Development of a maize meal based-drink fermented using kefir grains or yoghurt starter cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*)

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Abstract: This study aimed to develop a fermented maize meal drink using kefir grains or yoghurt starter cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*). Maize flour was first gelatinized into gelatinized maize meal slurry and this was partially replaced with pasteurized milk (80:20), cooled to 35°C (unfermented drink, UFD) and fermented with either kefir grains (KGD) or yoghurt cultures (YSD). The physicochemical properties, total soluble solids, sugar compounds, and storage stability indices of the derived beverages were evaluated. The fermented drinks showed low acidity levels (pH 3.90–4.15) and total soluble solids (< 5.25 Brix). The effects were due to the metabolic action of the cultured microbes to hydrolyze macromolecules in the drinks, as well as the formation of organic acids. As a result, biochemical changes were also noted for other assessed parameters. The YSD had the least alpha-lactose (35680.30 μ g g⁻¹) and fructose (168.94 μ g g⁻¹) contents, high sorbitol concentration (279.36 μ g g⁻¹), increased antioxidant activity (2.53 μ mol TEg⁻¹), maximum lightness, moderately thin consistency and good shelf stability indices. Although the KGD had appreciable properties, findings indicated that the YSD (with good indices in all the qualities investigated) could be a potential functional drink.

Keywords: Maize meal, kefir grains, yoghurt starter culture, fermented drinks, sugar compounds

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1 Introduction

The evolving global interest in consuming foods containing health-improving live microbes (probiotics) is one of the key drivers projected to increase the market growth of fermented foods and drinks by 846.73 billion US dollars between 2023 and 2027 (ReportLinker, 2022). Synbiotic drinks may be considered a group of fermented beverages comprising probiotics and prebiotics (non-

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digestible food constituents with selective metabolic functions) that are of nutritional and functional food interests (Salmer án, 2017; Sebasti án et al., 2019). Cereal grains are an essential substrate for producing symbiotic dinks due to the presence of utilizable nutrients that stimulate the fermentation of probiotics and vital prebiotic components (including noncarbohydrate substances) for promoting gut health (Marsh et al., 2014; Salmer án, 2017). Some of these grains, like maize, millet, oat, rice, spell and sorghum, have been used to develop synbiotic drinks (Kewuyemi et al., 2020; Ziarno and Cichońska, 2021).

Maize (*Zea mays* L.) is an African staple food grain that contributes starch, protein, essential amino acids, unsaturated fatty acids and good amounts of natural antioxidants to the daily diet (Erenstein et al., 2022). Bioprocessing maize through fermentation has been reported to yield affordable maize-based beverages and complimentary gruels characterized by enhanced nutrient bioavailability and biologically active compounds (Mashau et al., 2021; Mehlomakulu et al., 2023). However, maize beverages have been mostly fermented with lactic acid bacteria, and fewer reports are available on using other microbiota with probiotic potential (Menezes et al., 2018; Mehlomakulu et al., 2023).

Commercial starter cultures, including kefir grains and yoghurt lactic acid bacteria (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*), are used to produce fermented beverages (Guzel-Seydim et al., 2021; Lopusiewicz et al., 2022). Kefir grains comprise a symbiotic composition of microorganism genera and species, primarily lactic acid bacteria and yeasts embedded in a kefiran polysaccharide matrix (Guzel-Seydim et al., 2021). Food use of LAB and kefir grains is crucial for potential wellness values (Lopusiewicz et al., 2022). The objective of the current study was thus to develop a fermented maize meal drink cultured with either kefir grains or yoghurt cultures. The physicochemical properties, total soluble solids, sugar compounds, and storage stability of the prepared drinks were subsequently investigated.

2 Materials and methods

2.1 Materials and sample preparation

Maize grains (Zea mays), milk (Clover [Pty] Ltd, Roodepoort, South Africa) and sugar (Selati, RCL Foods [Pty] Ltd, Westville, South Africa) were purchased at Checkers Hyper Pty (Ltd), Johannesburg, South Africa. Kefir grains were sourced at Dischem Pty. (Ltd.), Johannesburg, South Africa, while starter cultures (Lactobacillus bulgaricus and **Streptococcus** thermophilus) were procured from CHR Hansen (YC-X11, Hoersholm, Denmark). Maize grains were converted into maize meal by cleaning to remove unfit grains and foreign materials and then dry milled (Perten Laboratory Mill 3600, Perten Instruments, Stockholm, Sweden). The obtained meal was stored in sterile polythene bags at (4°C) prior to further use. The starter cultures were also stored as recommended by the manufacturer. All chemicals and reagents used were of analytical grade.

