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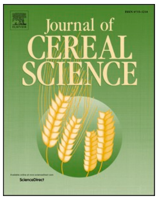

Effects of sourdough-type fermentation and sorghum type on the techno-functional properties of the batter used for kisra, a fermented flatbread

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ABSTRACT

Techno-functional properties of six sorghum types were determined during spontaneous sourdough batter-type fermentation for Kisra production. All sorghum types showed a progressive decrease in pH to about 3.3 with fermentation for 40 h, accompanied by an increase in titratable acidity and free amino nitrogen. Fermentation increased pasting viscosity at 16 and 24 h, but decreased at 40 h due to starch hydrolysis. Protein hydrolysis as shown by SDS-PAGE leads to disaggregation of flour particles and the release of starch granules to increase the pasting viscosity. Thus, starch was more freely available to absorb water to paste and produce a higher viscosity from zero to 40 h. The decrease in pasting viscosity from 24 h to 40 h of fermentation was related to starch hydrolysis, as there was a reduction in total starch in the batter and pitting of starch granules, as shown by scanning electron microscopy. Fermentation had a more significant effect on techno-functional properties than sorghum types. Low protein and high tannin contents among the sorghum type also showed higher pasting viscosity. The techno-functional changes of sorghum batter during fermentation, especially the high pasting viscosity, might be helpful for structure design of kisra and other gluten-free sorghum-based products.

1. Introduction

Kisra and injera made from sorghum are important staples in Sudan and Ethiopia. Kisra is a flatbread made from sorghum that is fermented with lactic acid bacteria and yeast (Sulieman and Ali, 2022). Fermented sorghum batter is baked into circular, thin sheets with a diameter of 30–45 cm and a thickness of 1–1.5 mm (Ejeta, 1981; Badi et al., 1990). The kisra texture should be soft, pliable and moist, but not spongy. Kisra is one of the staple foods in Sudan (AwadElkareem and Taylor, 2011). Kisra made from fermented sorghum has potential as the foundation for a good quality gluten free wrap. It would be conceivable to develop some new foods based on sorghum flour for consumers with gluten intolerance (Abd Elmoneim et al., 2005). Fermentation also has been shown to boost nutritional properties, palatability, and consumer appeal (Xiang et al., 2019).

There are three types of sorghum fermentation: spontaneous (also known as natural or wild), back-slopping and controlled fermentation (Capozzi et al., 2017). Spontaneous fermentation has been practiced for many years and consists essentially of adding water to sorghum and incubating the slurry under appropriate temperature and time (Capozzi et al., 2017)). Spontaneous fermentation is used in the current research work. There is limited literature on the techno-functional changes relating to the protein, starch and non-starch polysaccharides during the sorghum and kisra sourdough-type fermentation processes. The techno-functional property changes during fermentation seems to affect quality of flat bread during sour dough-type fermentation. For example, during fermentation, the starch content of the sorghum flour has been found to decrease indicating hydrolysis of starch (Ogodo, 2018). The endosperm protein matrix, which mostly comprises the kafirin prolamin protein, is partially hydrolyzed (Abd Elmoneim and Bernhardt, 2018). Reactive –SH groups were found to diminish in the fermented sorghum, suggesting that most of the cysteine-containing peptides released during proteolysis were metabolized by the lactic acid bacteria (Marengo et al., 2015).

The partial hydrolysis of protein and starch by fermentation can change the chemical composition of fermented batter. However, techno-functional properties, for example pasting, which represent the viscous characteristics during cooking and its relationship with starch and protein microstructure and their molecular properties is not well established. The influence of sorghum types on the properties of fermented sorghum batter is also not well understood. Sorghum cultivars have been classified based on tannin content and testa color as (i) Type I,

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lacking pigmentation and tannins, holds the lowest content (less than 0.28 g/kg) (ii) Type II, with a pigmented testa containing tannins, averages about 4.48 g/kg (iii) Type III with the highest tannin content (11.95 g/kg) due to its presence in both the testa and pericarp layers (Sedghi et al., 2012). Thus, the objective of this study was to determine the effect of sourdough fermentation on the techno-functional properties of sorghum batter with the aim of understanding and relating functional, molecular, and microstructural changes in different types of sorghum fermentation during production of kisra. The techno-functionality has been described as any food property, excluding its nutritional value, affecting its utilisation. Emulsification, solubility, water and oil binding, foam capacity and stability, gelation, and viscosity are among the techno-functional properties vital in food processing (Mshayisa et al., 2022). In this manuscript, Water absorption, protein solubility, swelling, and viscosity are the techno-functional properties that were determined.

2. Experimental

2.1. Materials

The sorghum used in the study are (i) Tabat, Lekgeberwa and Macia which are white Type I (non-tannin) (ii) Gadam, Wadahmed and Wafer which are a white Type II tannin. The sorghums were milled into whole-grain flour using a 2-stage process. First, size reduction was done using a laboratory-scale stone disc mill (Stone Flour Mill A100, Osttiroler Getreidemuehlen, Austria) to a coarse flour. Then, the coarse flour was further reduced in particle size using a laboratory-scale hammer fitted with a 0.5 mm opening screen (Laboratory Mill 3100, Perten, Huddinge, Sweden). All reagents were of analytical grade.

The sorghum flour samples was characterized in terms of moisture, protein, ash, lipid, total soluble and insoluble dietary fibre according to AOAC methods 950.46, 928.08, 923.03, 948.22 and 991.43 respectively (AOAC, 1995), tannin content was determined using the modified Vanillin-HCl method of Price et al. (1978), endosperm texture and damaged starch. The endosperm texture of the sorghum grains was determined according to Taylor and Taylor (2008). In this assay, 20 sorghum grains were cut in two lengthwise, to produce two even size halves, so that each half contains an equal portion of the germ. The halves grain were observed with the naked eye to estimate the relative proportion of corneous to floury endosperm. The Megazyme Starch Damage Assay Procedure, K-SDAM 05/2008, AACC Method 76.31.01, (AACC, 2000) was used to detect starch damage (Megazyme International Ireland Ltd., Co. Wicklow, Ireland). The data for raw material characterization are shown in Table 1.

2.2. Fermentation of sorghum batter

Sorghum batter was incubated at 37 °C for 40 h after mixing flour and water in a 1:1.5 ratio [w/w] (flour: water, 40 g flour: 60 g water) for spontaneous fermentation. Samples were taken at 0, 16, 24 and 40 h and divided into two portions. Sodium azide (0.2 % w/v) was added to the first portion to inhibit microbial activities for analyses of pH, titratable acidity (**TA**) and pasting properties. The latter was analysed as 'fresh' sample because viscosity is a result of both molecular and microstructure properties. Freeze drying can damage the microstructure of the food samples (Oyinloye and Yoon, 2020). The second portion was freeze-dried for analysis of soluble and insoluble dietary fiber, total starch, water absorption index, free amino nitrogen, SDS-PAGE and light microscopy. The freeze-dried fermented material was stored in airtight container at 4 °C.

2.3. Characterization of sorghum batter

2.3.1. Determination of pH and titratable acidity (TA)

To determine TA, 5 g batter was diluted in 20 mL distilled water, titrated with 0.1 M NaOH to pH 6.3, and expressed as % lactic acid (Abd Elmoneim and Bernhardt, 2018).

