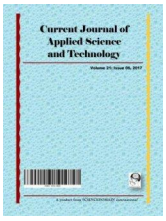

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Combination Effect of Germination and Fermentation on Functional Properties of Sorghum Flour

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Authors' contributions

This work was carried out in collaboration between both authors. Author AEOE designed the study, managed the analyses of the study and wrote the first draft of the manuscript. Author RB managed the literature searches, wrote the protocol and performed the statistical analysis. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: To understand the effect of the combination of the two traditional Sudanese processing methods, germination and fermentation, on the functional properties of sorghum flour.
Study Design: Factorial Experimental design.
Place and Duration of Study: Lehrstuhl für Biochemie, Universität des Saarlandes, Saarbrücken, Germany between May 2015 and July 2015.
Methodology: Fetarita sorghum was germinated for three days, and then the flour obtained was fermented for 8 h. The functional properties of produced flour were studied and ungerminated, and unfermented flour was used as a control.
Results: The germinated-fermented flour has low pH and high titratable acidity. The proteins of the germinated-fermented flour were more soluble in different buffers than the control. Germinated-fermented sorghum flour had the least gelation concentration of 8% compared to 18% for the control. Germination and fermentation significantly ($P \leq 0.05$) decreased the bulk density (loose and

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packed), water and alkaline water capacities. A significant increase in the oil binding capacity, emulsifying activities and stabilities, foaming capacity, foaming stability and wettability was reported in the treated flour. The produced flour has high dispersibility at all pH tested, and high dispersibility was observed at the alkaline pH. SEM results of the germinated-fermented flour support the development in functional properties.

Conclusion: Combination of germination and fermentation improves the functional properties of sorghum flour. This could help anticipate end-product quality and more applications in gluten-free products based on sorghum flour.

Keywords: Sorghum; germination; fermentation; functional properties; SEM.

1. INTRODUCTION

Sorghum is the main source of food in Africa and many other developing countries. Sorghum and millet represent the major source for energy and protein for the African population [1]. In Sudan, the main cereal crop is also sorghum, and different types of Sudanese foods are made from sorghum.

Some sorghum contains large amounts of micro and macronutrients, with high amounts of phenolic compounds such as phenolic acids, flavonoids, and condensed tannins [2]. Phenolic compounds have been reported to have health benefits to humans they decrease the risk of cardiovascular disease by improving endothelial function and inhibiting platelet aggregation [3].

The most used traditional processing methods in Africa are germination and fermentation. They are widely used for the preparation of foods and beverages. Germination is a common practice in the sorghum producing areas; the germinated sorghum grains are malted for the production of weaning foods, opaque beers and other traditional dishes. Germination promotes the development of hydrolytic enzymes, which are not present in non-germinated grain [4]. According to Elkhalifa and Bernhardt [5], germination was reported to have certain technological effects on the physicochemical properties of sorghum flour. Also, Elkhalifa and Bernhardt [6] revealed that sorghum grain germination affects the functional properties of the flour to a large extent. Dewar [4] reported that malting of sorghum will improve protein characteristics, increased vitamin C content, phosphorus availability, and synthesis of lysine and tryptophan.

The nutritional and functional characteristics of fermented sorghum have been investigated in many reports [7 and 8], and they reported that natural sorghum fermentation improves most of the nutrients in sorghum. Moreover, some independent studies reported that fermented sorghum flour had higher protein and starch digestibility *in vitro* than unprocessed sorghum flour [9]. Elkhalifa et al. [7] reported that traditional Sudanese method of fermentation had a great effect on the functional properties of sorghum flour. There is clear evidence that both germination and fermentation processing technologies can improve the nutritional quality in sorghum flour.

Recently, sorghum had got the attention of the scientists as a gluten-free cereal to be used in the diet of people suffering from celiac disease. Formation of a gluten-like protein network will not be possible when using untreated sorghum flour as the main component of the dough mixture [10]. Globulins and prolamins are the main protein fractions in sorghum they are present as compact aggregates in protein bodies surrounding the starch granules. Due to this structure pre-treatment of sorghum flour will be necessary to facilitate the formation of a gluten-like protein network.