2.2 Fermented drink preparation

The formulation presented in Table 1 was used to prepare fermented maize meal drinks inoculated with either kefir grains or yoghurt lactic culture. Briefly, 200 g of maize meal was made into a slurry by mixing it with 3 litres of water at 100°C. The mixture was gently stirred, allowed to cook and gelatinized for 10 min at 75°C. The cooked maize slurry was mixed with milk (previously pasteurized at 72°C and held for 15 sec). The recovered blend was rapidly cooled to 35°C and divided into three batches (1 litre of semi-liquid each). A portion was reserved as the control, while the remaining batches were inoculated with 0.1 g of kefir grains or yoghurt culture and were allowed to ferment until a pH between 3.90 and 4.15 was attained. Fermentation was terminated by refrigeration at $< 4^{\circ}$ C, sugar was added, and the samples were stored prior to further analysis.

Table 1 Formulation of maize meal drink and fermented maize

	meal drink	
	Ingredient	Percentage (%)
	D (11)	02.75
	Portable water	93.75
Maize meal drink	Maize meal	6.25
	Total	100
	Cooked maize meal	80
	slurry	
Fermented drink	Fresh milk	20
	Total	100

2.3 pH and titratable acidity (TA)

A calibrated pH meter (pH 510, Eutech Instruments Pte Ltd., Queenstown, Singapore) was used to measure the pH of the prepared beverages. The TA of the samples were examined by titrating a solution containing 2 g of sample in 20 mL distilled water against 0.1N NaOH using phenolphthalein as an indicator. The titre value was recorded, and TA was subsequently calculated (Adebo et al., 2018). The pH and TA values were obtained at sixhour intervals during fermentation.

2.4 Antioxidant activity

The spectrophotometric technique based on 2,2'-Azino-bis(3-ethylbenzthiazoline-6-sulfonic acid), also regarded as Trolox equivalent antioxidant capacity (TEAC), was adopted for the determination of the antioxidant capacity of the samples (Adebo et al., 2018). Concisely, solutions containing 20 µL methanol extract of the sample and 180 µL of the ABTS free radical were incubated for 300 sec at 37°C in the dark. Trolox served as a reference standard. The absorbance of reaction solutions was read on a microplate reader (iMark; Bio-Rad Laboratories, Johannesburg, South Africa) set at 734 nm. The antioxidant activities of the beverages were expressed as micromolar of Trolox equivalent per gram sample (µmol TE g⁻¹ sample).

2.5 Consistency and total soluble solids (TSS)

Consistency (cm min⁻¹) and TSS (Brix) of the drinks were measured using Bostwick consistometer CR-BC-24 (CR Instruments Ltd., Christchurch, United Kingdom) and refractometer (HI 96801, HANNA Instruments, Inc., Romania), respectively.

2.6 Determination of sugars and sugar-related compounds

The sugar compounds in the drinks were analyzed at the Central Analytical Facility of Stellenbosch University, South Africa, on a gas chromatograph (TRACE 1300 GC, Thermo Fisher Scientific, Millan, Italy) coupled to a flame ionization detector (FID). Into 20 mg of sample, 1 mL of 70% methanol was added, vortexed and extracted in the oven at 60°C for 4 h. Total of 250 µL extract was completely dried under a gentle stream of nitrogen, and 0.1 mL of 2% methoxyamine in pyridine was used to derivatize the sample at 40°C for 2 h. Furthermore, 0.05 mL N, O-Bis(trimethylsilyl)trifluoroacetamide was added and re-derivatized at 60°C for 30 min, vortexed and transferred to an insert and injected into a GC-FID instrument coupled to a CTC Analytics PAL autosampler. The separation of sugars was performed on a ZB-Semivolatile Zebron 7HG-G027-11-GGA capillary column (30 m, 0.25 mm, 0.25 µm film thickness). One (1) µL of the extract was injected in a 10:1 split ratio. The oven temperature was programmed as follows: 80 °C for 1 min and ramped up to 300 °C at a rate of 7 °C min⁻¹ for 2 min. The temperature of the FID was maintained at 300 °C. Helium was used as the carrier gas at a flow rate of 1 mL min⁻¹, and the injector temperature was maintained at 240°C.