2.3.2. Pasting properties

The pasting properties of non-fermented and fermented batters were determined using a Physica MCR 101 Rheometer (Anton Paar, Ostfildern, Austria). The batters were cautiously transferred into the rheometer cup. A thin layer of light paraffin oil was applied on top of the exposed sample surface to prevent loss of moisture though evaporation (Da Silva et al., 2011). The batters were subjected to a pasting cycle beginning with an initial stirring speed of 960 rpm at 37 °C for 1 s and then stirred at 160 rpm for the remaining period. The temperature was held at 50 min for 5 min then increased at a rate of 5.5 °C/min to 91 °C, this temperature was held for 10 min. The pastes were then cooled to 50 °C at a rate of 5.5 °C/min.

2.3.3. Water absorption index

With some modifications, the method described by Singh Gujral and Singh (2002) was used to determine the water absorption index of the

Table 1

Partial proximate composition, damaged starch, and tannin content of the different sorghum type.

Type	Type	Origin	Endosperm Texture	Moisture (%)	Fat (% db)	Insoluble dietary fiber (% db)	Soluble dietary fiber (% db)	Total dietary fiber (% db)	Ash (% db)	Damaged starch (% db)	Protein (% db)	Tannins (mg catechin equivalents/ 100 mg db.)
Tabat	I, Non-tannin (White)	Sudan	Mixed Endosperm	$5.6^{a} \pm 0.1$	$3.6^{b} \pm 0.1$	$8.7^{b} \pm 0.1$	$1.3^{b} \pm 0.0$	$10.0^{d} \pm 0.0$	$2.9^{a} \pm 0.0$	$5.5^{e} \pm 0.1$	$11.1^{c} \pm 0.0$	$0.1^{a} \pm 0.0$
Wadahmed	II, Tannin (White)	Sudan	Floury	$6.6^{b} \pm 0.1$	$3.6^{b} \pm 0.2$	$6.7^{a} \pm 0.2$	$1.9^{e} \pm 0.1$	$8.6^{c} \pm 0.2$	$2.9^{a} \pm 0.0$	$6.26^{d} \pm 0.1$	$11.1^{c} \pm 0.0$	$1.0^{b} \pm 0.0$
Wafer	II, Tannin (White)	Sudan	Floury	$7.8^{c} \pm 0.1$	$3.3^{a} \pm 0.0$	$6.7^{a} \pm 0.1$	$1.6^{d} \pm 0.2$	$8.3^{b} \pm 0.9$	$2.9^{a} \pm 0.0$	$2.80^{f} \pm 0.1$	$12.5^{e} \pm 0.0$	$1.4^{c} \pm 0.0$
Gadam	II, Tannin (White)	Zimbabwe	Floury	$14.4^{f} \pm 0.2$	$3.9^{d} \pm 0.0$	$6.7^{a} \pm 0.02$	$1.5^{c} \pm 0.1$	$8.2^{b} \pm 0.1$	$3.0^{b} \pm 0.0$	$8.7^{a} \pm 0.1$	$6.5^{a} \pm 0.2$	$1.9^{d} \pm 0.1$
Lekgeberwa	I, Non-tannin (White)	Botswana	Corneous	$10.0^{d} \pm 0.3$	$4.6^{e} \pm 0.0$	$6.5^{a} \pm 0.1$	$1.1^{a} \pm 0.2$	$7.6^{a} \pm 0.11$	$3.1^{b} \pm 0.0$	$8.1^{b} \pm 0.1$	$11.7^{d} \pm 0.1$	$0.1^{a} \pm 0.0$
Macia	I, Non-tannin (White)	South Africa	Intermediate	$12.2^{e} \pm 0.2$	$3.7^{c} \pm 0.0$	$6.3^{a} \pm 0.1$	$1.3^{b} \pm 0.0$	$7.6^{a} \pm 0.1$	$2.9^{a} \pm 0.0$	$7.4^{c} \pm 0.1$	$7.6^{b} \pm 0.0$	$0.13^{a} \pm 0.0$

[a-f] Mean values with different lower case letter in a column differ significantly from each other ($p < 0.05$). N = 3.

batters. Freeze-dried samples (1 g dry basis) were incubated in 10 ml distilled water in a shaking water bath (3169×*g*) for 30 min at 91 °C [to simulate the temperature during pasting], while vortexing the slurry every 5 min. The sample mixture was centrifuged for 15 min at 9154.3×*g*. The supernatant was decanted, and the wet pellet was weighed, and the water absorption index was calculated based as the weight of pellet (g) obtained per gram of dry sample weight.

2.3.4. *Total starch*

The assay is specific for α-glucans (including starch, glycogen, phytoglycogen and non-resistant maltodextrins). Total starch content was determined enzymatically using a Megazyme kit (amyloglucosidase/α-amylase) AOAC Method 996.11 (AOAC, 1995). In this method, starch is enzymatically hydrolyzed to glucose. Glucose was then quantified colorimetrically by the glucose oxidase-peroxidase reaction and the absorbance was read at 510 nm.

2.3.5. *Free amino nitrogen (FAN)*

Free amino nitrogen (FAN) was determined using the European Brewery Convention ninhydrin colorimetric assay using glycine as a reference amino acid (Convention, 2009). The results were expressed as mg FAN/100 g sorghum (dry basis).

2.3.6. *Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE)*

SDS-PAGE was performed under non-reducing and reducing conditions using 4–12% polyacrylamide gradient gels (8 × 8 cm x1.0 mm thick with 15 wells) (NuPAGE® Novex, Invitrogen. Carlsbad, CA). Invitrogen Mark12 Unstained Standard was used (3.5–260 kDa). Samples were loaded to 15 μg constant protein. Staining was with Coomassie Brilliant Blue R-250. After de-staining, the gels were photographed by scanning on an electrophoresis gel scanner.

2.3.7. *Light microscopy (LM)*

Light microscopy was used to examine the overall shape and size of the starch granules. Distilled water (1 mL) was added to 100 mg (dry basis) freeze-dried batter. The mixture was pasted at 90^{o} C for 15 min, with shaking every 5 min. The sample was then diluted 20 times. One drop of the suspension was placed on a slide and a coverslip was applied. A Nikon Optiphot Transmitted Light Microscope (Tokyo, Japan) with phase contrast optics and with iodine staining was used to examine the samples.

2.3.8. *Scanning electron microscopy (SEM)*

Freeze-dried specimens were placed on aluminum stubs with single side conductive carbon tape. The stubs were sputter coated with gold and then viewed by using a Zeiss 540 Crossbeam SEM (Oberkochen, Germany) at 5 kV.

2.4. *Statistical analyses*

The experiment was repeated three times. IBM SPSS 20 statistical software for Windows (SPSS, Inc., Chicago, IL) was used for data analysis. Two-way analysis of Variance was performed on the data, with fermentation time and sorghum types as the independent variables, to assess their effects on the measured values, which were the dependent variables. Fisher's least significant difference (LSD) test was used for mean separation at $p \leq 0.05$.

A principal component analysis (PCA) was performed to assess the relationship and correlation matrix between the fermented and non-fermented sorghum batter's physical, chemical, and pasting properties. The independent variables were fermentation time and sorghum types. The dependent variables were the measured values.