Functional properties were defined as physicochemical properties that give information on how food ingredients will behave in a food system [11]. In order to determine the nutritional, sensory, physicochemical and organoleptic properties of a final product it is important to determine its functional properties. Determination of functional properties of proteins is very important to determine the suitability and applicability of a protein in certain food systems and products [12]. Therefore, the functional properties of sorghum proteins can be used to define how flour proteins can be used to supplement or replace gluten protein sources. There is, however, limited information on the functional properties of sorghum flour produced from germinated-fermented sorghum and this information is essential for determining potential uses of this product in food formulations; as

the use of sorghum as a food source has not been utilised fully, especially in developed countries.

The objective of this study was to understand the effect of the combination of the two traditional Sudanese processing methods, germination and fermentation, on the functional properties of sorghum flour as a means for expanding the use of this crop.

2. MATERIALS AND METHODS

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2.1 Samples

Sorghum cultivar Fetarita was obtained from Gadarif, Sudan, and harvested in the year 2014. The material was carefully cleaned and stored at 4°C.

2.2 Sorghum Germination

Sorghum seeds were germinated in Sudan for 72 h according to the traditional Sudanese wife method of germination as described by Elkhalifa and Bernhardt [5].

2.3 Sorghum Fermentation

The germinated seeds were ground in a hammer mill to pass through a 0.4 mm screen, and the flour was fermented in Sudan for 8 has described by Elkhalifa et al. [13]. At the end of the fermentation time (8 h at 35 - 37°C) the sample was placed in a hot air oven (Heraeus UT 5042, Germany), dried at 56°C for 16 h, and ground into flour in a hammer mill to pass through a 0.4 mm screen. The same milling procedure was used to prepare control flour (from un-germinated and un-fermented sorghum).

2.4 pH Determination and Titratable Acidity

The pH of the two samples was determined by using a glass electrode pH meter (PUSL München, Germany). Titratable acidity, like lactic acid, was determined with 0.1M NaOH.

2.5 Protein Solubility

Proteins were extracted by suspending 500 mg of a sorghum flour sample in 10 ml of 50 mM phosphate buffer, 0.1M NaCl, pH 7.0, at 25°C for 30 min. When indicated, the extraction buffer contained 8M urea and 10mM dithiothreitol (DTT). The amount of soluble proteins was measured according to Bradford [14].

2.6 Least Gelation Concentration

The least gelation concentration was determined by the method described by Elkhalifa and Bernhardt [6].

2.7 Loose and Packed Bulk Densities

Loose and packed bulk densities of the flour samples were determined using the following method. For packed bulk density ten grams of the tested flour was placed in a 25 ml graduated cylinder and packed by gentle tapping of the cylinder on a bench top, ten times, from a height of 5–8 cm to eliminate air spaces between the flour in the cylinder. The final volume of the test flour was measured and expressed as g/ml. For loose bulk density (LBD) space was not eliminated by trapping.

2.8 Carr Index

The flowability of the samples was determined by calculating the Carr's index as described by Otutu et al. [15].

$$\text{Carr Index } (\%) = \frac{\text{Packed bulk density} - \text{Loose bulk density}}{\text{Packed bulk density}} \text{X } 100$$

2.9 Water- and Oil-absorption Capacity

One gram of each flour sample was weighed into a pre-weighed centrifuge tube and 10 ml of distilled water were added. Samples were vortexed for one min and allowed to stand for 30 min at 25 ± 2°C before being centrifuged at 4000g for 25 min. Excess water was decanted by inverting the tubes over absorbent paper and samples were allowed to drain. For oil absorption, 10 ml refined sunflower oil were used. The weights of water and bound oil samples were determined by difference.

2.10 Alkaline Water Retention Capacity

One gram of each sample was transferred into a test tube and weighed (W_1). Five ml of 0.1M $NaHCO_3$ were added and mixed for 30s (Fisher Vortex Genie 2TM Mixers). The sample was then allowed to stand at 25 ± 2°C for 20 min, centrifuged (4000 g, 15 min) and drained for 10 min at an angle 10–15° to the horizontal. Test

tube with the content was then weighed (W_2) and the alkaline water retention calculated as follows alkaline water retention capacity (g/g) of sample = $W_2 - W_1$.

2.11 Emulsifying Activity and Stability

Emulsifying activity and stability were determined using the method described by Elkhalifa and Bernhardt [6].

The emulsifying activity was determined using the following formula:

$$\text{Emulsion activity} = \frac{\text{height of emulsion layer}}{\text{height of whole layer}} \text{ X } 100$$

The emulsifying stability was calculated by using the following formula:

$$\text{Emulsion stability} = \frac{\text{height of emulsion layer after heating}}{\text{height of whole layer}} \text{ X } 100$$

Height was measured in centimeters using a transparent graduated ruler.