2.7 Colour profile

The colour of the drinks was profiled using a calibrated colourimeter system (Konica Minolta, Inc., Tokyo, Japan). The CIELab colour co-ordinates presented were L* (lightness), +/-a*(redness/greenness), +/-b* (yellowness/blueness) and ΔE^* (total colour difference) (Ramashia et al., 2021). The chroma (C*) and hue angle (H^o) were calculated using the reported Equations 1 and 2 by (Ramashia et al., 2021).

Chroma (C*) =
$$\sqrt{(a^*)^2 + (b^*)^2}$$
 (1)

Hue
$$(H^{\circ}) = tan^{-1} \left\{ \frac{b^{*}}{a^{*}} \right\}$$
 (2)

2.8 Storage stability test

The storage stability test of the prepared drinks was carried out by storing capped plastics containing the drinks at 4°C for 28 days. The changes in the drinks were monitored based on the differences between pH, TA and consistency over the storage duration.

2.9 Statistical analysis

The one-way analysis of variance based on the Duncan multiple range tests was used to establish statistical differences between triplicate determinations, and mean values \pm standard deviation was expressed at a 5% ($p \le 0.05$) probability level (IBM SPSS statistics version 22, New York, USA).

3 Results and discussion

3.1 pH, TA and antioxidant activity of unfermented and fermented maize meal drinks

The fermented maize meal drinks inoculated with kefir grains or starter cultures differ with fermentation temperatures and times. The temperatures used were selected based on the ability of the culture inoculums to grow and attain the desired pH level (Altay et al., 2013).

Thus, the gelatinized maize slurry inoculated with kefir grains was fermented at 25°C, and its fermentation took a period of 24 h. The yoghurt culture-induced maize slurry fermentation was done at 35°C for 12 h. Figure 1 shows the pH and TA of the unfermented and fermented maize meal slurry over time. The pH of the unfermented maize drink (UFD) remained neutral (6.88), while the kefir grain fermented maize drinks (KGD) and voghurt starter fermented maize drinks (YSD) decreased from neutral (6.88) to acidic (4.12 and 3.93, respectively). The reduction in pH was mainly due to the metabolic rate of the inoculated cultures to utilize macromolecules such as carbohydrates as their source of carbon and hydrolyze sugars such as lactose by the presence of β -galactosidase to liberate lactic acid (and other organic acids), which resulted in decreased pH values (Brancher et al., 2014). Unsurprisingly, a decrease in the pH of the slurries concomitantly brought about an increase in TA (Figure 1). The differential fermentation rate (as shown with variation in pH and TA) of the inoculated cultures in the drinks may be associated with the report that indicated kefir fermentation occurs slowly due to the symbiotic interaction of the microorganisms in kefir grains (Singh and Shah, 2017).

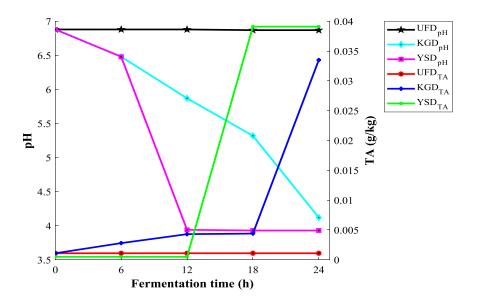
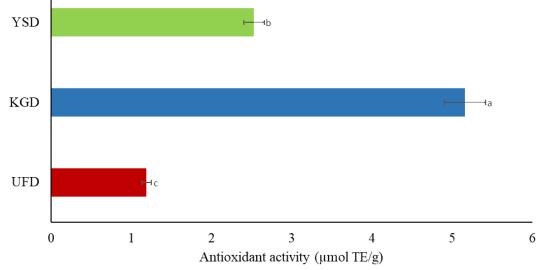


Figure 1 pH and TA of the unfermented and fermented maize meal drinks over six hours intervals Note: UFD – unfermented maize drink, KGD – kefir grain fermented maize drink, YSD – yoghurt starter fermented maize drink

The TEAC of the unfermented and fermented maize meal drinks is presented in Figure 2. The UFD showed less antioxidant activity (1.19 μ mol TE g⁻¹) compared to the fermented counterparts KGD (5.16 μ mol TE g⁻¹) and YSD (2.53 μ mol TE g⁻¹). As such, the fermentation of the maize beverages suggests the degradation of the grain cell wall and ensuing enzymatic activities of the inoculated cultures resulted in the release of bound polyphenols with potent antioxidant activities (Adebo and Gabriela Medina-Meza, 2020). Irrespective of the inoculum used, the fermentation of the maize drinks brought about significant increases in the antioxidant activity of the resultant beverages. The KGD had a twofold antioxidant activity increase than the YCD. In reference to the coexistence of LAB and yeast in kefir grains, the observation is consistent with earlier reports that have proposed that the antioxidant activity of yeasts is greater than that of LAB due to the high presence of some components (such as β -glucan, intracellular enzymes and peptides) in the yeast cell wall that plays significant antioxidant roles (Gil-Rodr guez et al., 2015; Freire et al., 2017; Ferreira et al., 2022).