3. Results and discussion

3.1. *pH and titratable acidity*

As expected, all sorghum types showed a progressive decrease in batter pH during fermentation up to 40 h (Table 2). The pH of the fermented batters declined to 3.3–3.5 and the initial range of batters 5.8–6.13. The drop in pH was accompanied by a rise in titratable acidity. The pH showed an exponential decrease up to about 24 h and followed by a plateau from 24 to 40 h. This may be due to the decrease in the total number of bacteria after 28 h of fermentation due to the accumulation of metabolic products for example ethanol, carbon dioxide and antibacterial substances that inhibit microbial growth (Mengesha et al., 2022). Spontaneous lactic acid fermentation involves lactic acid bacteria (LAB) and yeasts (Houngbédji et al., 2018). LAB acidify the dough (lactic and/or acetic acid) and contribute to a basic acidic flavor. Yeasts further develop flavor (higher alcohols and esters) and leaven the wheat flour dough with carbon dioxide (De Vuyst et al., 2023). Antimicrobial for example organic acids and bacteriocins produced by lactic acid bacteria can also reduce microbial growth (Chikindas et al., 2018). On the other hand, more fermentable sugars led to better growth and more organic acids, while less nutrient at end of fermentation resulted in more death and injured cells (Barbieri et al., 2020). The reduction in pH agrees with the work conducted by Abd Elmoneim et al. (2018) for the fermented sorghum flour. There were differences in titratable acidity values between the different sorghum types. The Wafer sorghum type exhibited the highest TA (1.34 %) value, whereas Macia exhibited the lowest TA (1.01 %). This is possible because of the differences in buffering effects of the different varieties based on their chemical composition (Taylor and Taylor, 2002). This is further discussed in section 3.8.

3.2. *Pasting properties*

Fermentation period significantly ($p < 0.05$) affected the peak and final viscosity of the fermented sorghum batters (Fig. 1, supplementary Table III). The fermented batters all had significantly ($p < 0.05$) higher pasting viscosity than non-fermented ones. Gadam had the highest pasting peak viscosity at least twice that of the other varieties at time zero. All sorghum types had higher peak and final viscosities at 16 h and 24 h fermentation than at 40 h. The fermented batters of Tabat, Wafer and Wadahmed showed a lower pasting temperature compared with Gadam, Macia and Lek. Adiandri and Hidayah (2019) similarly reported that increasing viscosity of fermented sorghum flour with fermentation time. They suggested that the starch-degrading bacteria lead to porosity in starch granules, and the latter absorb more water, thereby causing the granules to swell and consequently increasing the viscosity. In contrast, Bian et al. (2022) found that the pasting viscosity of non-fermented proso millet flour was higher than that of fermented millet flour. They attributed this to the weakening of the amylopectin structure as the molecular weight was reduced, resulting in less water absorption, thus a reduced pasting viscosity.

. The increase in pasting viscosity during fermentation, despite seemingly contradictory prior research, warranted further investigation in this study. Of possible relevance is that the Gadam, Macia, and Lek varieties, which had the highest pasting viscosities, had the lowest protein contents, 6.5, 7.6 and 9.7%, respectively. This agrees with the suggestion by Marchini et al. (2021) that the presence of protein in sorghum flour could inhibit the swelling of starch granules, thus producing a paste with lower viscosity. This is further discussed in later section 3.8 to explain the differences between the sorghum varieties. The sorghum endosperm texture did not seem to affect the pasting properties, as even though Gadam, Wafer, and Wadahmed have floury endosperm texture, Gadam sorghum type recorded the highest pasting viscosity.

Table 2
Effects of fermentation time and sorghum type on the pH and titratable acidity of the sorghum batters.

Sorghum type	pH				TA			
	0 h	16 h	24 h	40 h	0 h	16 h	24 h	40 h
Tabat	6.13 ± 0.01	4.50 ± 0.02	3.60 ± 0.02	3.40 ± 0.04	0.00	0.40 ± 0.10	1.0 ± 0.01	1.1 ± 0.20
Wadahmed	5.96 ± 0.02	4.30 ± 0.03	3.53 ± 0.01	3.40 ± 0.02	0.00	0.40 ± 0.02	0.9 ± 0.03	1.3 ± 0.10
Wafer	5.90 ± 0.03	4.34 ± 0.01	3.50 ± 0.03	3.30 ± 0.01	0.00	0.74 ± 0.04	1.11 ± 0.10	1.34 ± 0.10
Gadam	5.90 ± 0.03	3.80 ± 0.01	3.40 ± 0.04	3.42 ± 0.10	0.00	0.70 ± 0.10	1.02 ± 0.03	1.24 ± 0.10
Lekgeberwa	5.80 ± 0.01	4.10 ± 0.10	3.62 ± 0.01	3.52 ± 0.03	0.00	0.63 ± 0.10	1.20 ± 0.10	1.30 ± 0.03
Macia	6.03 ± 0.10	4.20 ± 0.04	3.50 ± 0.01	3.40 ± 0.04	0.00	0.40 ± 0.04	0.82 ± 0.10	1.01 ± 0.10

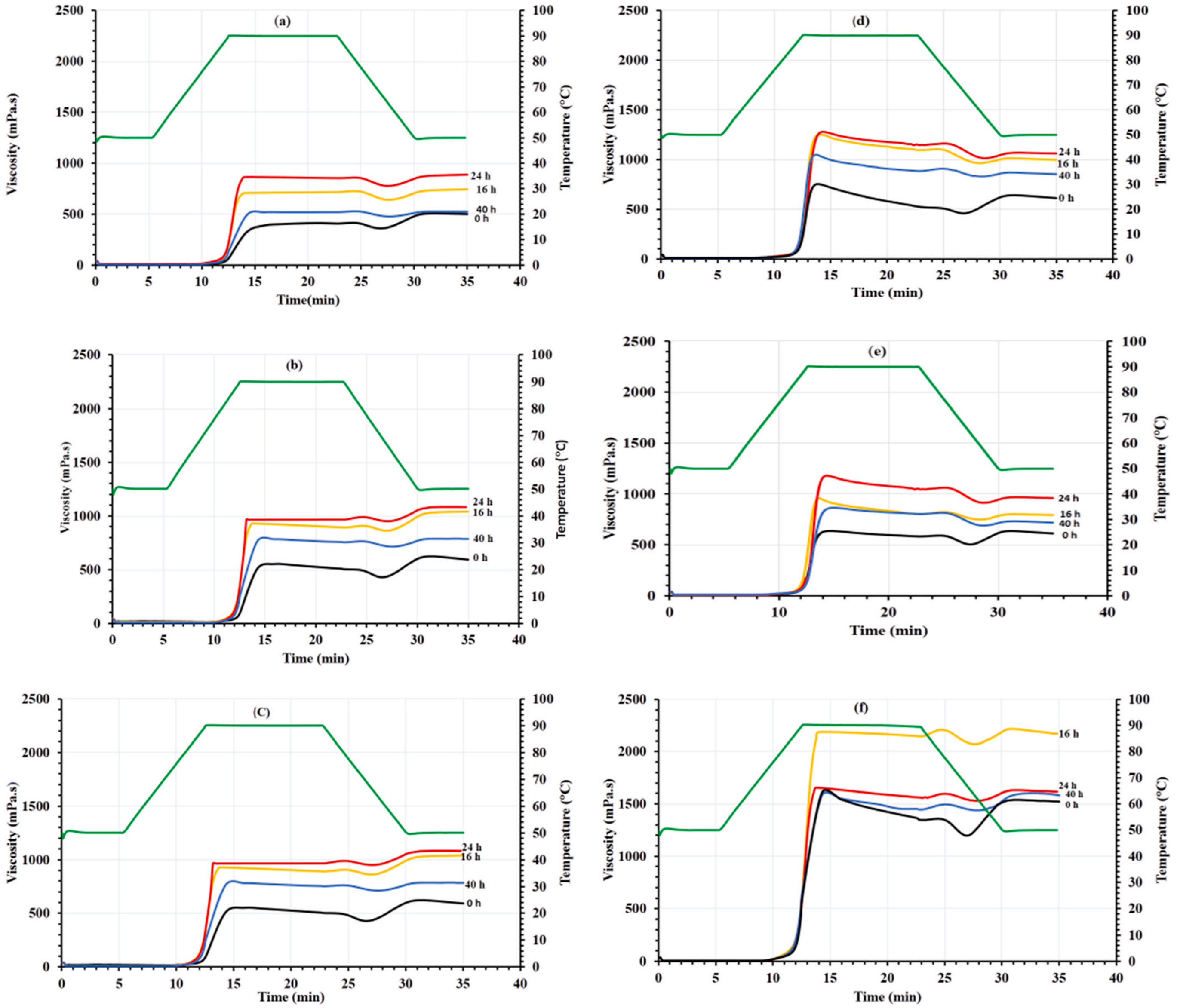

Fig. 1. Effects of fermentation the pasting properties of the batters produced from Tabat (a), Wad Ahemd (b), Wafer (c), Macia (d), Lekgeberwa (e), Gadam (f).