2.12 Foaming Capacity and Stability

For the determination of the foaming capacity of the different sorghum samples, 2 g of the material was weighed and transferred into a standard electric blender (Kenstar Supermix, Kenstar Company, Mumbai, India). A 100 ml sample of distilled water was added and the suspension was blended at 15,000 g for 6 min at 25°C. The contents were immediately transferred quantitatively to a 250 ml measuring cylinder and the volume of the foam recorded. Foaming capacity was expressed as the percentage increase in volume. Foam stability was determined as a function of time after 1 h, by monitoring the fall in the foaming capacity value.

2.13 Determination of Wettability

One gram of each flour sample was measured into a 10 ml measuring cylinder. The cylinder was inverted at 10 cm above the water contained in 600 ml beaker. The finger was used to close the cylinder disallowing the flour sample from falling. By removing the finger and giving the cylinder a gentle tap, the flour sample was discharged into the water surface. The time taken by the sample to get completely wet was recorded as the time of wettability.

2.14 Dispersibilty

The dispersibility of flour at selected pH levels (3, 7 and 10) was measured according to the method of Kulkarni et al. [16].

2.15 Scanning Electron Microscopy (SEM)

Sorghum samples were mounted on aluminum pin stubs using conductive self-adhesive carbon label. The specimens were sputtered coated with a layer of gold approximately 50 nm thick in a sputter coater. All samples were examined in a JSM-7000F scanning electron microscope (JEOL GmbH, Germany) at an accelerating voltage of 20 kV.

2.16 Statistical Analysis

Unless otherwise indicated, three separate batches, for a particular treatment, were taken and analysed separately and the figures were then averaged. Data were assessed by analysis of variance (ANOVA) and by Duncan's multiple range test with a probability $P \leq 0.05$, using SAS/STAT software, as reported by Marti et al. [17].

3. RESULTS AND DISCUSSION

3.1 pH and Titratable Acidity

Germination and fermentation significantly ($P \leq 0.05$) reduced the pH and increase the titratable acidity of the sorghum flour from 6.4 to 4.4 and from 3.4 to 14.9%, respectively Fig. 1. In agreement with the previous studies who reported a decrease the pH and increase in the titratable acidity after fermentation [13] and after fermentation and germination [8]. Elkhalifa et al. [13] attributed these changes to the production of lactic acid by lactic acid bacteria after fermentation; while Mbaeyi-Nwaoha and Onweluzo [18] attributed the low pH after germination of sorghum to partial hydrolysis of carbohydrates which occurred during germination. Low acidic pH will increase the nutritional value of the sorghum flour by increasing its digestibility and it would be stored after preparation without encouraging the growth of toxigenic microorganisms. Therefore, the pH of flours is an important parameter which affects the flavour and shelf life of products. The lower the pH of a product the better it keeps.

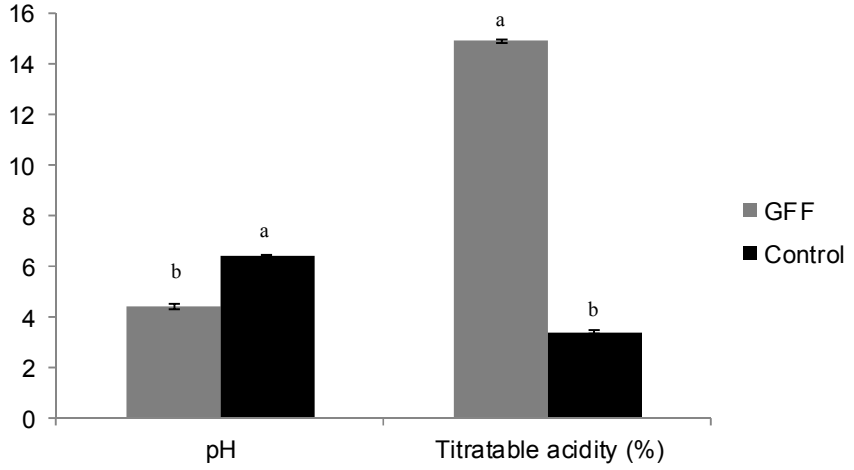

Fig. 1. pH and titratable acidity (%) of germinated-fermented (GFF) sorghum flour
The error bars represent mean values ± SD from three replicates of each sample. Bars having different letters within the same group (GFF and control) are significantly different (P ≤ 0.05)