Note: Means represented as bars, indicating different alphabet are significantly different at $p \le 0.05$. UFD – unfermented maize drink, KGD – kefir grain fermented maize drink, YSD – yoghurt starter fermented maize drink

3.2 Consistency and TSS of the unfermented and fermented maize meal drinks

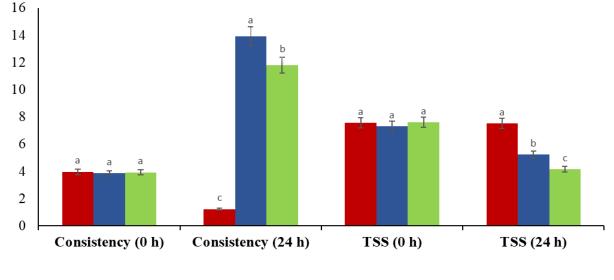
According to Maleke et al. (2022), reports on the consistency of fermented beverages using the Bostwick consistemeter or similar equipment to measure consistency are scarce. Consistency is a measure of the distance (cm) that the unfermented and fermented drinks flow in a given time interval (min). A lower consistency signifies a more viscous/thicker drink, while a higher consistency means a less viscous/thin and free-flowing drink. Figure 3 depicts the consistency before and after the fermentation of maize meal drinks. There were insignificant differences ($p \le 0.05$) between the beverages

before fermentation (3.86–3.97 cm min⁻¹). On the contrary, significant changes were noted after fermentation with either kefir grains (13.93 cm min⁻¹) or yoghurt starter cultures (11.81 cm min⁻¹). The reduction in the TSS (7.60–4.17 Brix) may be associated with the thin and free-flowing consistency of the fermented drinks (KGD and YSD). A thin consistency is an attribute of fermented beverages (Marsh et al., 2014).

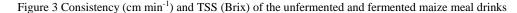
The soluble solids expressed as degrees Brix is a sweetness indicator of the maize meal drinks (Kumar et al., 2020). The changes in the TSS of the drinks before and after fermentation are depicted in Figure 3. While no significant differences ($p \le 0.05$) in the TSS of the drinks

were noted before fermentation, there were significant differences ($p \le 0.05$) in the TSS of the beverages after fermentation with either kefir grains (7.33–5.23 Brix) or yoghurt cultures (7.60–4.17 Brix). The TSS of the raw maize meal drink (> 7.50 Brix) remained unchanged before and after fermentation. Both fermented drinks had reductions in the TSS values, and the degrees Brix for the drink inoculated with yoghurt cultures was lower (4.17 Brix) than the drink cultured with kefir grains (5.23 Brix).

The varying decreases in the TSS of the fermented beverages relate to the extent of carbohydrate utilization by the respective microbes in the starter cultures to induce the formation of organic acids (Norberto et al., 2018; Kumar et al., 2020). Similarly, fermented beverages containing processed food grains have been demonstrated to show reductions in TSS (Tu et al., 2019; Kumar et al., 2020).



■UFD ■KGD ■YSD



Note: Means represented as bars, indicating the same alphabet, are not significantly different at $p \le 0.05$. UFD – unfermented maize drink, KGD – kefir grain fermented maize drink, YSD – yoghurt starter fermented maize drink

3.3 Sugar compounds in the unfermented and fermented maize meal drinks

The sugar compounds in the unfermented and fermented maize meal drinks consist of monosaccharides, disaccharides, and sugar alcohols (Table 2). The monosaccharides were largely present, and the concentrations varied in the maize meal drinks. Mannose and glucose were abundant in the monosaccharide group, and the sugar levels significantly increased in the drinks inoculated with either kefir grains (1170.84–2772.25 μ g g⁻¹, glucose) or yoghurt cultures (222.89–17150.86 μ g g⁻¹, mannose). In the same vein, the YSD had a high amount of arabinose, while KGD showed better concentrations of rhamnose (115.22 μ g g⁻¹) and ribose (104.96 μ g g⁻¹). The higher release of monosaccharides in the fermented

maize meal drinks may be due to the increased hydrolysis of fibre constituents in the drinks, as reported for fermented liquid cereal-co-products during 12 and 24 h fermentation periods (Rho et al., 2020). Existing literature has also shown increases in the concentrations of monosaccharides (arabinose, glucose, and xylose) at early fermentation stages (0 to 24 h) (Tu et al., 2019; Rho et al., 2020).