3.3. *Water absorption index [WAI]*

The WAI at 96 °C of the freeze-dried batters increased with fermentation time from 0 to 16 h for all sorghum varieties (Table 3). With the exception of Macia, there was a further increase in WAI up to 24 h followed by a decrease to 40 h. This increase in WAI followed by a decrease throughout the fermentation parallels the increase and the decrease in pasting peak viscosity during fermentation. The WAI effects observed in this study are generally similar to those of Elkhalifa et al. (2004) who found an increase in WAI (determined at 85-100 °C) with sorghum batter during fermentation. The increase in WAI study is possibly due to more available starch to bind water as the protein matrix was degraded during fermentation. Hydrolysis of the protein matrix is indicated by increased free amino nitrogen with fermentation time (Table 5). High pasting viscosity is associated with high water absorption (Falade and Okafor, 2015). The decrease in WAI from 24 to 40 h is also related to lower pasting peak viscosity.

Table 3

Effects of fermentation and sorghum type on the Water Absorption Index (g/g) (measured at 96 $^{\circ}$C) of the freeze dried batters.

Cultivar	Time of incubation			
	0 h	16 h	24 h	40 h
Tabat	$5.3^{Ae} \pm 0.03$	$6.3^{Cd} \pm 0.10$	$6.8^{Dd} \pm 0.10$	$6.1^{Bc} \pm 0.10$
Wadahmed	$6.4^{Ac} \pm 0.01$	$6.5^{Bc} \pm 0.03$	$6.7^{Ce} \pm 0.02$	$6.4^{Ab} \pm 0.02$
Wafer	$5.0^{Af} \pm 0.02$	$5.9^{Ae} \pm 0.02$	$6.2^{Bf} \pm 0.01$	$5.9^{Ad} \pm 0.02$
Gadam	$5.9^{Cd} \pm 0.01$	$7.1^{Be} \pm 0.02$	$7.3^{Dc} \pm 0.10$	$5.6^{Ae} \pm 0.01$
Macia	$7.5^{Ba} \pm 0.01$	$8.1^{Cb} \pm 0.10$	$8.1^{Ca} \pm 0.10$	$7.1^{Aa} \pm 0.02$
Lek	$6.7^{Bb} \pm 0.10$	$8.4^{Da} \pm 0.10$	$7.8^{Cb} \pm 0.10$	$6.4^{Ab} \pm 0.01$

$^{a-f}$ Mean values ($n = 3$) with different lower case letter superscripts in a column differ significantly from each other ($p < 0.05$).

$^{A-D}$ Mean values ($n = 3$) with different upper case letter superscripts in a row differ significantly from each other ($p < 0.05$).

3.4. Total starch

As expected, during fermentation the starch content of batters decreased with increasing fermentation time but the decrease between 24 and 40 h was less than from 0 to 24 h (Table 4). A reduction in starch content with increasing fermentation time was previously reported by Ogodo et al. (2019) with fermentation of sorghum flour.

The decrease in starch content is a result of the amylase enzyme produced by Lactobacilli bacteria that break down starch molecules into dextrins and simple sugars (Putri et al., 2021). The smaller decrease in the total starch content between 24 h and 40 h of fermentation also suggests a slowing down in the rate of fermentation, as indicated by the more limited pH and lactic acid changes (Table 2). The reduction in starch content during fermentation was expected to decrease the pasting viscosity, but Fig. 1 shows the opposite effect to place. This is discussed further in later section (3.8).

3.5. Free amino nitrogen (FAN)

The increase in FAN during fermentation (Table 5) was due to proteolysis (Dlamini et al., 2015). The increase of FAN, probably by the endogenous microflora hydrolyzing proteins into peptides and amino acids into soluble low molecular weight ones (Duodu and Dowell, 2019). There was a higher content of FAN in the batters of the white non-tannin sorghums (Tabat, Macia and Lek) than the white type II tannin varieties (Wadahmed, Wafer and Gadam). This is because tannins nave been associated with reduced protein hydrolysis in sorghum (dos Santos DAlmeida, 2021) due to binding to storage proteins, probably through hydrogen bonding and hydrophobic interaction (Jakobek, 2015). These protein and tannin complexes have limited digestion. Additionally, tannins can also inhibit enzymes (Barrett et al., 2018) and thus impede protein hydrolysis.

Table 4

Effects of fermentation and sorghum type on the total starch content (g/100 g) of the sorghum batters.

Type	Time of incubation			
	0 h	16 h	24 h	40 h
Tabat	$69.7^{Dc} \pm 1.0$	$53.5^{Cd} \pm 0.7$	$40.8^{Be} \pm 0.3$	$38.9^{Ae} \pm 0.2$
Wadahmed	$63.8^{De} \pm 0.7$	$51.8^{Ce} \pm 0.3$	$45.9^{Bd} \pm 0.1$	$44.1^{Ad} \pm 0.1$
Wafer	$74.7^{Da} \pm 0.1$	$61.2^{Cb} \pm 0.1$	$47.4^{Bc} \pm 0.6$	$45.0^{Ac} \pm 0.2$
Gadam	$67.3^{Ed} \pm 0.1$	$60.2^{Dc} \pm 0.2$	$55.9^{Ba} \pm 0.6$	$53.5_{Aa} \pm 0.6$
Lek	$68.9^{Dd} \pm 0.1$	$60.1^{Bc} \pm 0.1$	$53.5^{Ab} \pm 0.3$	$51.1^{Ab} \pm 0.1$
Macia	$70.8^{Cb} \pm 0.3$	$62.5^{Ba} \pm 0.4$	$53.7^{Ab} \pm 0.2$	$50.1^{Ab} \pm 0.1$

$^{a-f}$ Mean values ($n = 3$) with different lower case letter superscripts in a column differ significantly from each other ($p < 0.05$).

$^{A-D}$ Mean values ($n = 3$) with different upper case letter superscripts in a row differ significantly from each other ($p < 0.05$).

Table 5

Effects of fermentation and sorghum type on the free amino nitrogen contents (mg/100 g) of the batters.