3.2 Protein Solubility

The solubility of proteins of sorghum flours was determined by extraction in buffers with different dissociation ability towards covalent and non-covalent inter-protein bonds and it was presented in Fig. 2. There is a correlation between the physicochemical properties of proteins, including their aggregation state, and their behaviour during food processing [19]. The results showed that the combination of germination and fermentation significantly increased the protein solubility of sorghum flour (Fig. 2). Addition of urea or DTT to the buffer/saline extractant resulted in a significant increase in the amount of solubilised protein in the treated sorghum sample, while the addition of DTT to the buffer did not change the solubilization of the sorghum proteins of the untreated sorghum flour. However, when urea and DTT were concurrently present in the buffer solution, the amount of soluble proteins was twice as much that measured in their absence in the two sorghum samples. Elkhalifa et al. [13] reported that addition of urea or DTT to the buffer/saline solution significantly increase the amount of soluble protein of untreated sorghum flour, while in this study only the presence of urea increases the soluble protein in the untreated sorghum flour. This may be due to the fact that in this study high tannin sorghum cultivar was used. Combined processing (germination and fermentation) of sorghum significantly reduced the tannins content [20]. Therefore, the sorghum flour from the processed sorghum was more soluble in the presence of DTT than the untreated sorghum. Elkhalifa et al. [13] reported that the inter-protein disulphides bonds become available to the action of disulphide reductants when the structure of the involved protein is unfolded. This is due to the fact that these bonds are relevant to the structure of the protein network in sorghum flour.

Marengo et al. [8] observed that upon fermentation of low tannins sorghum flour buffer-soluble proteins (albumins and globulins) were most affected, and also they observed a marked decrease in non-covalent aggregates and for aggregates stabilised by disulfide bonds; indicating that proteins are the primary nutrient required for microbial growth [8]. In the same study, Marengo et al. [8] reported a less decrease of all classes of proteins in sprouted sorghum flour.

3.3 Least Gelation Concentration (LGC)

The results of the least gelation concentration are shown in Table 1. Flour from germinated-fermented sorghum formed a firm gel at a significantly ($P \leq 0.05$) lower concentration (8%) compared to the control flour (18%). Variations in the gelling properties may be due to the change in the ratios of the different ingredients such as proteins, carbohydrates and lipids occurred during germination and fermentation, protein hydrolysis as well as enzymatic hydrolysis of

carbohydrates into amylose and amylopectin molecules, indicating that interaction between such components may also have a significant role in functional properties. The lower the LGC, the better is the gelating ability of the protein ingredient and the swelling ability of the flour will enhance [21]. Elkhalifa et al. [7] reported the same value for the control and 8% the least gelation concentration for flour from 8 h fermented sorghum, while Elkhalifa and Bernhardt [6] reported 12% the least gelation concentration for flour from sorghum germinated for three days. The low gelation concentration of the treated sorghum flour may be an asset for the formulation of curd or as an additive to other gel forming materials in food products.

Table 1. Gelation properties of germinated and fermented sorghum flour

Flour concentration % (w\v)	Flour from germinated-fermented sorghum (GFF)	Control
2	-	-
4	-	-
6	±	-
8	+	-
10	+	±
12	+	±
14	+	±
16	+	±
18	+	+
30	+	+
LGC	**8**	**18**

–, Not gelled; ±, gelled slightly; +, gelled, LGS, least gelation concentration

3.4 Loose and Packed Bulk Densities

Bulk density is a measure of heaviness of a flour sample. The bulk density of flour samples influences energy density, texture, and mouth feel. Germination and fermentation significantly ($P \leq 0.05$) decreased the bulk density (loose and packed) by 11% and 18%, respectively (Table 2). Elkhalifa et al. [7] and Elkhalifa and Bernhardt [6] reported a reduction the packed bulk density after fermentation and germination of sorghum. The packed bulk density reported for the control in this study is relatively similar to what reported earlier by Elkhalifa et al. [7] and Elkhalifa and Bernhardt [6].

