of Molecular Sciences, SLU, for her valuable help with scanning electron microscopy.

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