Type	Time of incubation			
	0 h	16 h	24 h	40 h
Tabat	$30.6^{Ab} \pm 0.2$	$47.2^{Bbc} \pm 0.6$	$66.3^{Ca} \pm 0.7$	$94.4^{Da} \pm 4.5$
Wadahmed	$25.2^{Ab} \pm 0.3$	$41.2^{Bc} \pm 0.7$	$54.1^{Cc} \pm 1.7$	$76.2^{Dc} \pm 1.3$
Wafer	$21.8^{Ac} \pm 0.2$	$35.9^{Bd} \pm 0.7$	$45.9^{Cd} \pm 1.3$	$100.3^{Da} \pm 1.7$
Gadam	$22.9^{Ac} \pm 1.7$	$35.5^{Bd} \pm 0.1$	$42.0^{Cd} \pm 1.3$	$64.8^{Dd} \pm 1.7$
Lek	$31.2^{Ab} \pm 0.4$	$55.6^{Ba} \pm 1.2$	$59.7^{Cb} \pm 2.3$	$82.6^{Db} \pm 2.8$
Macia	$38.4^{Aa} \pm 0.5$	$50.0^{Bb} \pm 3.4$	$65.5^{Ca} \pm 1.1$	$95.5^{Da} \pm 4.0$
Macia	$38.4^{Aa} \pm 0.5$	$50.0^{Bb} \pm 3.4$	$65.5^{Ca} \pm 1.1$	$95.5^{Da} \pm 4.0$

$^{a-f}$ Mean values ($n = 3$) with different lower case letter superscripts in a column differ significantly from each other ($p < 0.05$).

$^{A-D}$ Mean values ($n = 3$) with different upper case letter superscripts in a row differ significantly from each other ($p < 0.05$).

3.6. SDS-PAGE

SDS-PAGE analysis showed relatively small proteins with a relative molecular size (Mr) of 16–23 kDa, consistent with sorghum kafirin prolamins (Fig. 2) under both reducing and non-reducing conditions. These were α-kafirins (M_r 25 and 23 kDa) and β-kafirins (M_r 20, 18 and 16 kDa) (Gallo et al., 2024). In addition, there were bands at 60 and 54.2 kDa, suggesting the presence of kafirin oligomers or dimers (Xiao et al., 2015). A few faint bands of higher molecular weight were also present. In general, the protein bands including the monomeric kafirins (α- and β-classes) were more predominant before fermentation (0 h) as they show higher intensity (staining) compared to at 40 h fermentation. The decrease in band intensity after fermentation may be due to their hydrolysis by proteases from microorganisms in the fermented batter (Correia, 2010). This is also supported by an increase in free amino nitrogen during fermentation (Table 5).

3.7. Morphological analysis of starch granules light microscopy and scanning electron microscopy

Light microscopy micrographs (Fig. 3) showed that the aggregated blue-black particles of starch granules were larger in size, about 100–1000 μm, at 0 h fermentation and decreased in size during fermentation. The size was mostly <500 μm at 16 h fermentation, and mostly less than 100 μm at 24 h and 40 h fermentation. The particles observed at 0 h and 16 h were mostly starch as they had stained blue-black with iodine solution and in the form of aggregates due to the starch granules being surrounded by endosperm protein matrix (De Mesa-Stonestreet et al., 2010). The blue-black particles at 24 and 40 h mostly showed individual and disrupted starch granules. The microstructural changes of the hydrated flour particle suggest that starch was liberated from flour particles, thus they were available to paste and form a higher viscosity.

Fermentation changed the microstructure of the starch granules as shown by SEM (Fig. 4). The presence of surface pores (P) that are inherent to maize starch granules are visible before fermentation. After 24 h of fermentation, the starch granules had large “pin” holes on their surfaces. Similar observations for the sorghum starch granules after treated by pepsin and α-amylase enzymes were made by Benmoussa et al. (2006). The pores seem to be starting point for the pin holes as enzymes can diffuse and hydrolyse starch. It is suggested that starch granule hydrolysis was responsible for observed lower pasting viscosity from 16 to 24 h of fermentation (Fig. 1). Alpha-amylases can erode the entire starch granule surface or digest channels from selected points on the surface towards the center of the granule (Gonzalez Conde, 2023).

3.8. Principal component analysis

Fig. 5.1 shows PCA for the different types of sorghum before

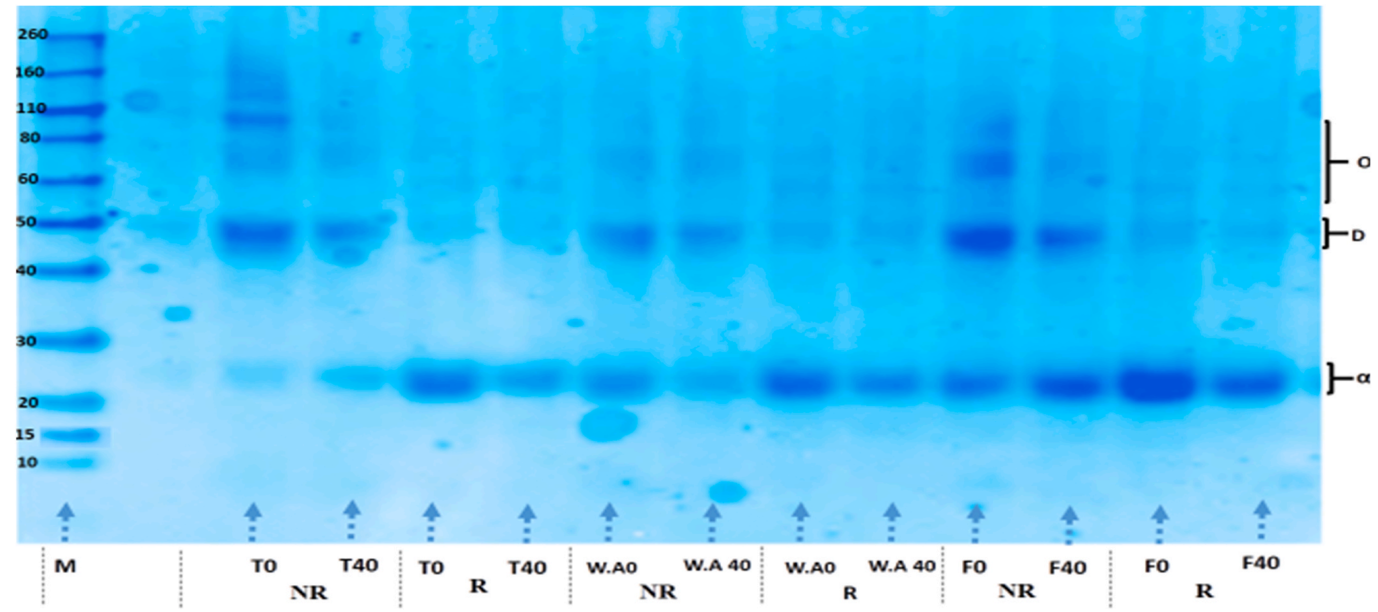

Fig. 2 (a). SDS-PAGE 0 and 40 h fermented sorghum batters, under non-reducing (NR) and reducing (R) conditions: T: Tabat; W: Wadahmed; F: Wafer; D: dimers; O: oligomers; Lane M: Molecular weight standards (kDa).