The reduction in bulk density (loose and packed) observed may be due to the breakdown of complex compounds such as starch and proteins as a result of the modification that occurred during germination and fermentation. According to Wilhelm et al. [22], the bulk density of a food material affects its mouth feel as well as the type of packaging material used in its packaging. Furthermore, foods, especially cereals, with high bulk densities are a disadvantage nutritionally. This is because a small quantity will yield very thick porridge which has very little nutrients whereas more quantities of less dense flour will be required to obtain the same thickness. Furthermore, a less dense food material will be more portable. The loose and packed densities also provide useful information for the flowability of the flours by calculating the Carr index. The calculated Carr index for the treated sorghum flour was 34.3%, while it was 39.2% for the control. Lower Carr index implies better

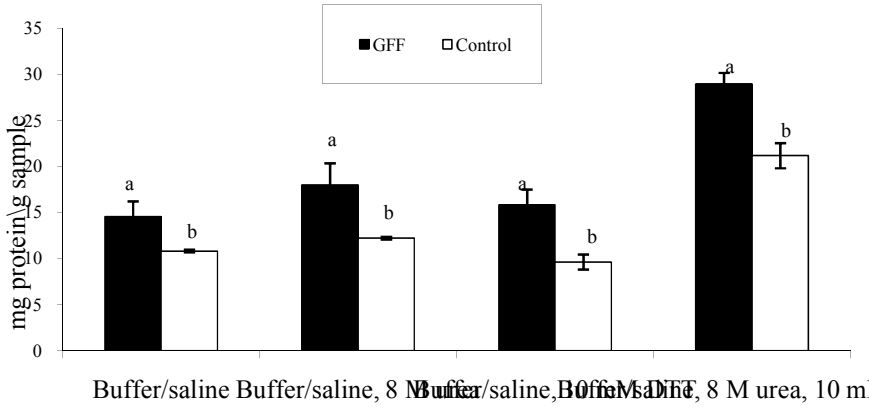

Fig. 2. Solubility of proteins from sorghum flours, germinated-fermented (GFF) and control, in different saline buffers with or without urea and DTT

The error bars represent mean values ± SD from three replicates of each sample. Bars having different letters within the same group (GFF and control) are significantly different ($P \leq 0.05$)

flowability of the flours. Otutu et al. [15] observed that germination increase the flowability of the sorghum starches by decreasing the Carr index.

3.5 Water- and Oil-absorption Capacity

Water binding capacity represents the volume occupied by the starch after swelling in excess water, which maintains the integrity of starch in aqueous dispersion [21]. It is an indication for the incorporation of flours into aqueous food formulations with dough handling. Combination of germination and fermentation significantly ($P \leq 0.05$) decreased the water absorption capacity of sorghum flour by 33% (Table 2). Elkhalifa and Bernhardt [6] reported that germination increase the water absorption capacity of sorghum flour after three days of germination. In another study Elkhalifa et al. [7] revealed that fermentation of sorghum flour significantly decreased the water-binding capacity of sorghum. Lin et al. [23] reported water absorption for soy flour and two commercial soy protein concentrates by 130%, 227.3% and 196.1%, respectively. Water absorption capacity gives an indication of the amount of water available for gelatinisation. Lower absorption capacity is desirable for making thinner gruels [7].

The trend of the oil absorption capacity differed from those of the water absorption capacity (Table 2). Germination and fermentation significantly ($P \leq 0.05$) increased the oil absorption capacity of the sorghum flour by 11%. Elkhalifa et al. [7] reported that fermentation increase the oil-binding capacity of sorghum flour after 8 h of fermentation and Elkhalifa and Bernhardt [6] also observed an increase in the oil absorption capacity of sorghum flour after three days of germination by 19%. Deepali et al. [24] stated that germination-induced increased oil absorption capacity may be due to solubilisation and dissociation of proteins leading to exposure of non-polar constituents from within the protein molecule. Oil absorption capacity is the ability of the flour protein to physically bind fat by capillary attraction and it is of great importance since fats act as flavour retainer and also increases the mouth feel and flavour retention of the foods [21]. Singh et al. [25] observed higher oil absorption capacity in germinated rice flours, when compared to non-germinated rice flours, and attributed this to be due to the fact that germination may have caused dissociation and partial unfolding of polypeptides that expose the hydrophobic sites of the amino acids which aids hydrophobic association of the peptide chains with the lipid droplets. High oil absorption capacity of germinated-fermented sorghum flour suggests that the flour could be used to produce gluten-free bakery products where the high amount of oil is required. Water and oil absorption capacities are useful indices of the ability of the protein in the material to prevent fluid loss from a product during food storage or processing.

3.6 Alkaline Water Retention Capacity (AWRC)

It is a parameter that is related to cookie diameter. The AWRC much more informative, when combined with baking. The higher AWRC value indicates smaller cookie diameter and vice-versa [26]. Results showed that combination of traditional food processing of sorghum germination and fermentation significantly ($P \leq 0.05$) decreased the alkaline water retention by one third (Table 2).