Among the monosaccharides, only fructose was reduced after fermentation and the lowest concentration was recorded for the YSD. The reduction in fructose (428.09–168.94 μ g g⁻¹) and fructose-containing disaccharide (sucrose, 13168.92–84.78 μ g g⁻¹), as well as the decrease in the TSS values of the fermented maize drinks, imply that microbial consortiums in the starter

cultures utilized available monosaccharides as carbon sources (Kawai et al., 2020; Ziarno and Cichońska, 2021). The observed reductions in fructose and sucrose concentrations of the fermented maize meal drinks align with earlier reports for kefir grain fermented beverages (Mart ńez-Torres et al., 2017; Tu et al., 2019). The decreases in alpha-lactose and fructose concentrations in YSD may suggest a preferred drink to individuals with lactose intolerance and lessen the undesirable cardiometabolic effects of fructose (Malik and Hu, 2015; Norberto et al., 2018).

Sugar alcohols (e.g., arabitol, mannitol, myo-inositol, sorbitol etc.) are an important sugar group and

derivatives of major saccharides that contribute to the sweetness of food products with fewer calories (Goldfein and Slavin, 2015). The KGD showed better concentrations of arabitol (101.78 μ g g⁻¹) and mannitol (183.59 μ g g⁻¹), whereas YSD had improved levels of myo-inositol (108.61–154.43 μ g g⁻¹) and sorbitol (176.91–279.36 μ g g⁻¹) (Table 2). The possible saccharolytic activity differences of microorganisms involved in carbohydrate metabolism might have stimulated increased concentrations of the observed hydrogenated monosaccharides (Ghosh et al., 2015; Das et al., 2019). The YSD with a high sorbitol level may act as a stimulant laxative (Goldfein and Slavin, 2015).

Sugars	UFD	KGD	YSD
Monosaccharides			
Arabinose	$66.88^{a} \pm 0.08$	80.95 ^b ±0.11	86.19°±0.13
Fructose	428.09 ^c ±0.02	335.15 ^b ±0.08	168.94 ^a ±0.05
Glucose	1170.84 ^a ±0.14	2772.25 ^c ±0.08	2468.15 ^b ±0.05
Mannose	222.89 ^a ±0.11	6643.65 ^b ±0.04	17150.86 ^c ±0.17
Rhamnose	70.15 ^a ±0.15	115.22 ^c ±0.09	79.74 ^b ±0.18
Ribose	$73.00^{a} \pm 0.07$	104.96 ^b ±0.03	103.14 ^b ±0.09
Disaccharides			
Alpha-lactose	39840.86 ^b ±0.18	52511.65 ^c ±0.09	35680.30 ^a ±0.11
Sucrose	13168.92 ^c ±0.20	$84.78^{a} \pm 0.09$	1246.97 ^b ±0.16
Sugar alcohols			
Arabitol	50.57 ^a ±0.06	101.78 ^b ±0.12	ND
Mannitol	ND	183.59 ^b ±0.10	92.01 ^a ±0.03
Myo-inositol	108.61 ^a ±0.06	112.17 ^{ab} ±0.04	154.43 ^b ±0.04
Sorbitol	176.91 ^a ±0.02	217.36 ^b ±0.13	279.36°±0.18

Note: Means with different superscripts under the same column are significantly different $p \le 0.05$. UFD – unfermented maize drink, KGD – kefir grain fermented maize drink, YSD – yoghurt starter fermented maize drink, ND – not detected

Table 3 Colour attributes	of unferment	ed and fermentee	d maize meal drinks
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Colour parameters	UFD	KGD	YSD
L*	2.27 ^a ±0.01	2.62 ^b ±0.00	4.04°±0.02
-a*	$-0.99^{a} \pm 0.01$	-1.25 ^b ±0.01	-1.72 ^c ±0.01
-b*	-1.55 ^a ±0.01	-2.04 ^b ±0.01	-1.21 ^c ±0.01
ΔE	2.93 ^a ±0.01	3.54 ^b ±0.02	4.56 ^c ±0.01
Chroma	1.84 ^a ±0.02	2.39 ^c ±0.01	2.10 ^b ±0.02
Hue	57.43 ^b ±0.01	58.50 ^b ±0.02	35.13 ^a ±0.01

Note: Means with different superscripts under the same column are significantly different $p \le 0.05$. L* – lightness, -a* - greenness, -b* – blueness, ΔE – total colour difference, UFD – unfermented maize drink, KGD – kefir grain fermented maize drink, YSD – yoghurt starter fermented maize drink

3.4 Colour attribute of the unfermented and

fermented maize meal drinks

The colour attributes of the unfermented and

fermented maize meal drinks are presented in Table 3.