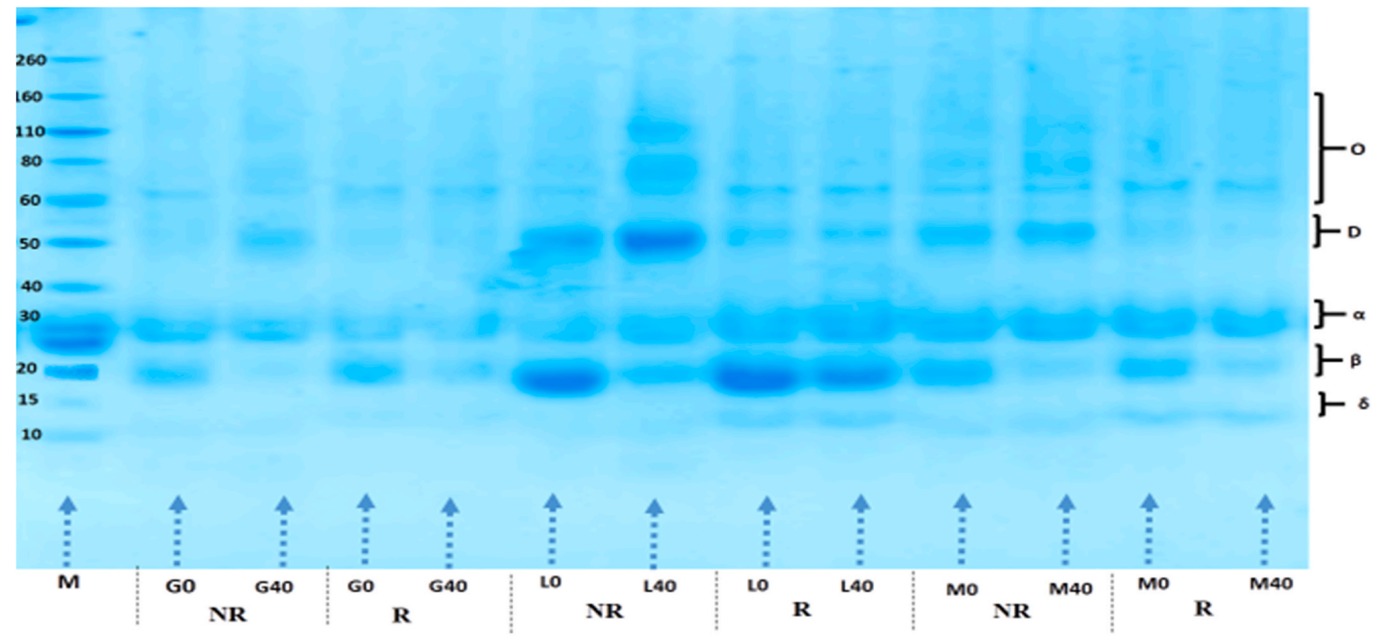

Fig. 2 (b). SDS-PAGE 0 and 40 h fermented sorghum batters, under non-reducing (NR) and reducing (R) conditions: G: Gadam; L: LEK; M: Macia; D: dimers; O: oligomers; Lane M: Molecular weight standards (kDa).

fermentation. The first and second principal components (PC1 and PC2) explained 70% of the total variance, with PC1 and PC2 explaining 43.79% and 24.59%, respectively. The PC1 showed a strong positive relationship between peak viscosity, final viscosity, floury endosperm and water absorption index and is associated with Gadam sorghum type. These functional properties however have a negative relationship between the protein content and total starch. The functional properties of the flour are related to its intrinsic factors like chemical and physical properties. The peak and the final viscosity were inversely related protein content because the starch granules were encapsulated in the protein matrix. There was little access of water to the starch hence less starch-water interaction during pasting. The peak viscosity and grain polyphenol content were associated and negative correlation with the setback (Beta et al., 2001). Pasting properties and soluble dietary fiber were associated, and this is in agreement with the suggestion by Heyman et al. (2014) who also showed that the soluble dietary fiber played an essential role in starch swelling and could form hydration film by coating the starch granules to increase the hydration volume. The above thus suggests that various chemical properties and composition affect the functional properties, but it looks like low protein content showed the most prominent in terms of pasting properties for non-fermented sorghum flour.

Fig. 5.2, the first and second principal components explained 64.54 of the total variances, with PC1 and PC2 explaining 48.86% and 26.78%, respectively. PC1 separates the sample according to fermentation time (0–40 h) in relation to the chemical properties. It is noted that most

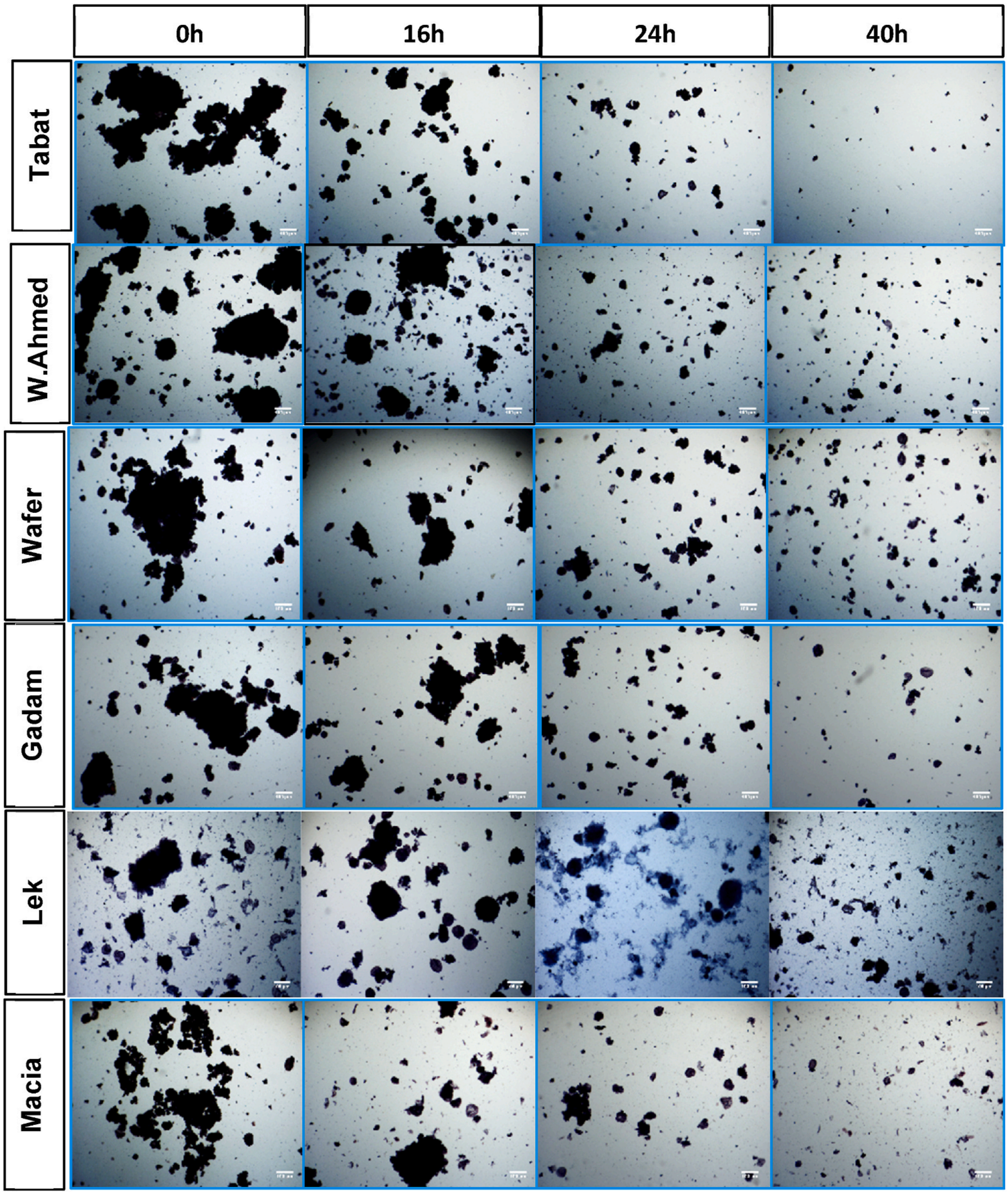

Fig. 3. Light microscopy images of starch granules from non-fermented and fermented sorghum batters. Scale bar = 100 μm.