3.7 Foaming Capacity and Stability

According to previous studies [6 and 7] and confirmed in this study, the untreated sorghum flour did not show any foaming capacity (FC). Treated sorghum, germination and fermentation, shows some foaming capacity (9%, Table 2). Elkhalifa and Bernhardt [6] reported 11.5% FC of sorghum flour after five days of germination. While fermented sorghum flour has no foaming capacity as reported by Elkhalifa et al. [7]. Elkhalifa and Bernhardt [6] explained the improvement in the FC of the germinated sorghum to the fact that during germination, the amount of solubilised proteins increased, resulting in improved FC.

Flour from germinated-fermented sorghum has 62.25% foaming stability (FS) after 60 min, which is to some extent similar to that reported by Elkhalifa and Bernhardt [6] for three days germinated sorghum (64.25%). Foam formation and stability are dependent on different parameters such as protein type, pH, surface tension, viscosity and processing method [27]. Eltayeb et al. [28] reported that proteins in flours are surface active and that is why flours are able to produce foam. According to Elkhalifa and Bernhardt [6] germination may have lead to surface denaturation of the proteins and reduced the surface tension of the molecules, which gave good foamability. Foam stability is important since the usefulness of whipping agents depend

Table 2. Selected functional properties of germinated-fermented sorghum flour (GFF)

Samples	Bulk density (g\ml)	Loose density (g\ml)	Water absorption capacity (%)	Oil absorption capacity (%)	Alkaline water retention (%)	Foaming capacity (%)	Foaming stability (%)	Wettability time (s)
GFF	0.61 [b] ± 0.01	0.40 [a] ± 0.01	105.00 [b] ± 5.66	107.50 [a] ± 6.36	92.00 [b] ± 2.83	9.00 ± 1.41	6.25 ± 0.35	71.10 [b] ± 3.82
Control	0.74 [a] ± 0.03	0.46 [a] ± 0.00	157.50 [a] ± 16.26	96.50 [b] ± 0.71	139.00 [a] ± 7.07	-	-	84.90 [a] ± 2.97

–, No values were recorded.

Values are mean ± SD. Values with the same superscript letter in a given column are not significantly different (P ≤ 0.05)

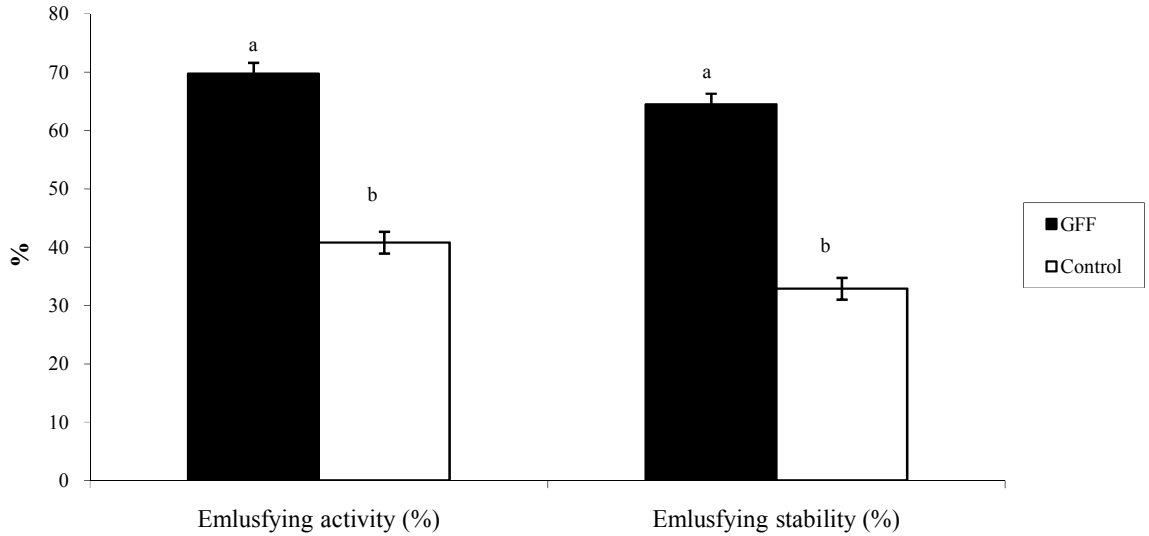

Fig. 3. Emulsifying activity and stability of sorghum samples, germinated-fermented (GFF) and control

The error bars represent mean values ± SD from three replicates of each sample. Bars having different letters within the same group (GFF and control) are significantly different (P ≤ 0.05)

on their ability to maintain the whip as long as possible [23]. The development in foam capacity and stability of sorghum flour due to germination and fermentation may find application of this flour in gluten free products.