The significant differences $(p \le 0.05)$ in the colour attributes of the prepared beverages correspond with the variation in the drinks' total colour differences. While the YSD had the maximum lightness and the minimum greenness, the KGD showed less lightness and blueness. The observation is in accordance with the qualitative colour attributes. On the one hand, the KGD had improved saturation as indicated by high chroma and hue angle closer to yellow ($< 90^{\circ}$). On the other hand, the YSD with less chroma suggests less saturation (i.e., closer to lightness). The high lightness intensity of the YSD may be due to the bleaching effect impacted by hydrogen peroxide (H₂O₂) produced during heterolactic fermentation (Agarry et al., 2010). In contrast, insignificant differences ($p \le 0.05$) were observed between the hue angle (light yellow) of the KGD and UFD. Paredes et al. (2022) also noted a non-significant effect on the hue angle of a novel functional kefir grain fermented beverage within 24 h.

3.5 Storage stability of the unfermented and fermented maize meal drinks

As the proliferation of microbes in cultured cerealbased drinks may continue during storage, thus the consideration of determining microbial growth factors such as physicochemical parameters is crucial (Marsh et al., 2014). The shelf life stability of the unfermented and fermented maize meal drinks was evaluated based on possible changes in pH, TA and consistency over 28 days (Table 4). The result revealed insignificant differences ($p \le 0.05$) in each parameter per drink sample during the storage period. The non-significant variations in the storage stability parameters of the fermented beverages imply that the drinks may be shelf-stable for four weeks at refrigeration temperature (4°C).

4 Conclusion

The results of the physicochemical properties, total soluble solids, sugar compounds, and storage stability indices of the unfermented and fermented maize meal drinks were presented in this study. The analytical data revealed that the fermented beverages were within the acidic range and had reduced total soluble solids. The inoculated drink with kefir grains had the highest antioxidant activity. In contrast, yoghurt starter cultured drink showed the least alpha-lactose and fructose contents, high sorbitol concentration, brighter colour, moderately thin consistency and better shelf stability indices. These findings for the voghurt starter fermented maize drink are essential for wellness and consumer acceptability. Future studies are recommended to profile the metabolites in the drink and determine the beverages' sensory characteristics and storage stability based on microbial growth as spoilage indicators.

Sample	Parameters _	Time (Days)			
		7 days	14 days	21 days	28 days
UFD	pН	6.86 ^b ±0.01	6.86 ^b ±0.01	6.85 ^b ±0.01	6.85 ^b ±0.02
	ТА	0	0	0	0
	Consistency	1.28°±0.01	1.29°±0.02	1.22°±0.03	1.26 ^c ±0.02
KGD	рН	4.11 ^a ±0.05	4.10 ^a ±0.06	3.96 ^a ±0.04	3.85 ^a ±0.03
	ТА	0.03 ^a ±0.12	0.03 ^a ±0.01	0.03 ^a ±0.02	0.03 ^a ±0.01
	Consistency	13.9 ^a ±0.04	13.92 ^a ±0.00	13.92 ^a ±0.08	12.56 ^a ±0.09
YSD	pH	3.93 ^a ±0.12	3.92 ^a ±0.19	3.92 ^a ±0.05	3.80 ^a ±0.06
	ТА	0.04 ^a ±0.00	0.04 ^a ±0.01	0.04 ^a ±0.01	$0.04^{a}\pm0.02$
	Consistency	11.91 ^b ±0.04	11.82 ^b ±0.01	11.80 ^b ±0.01	11.80 ^b ±0.01

Table 4 Shelf-life stability of unfermented and fermented maize meal drinks based on pH, TA and consistency over 28 days

Note: Means of the same alphabet across the column are not significantly different $p \le 0.05$. UFD – unfermented maize drink, KGD – kefir grain fermented maize drink, YSD

- yoghurt starter fermented maize drink

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