sorghum types behave similarly during fermentation as shown by PC1. The general trend as already discussed in the above sections is that pH, total starch, setback decreased, but acidity and free amino nitrogen increased with fermentation time. The highest peak and final viscosity is at 16 and 24 h of fermentation. Most sorghum types followed the same trend during fermentation except Gadam. This is differentiated in PC2. The tannin content may play a role as this type had the highest tannin content (Table 1). Through a type of processes, tannins in tannin sorghums inhibit microbial fermentation. On the one hand, proteins and other nitrogen molecules could cause tannin to react and precipitate. Chao et al. (2017) found that addition of tannin at 2 g/L reduce the rate of glucose metabolism during fermentation. Tannin has the ability to bind metal ions, limiting their accessibility to bacteria for growth (Chao et al., 2017). Fig. 6 schematically proposes the changes as a result of fermentation of sorghum up to 40 h. At 0h, starch is encapsulated in the protein matrix. There is little water access to the encapsulated starch, hence less starch-water interaction to lower water absorption during pasting, leading to the observed low peak and final viscosities. After 16h and 24h of fermentation, proteases from the fermenting microbes hydrolyzed the protein to disrupt the protein matrix which was evident by an increase in FAN, SDS PAGE and microstructural changes. Starch was then released from the protein matrix and interacted with water molecules to form a 3D network during pasting which explains the high WAI at 96 °C, peak and final viscosities. This is shown by light microscopy showing aggregated particles at 0h compared to individual starch granules at time 16 and 24h. The WAI, peak and final viscosities drop after 40h could be the result of the hydrolysis of starch by alpha-amylases produced by the fermenting microbes which was evident by the high acidity of the fermenting medium as well as more starch disruption. The hydrolysis of starch by enzyme action and low pH resulted in the production of low molecular weight molecules, such as dextrins, disaccharides, and monosaccharides and organic acids resulting in low WAI and pasting viscosity.

The above-described techno-functional properties of fermented sorghum batter suggest that fermentation can impact of kisra quality. As such, a high pasting viscosity due to fermentation suggests that the release starch molecules more specifically amylose can interact with amylopectin to produce a network during kisra baking. During cooling

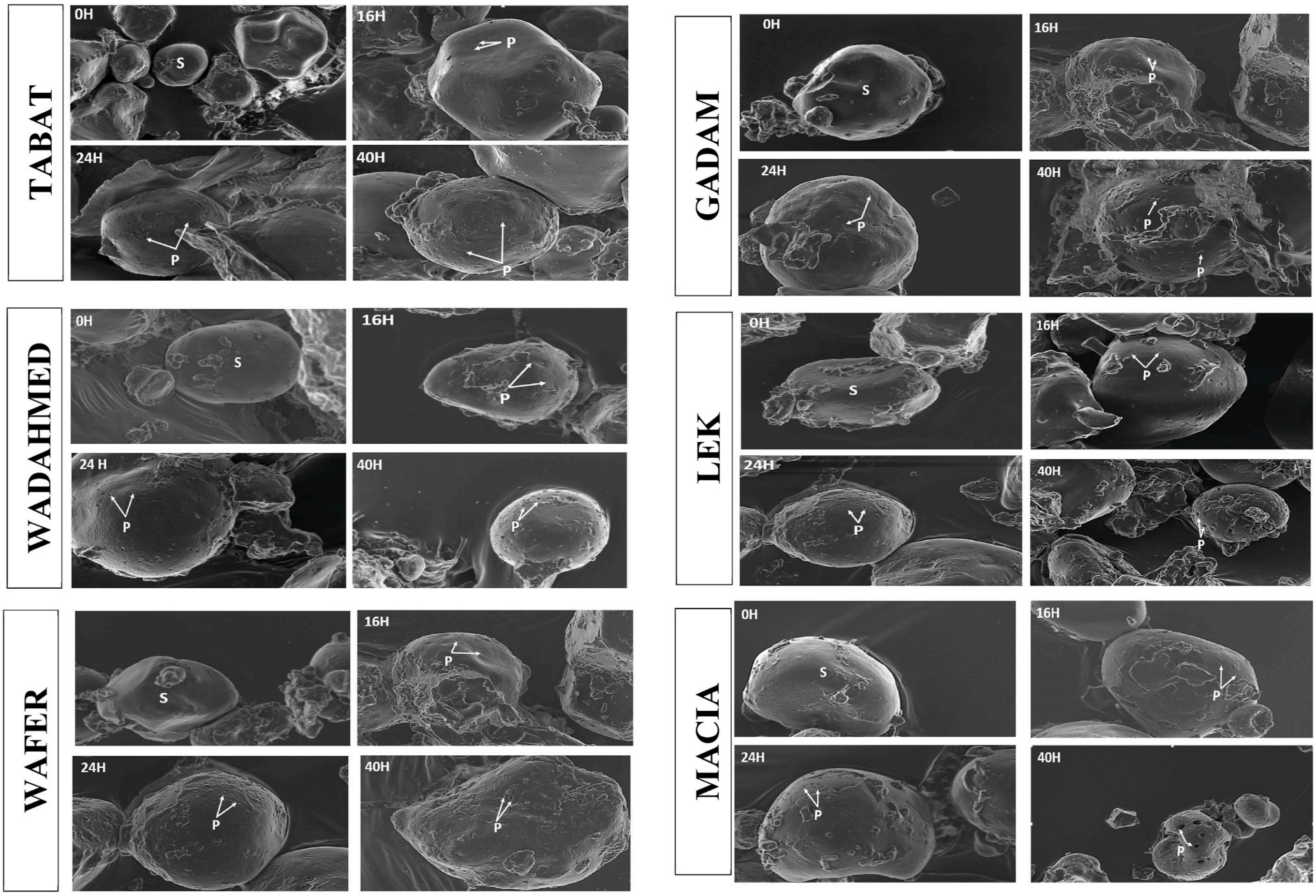

Fig. 4. Scanning electron micrographs of starch granules from non-fermented and fermented sorghum batters. S: smooth surface; P: Pores. Scale bar = 2 μ

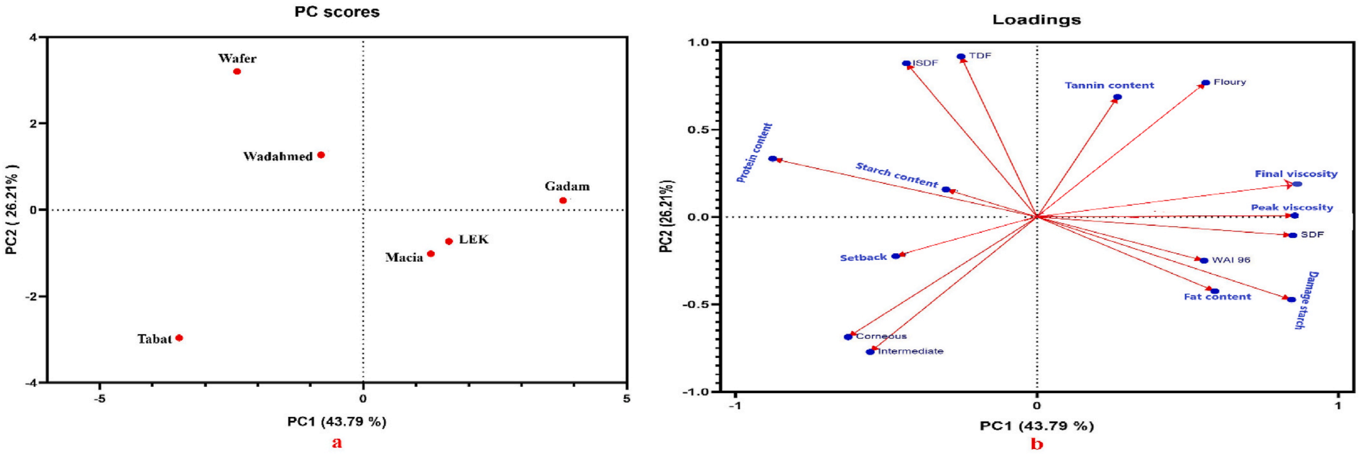

Fig. 5a. Principal component analysis biplot sorghum varieties and their grain and non-fermented batter physico-chemical characteristics. WAI: water absorption index; TDF: total dietary fiber; SDF: soluble dietary fiber; I SD: insoluble dietary fiber.