3.6 Wettability

Generally, wettability depends on different parameters such as: particle size, density, porosity, surface charge, surface area, the presence of amphipathic substances and the surface activity of the particles [29]. Ease of wettability is important in food formulations and is affected by surface polarity, topography, texture and area, and by the size and microstructure of the protein particles [30]. The wettability time of the sorghum flours is presented in Table 2. Results show that the control sorghum flour took longer time (84.9s) to completely be wet in cold water than the germinated-fermented sorghum flour (71.10s). This may be due to the changes that took place during germination and fermentation of sorghum, mainly the increase in total sugars as reported by Elkhalifa et al. [31]. Higher sugar content causes shorter wetting time [32]. Mbaeyi-Nwaoha and Onweluzo [18] reported a mean wetting time between 31.3-92.5 seconds for germinated sorghum flours. They reported that sprouting may have induced a change in the texture of the hydrophilic components of sorghum flours to have influenced the ease of wetting of the flour. Generally, the determination of wettability will provide a useful indication of the degree to which the flour is likely to possess instant characteristics. The flour with the lowest time of wettability will dissolve in water faster and would perform better in texture and comminuted meats and baked products [33].

3.7 Emulsifying Activity and Stability

Combination of germination and fermentation significantly ($P \leq 0.05$) increase the emulsifying activity and stability of sorghum flour by 70% and 96%, respectively (Fig. 3). Elkhalifa and Bernhardt [6] reported an increase in the two properties by 33% and 21% after three days of germination. Fermentation was also reported to increase the emulsifying activity and stability of sorghum flour as reported by Elkhalifa et al. [7]. The increase observed in emulsion properties could be due to an increase in the area of stabilized oil droplet at the interface which is a function of the food components [34]. According to Sikorski [35] proteins assist emulsification by reducing surface tension while some types of polysaccharides can help stabilise the emulsion by increasing the viscosity of the system.

Increasing emulsion activity (EA), emulsion stability and fat binding during processing are primary functional properties of protein in such foods as mayonnaise, salad dressing and frozen desserts.

3.8 Dispersibility

The dispersibility of flour in water indicates its reconstitutionability. The higher the dispersibility, the better the reconstitution property [16]. Higher dispersibility enhances the emulsifying and foaming properties of proteins, which was observed during the making of different food products, e.g. bread, macaroni and cookies [30]. Results revealed that flour from germinated-fermented sorghum has higher dispersibility than the control at all pH tested (Fig. 4) and it is high dispersibility was observed at the alkaline pH, while the control flour has its high dispersibility at the neutral pH. The lowest dispersibility of the two samples occurred at the acidic pH, this may be due to nearness to iso-electric point of proteins in these flours. Both sorghum flours (processed and control) had an excellent dispersibility in distilled water 78.8% and 75.8%, respectively. Kulkarni et al. [16] studied the dispersibility of the sorghum malt based weaning food formulations and a commercial weaning food. They reported 63 to 79% dispersibility for their formulations with different proportion of constituents and a very poor dispersibility (40%) in case of commercial preparation.

3.9 Scanning Electron Microscopy (SEM)

Scanning electron micrographs are shown in Fig. 5 a and b for sorghum flours, control and germinated-fermented, respectively. Sorghum flour control (Fig. 5 a) constitute mainly compacted starch granules, completely enclosed in a very compact protein matrix with size greater than 10 mm, mixed with protein bodies with a diameter less than 5 mm; the protein bodies were seen attached to the protein matrix. These micrographs are very similar to previously published work [13]. The SEM micrograph of the germinated-fermented sorghum flour (Fig. 5 b) shows the effect of the amylase and proteolytic occurring during germination and fermentation have a profound impact on the structure of the

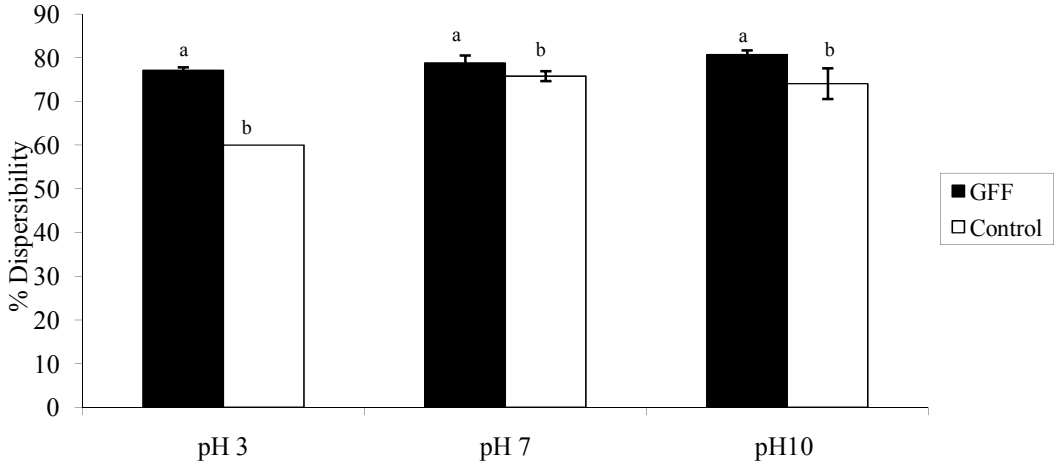

Fig. 4. Dispersibility (%) at different pHs of flour from germinated-fermented sorghum (GFF) and control

The error bars represent mean values ± SD from three replicates of each sample. Bars having different letters within the same group (GFF and control) are significantly different (P ≤ 0.05)

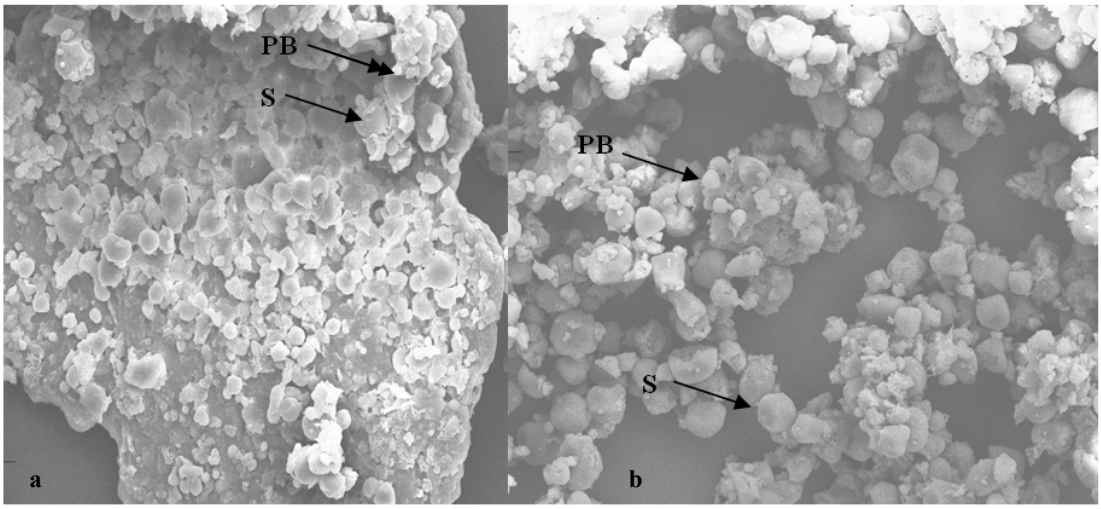

Fig. 5. SEM of sorghum flour, control, (a) and germinated fermented sorghum flour (b), PB, protein bodies, S, starch granules

protein coating leading to the release of the small starch granules and clear proteins bodies due to degradation of the proteinaceous matrix that holds starch granules. Also, it can be seen that there is some kind of starch degradation due to the activated enzymes during germination and fermentation. Lactic acid fermentation has also been noticed to result in swelling of the starch granules in wheat [36]. The effects were assigned to enzymatic actions of the microorganisms The SEM results explain most of the improvement in the functional properties observed in germinated-fermented sorghum.

4. CONCLUSION

This study indicates that combination of germination and fermentation can be a tool to improve the functional properties of sorghum flour. In addition to the reported improvement of nutritional properties as the influence of germination and fermentation; treated sorghum flour can be used for supplementation or preparation of gluten-free foods and expanding the uses of this crop outside it is local geographical areas, where sorghum-based foods represent a major staple.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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