of the gelatinized/pasted starch, starch molecules form a gel structure with water which is stabilized by the intra- and intermolecular interactions of amylose and amylopectin (Pulgarín et al., 2023). A high water absorption index may also indicate that kisra will possibly have a higher water content, thus a softer thin flat bread can be produced (Ghasemi et al. 2022). The lower setback viscosity may suggest a lower starch retrogradation. A lower starch retrogradation have been correlated with lower staling rate for white wheat bread (Barros et al., 2018).

4. Conclusions

Lactic acid fermentation change the techno-functional, micro and molecular structures of sorghum batter. These changes properties should be related to the quality characteristics of kisra flatbread. However, this relationship now needs to be determined. Fermentation increases the pasting viscosity and water absorption index of the batters. Notably the fermentation results hydrolysis of endosperm matrix protein around the starch granules. The increased viscosity of the sorghum flour batter

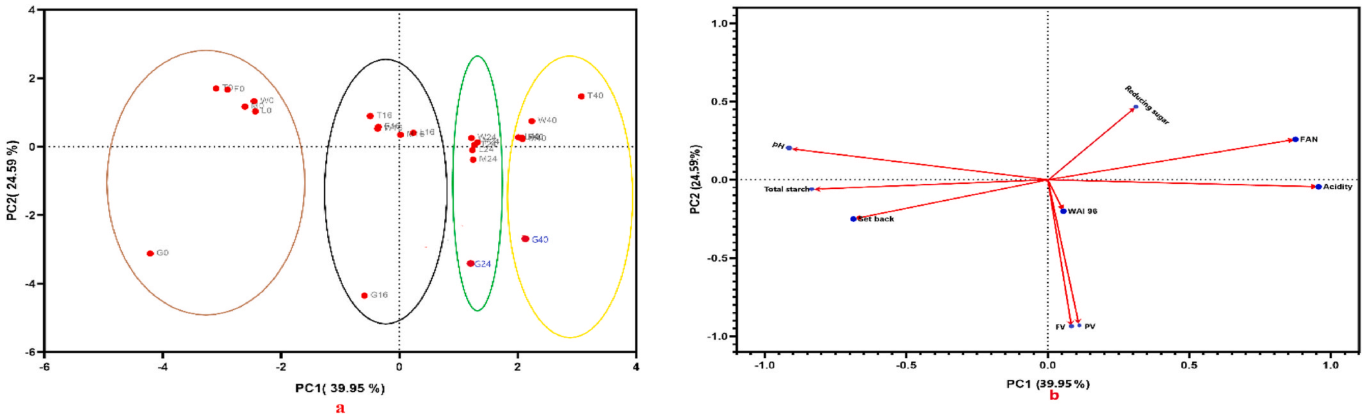

Fig. 5b. Principal component biplot of sorghum varieties during the batter fermentation and the physico-chemical properties of their batters. T: Tabat; F: Wafer; W: Wadahmed; G: Gadam; M: Macia; L; Lekgeberwa; WAI: water absorption index; PV: peak viscosity; FV: final viscosity.

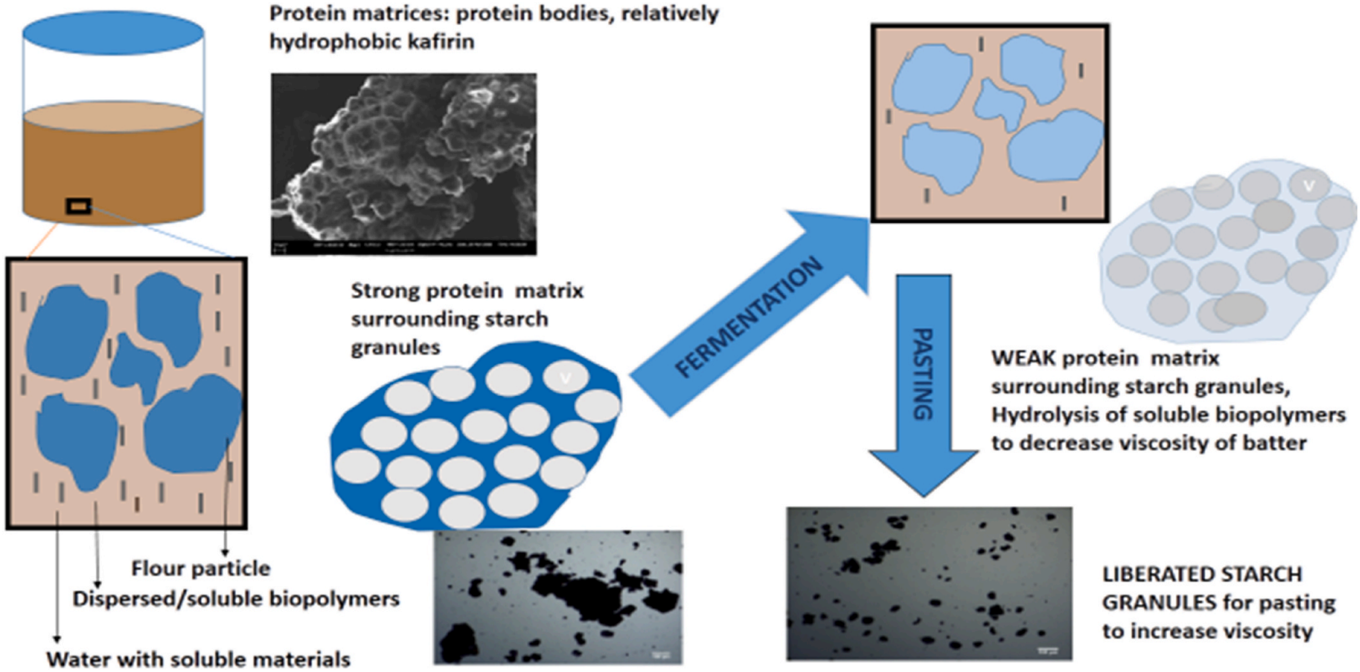

Fig. 6. Schematic diagram of the variation in the physico-chemical properties of sorghum batters during fermentation.

during fermentation is due freeing of the starch granules that were embedded in the protein matrix, leading to increased accessibility of the starch to water. Th knowledge changes of batter physicochemical properties such as batter pasting viscosity are related to sorghum grain characteristics such as protein and tannin content. The results also indicate that kisra sourdough fermentation can be optimized at 16 h and 24 h to obtain desired batter characteristic before baking.

CRediT authorship contribution statement

Sami Sidahmed Ali: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – original draft. **John RN. Taylor:** Methodology, Supervision, Writing – review & editing. **Mohammad Naushad Emmambux:** Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

Authors declare that they have no conflict of interest.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jcs.2024.103937.

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