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Comparative Evaluation of Health-Promoting Compounds, Physicochemical and Microbiological Properties of Sorghum [*Sorghum bicolor* (L.) Moench] Based *Mahewu* Produced by Different Traditional Brewers in Thohoyandou, South Africa

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Abstract: Sorghum *(Sorghum bicolour* (L.) Moench) is an emerging reliable alternative for *mahewu* production. The aim of this study was to evaluate the health-promoting compounds, physicochemical and microbiological properties of sorghum-based *mahewu* produced by different traditional brewers in Thohoyandou, South Africa. A total of 18 *mahewu* samples produced by six traditional brewers (TB1–TB6) were collected and compared for antioxidant, physicochemical, and microbiological properties. Commercial sorghum *mahewu* was used as a control sample. The total phenolic content of the *mahewu* samples varied from 27.37 to 65.89 GAE /g, with commercial *mahewu* having a lower value. The flavonoid content ranged from 0.18 to 0.30 GAE/g, and commercial *mahewu* had a higher value. The DPPH scavenging activity and FRAP of *mahewu* samples ranged from 44.62% to 49% and 1.47 to 2.36 mg GAE/g, respectively. Commercial *mahewu* had a higher DPPH value but a lower FRAP value. The pH of *mahewu* varied significantly, ranging from 3.38 to 3.66, but was within the acceptable range. The °Brix values varied from 9.68 to 17.49, with traditional *mahewu* samples having higher values than commercial *mahewu*. Total titratable acidity ranged from 0.63 to 1.17%. The viscosity ranged from 444.33 to 1297.00 cP, with commercial *mahewu* having a higher value. There was a significant variation in the color of the *mahewu* samples with respect to L*, a*, b*, C, Hue, and ΔE. The growth of yeasts and molds varied from 7.95 $\log_{10}$ to 8.99 $\log_{10}$ (cfu/mL) in traditional *mahewu* samples, and coliforms ranged from 3.68 to 5.96 $\log_{10}$ (cfu/mL) and were not isolated in commercial *mahewu*. The total plate count ranged from 7.914 to 8.978 $\log_{10}$ (cfu/mL). The microbiological results show that traditional brewers are meeting the legal limit and can increase their products for commercialization.

Keywords: *mahewu*; sorghum; physicochemical properties; bioactive compounds; microbiological quality

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1. Introduction

Various climate change models have predicted a future increase in the prevalence and ferocity of drought in sub-Saharan Africa. In this context, the production of cereal grains such as maize (*Zea mays* L.) is expected to decrease dramatically in the next decades due to this climate change [1,2]. This will endanger food and nutrition security, especially for rural households that rely on maize production. Thus, scientists need to search for a substitute for maize in food production and processing with a special focus on climate-friendly crops such as sorghum (*Sorghum bicolor*) [3]. Sorghum is a reliable emerging alternative for *mahewu* production because it can grow in harsh conditions, it is rich in phytochemicals, and it is nutritionally superior to maize [4]. The presence of phytochemicals in the grain of sorghum includes phenolic acids, flavonoids, and condensed tannins [5–7]. The polyphenolic compounds of sorghum grains have higher antioxidant ability than those

of maize, wheat, and rice [8,9]. Furthermore, sorghum contains 8–18% protein, 1 to 5% fat, 19% dietary fiber, and 70 to 80% carbohydrate [10]. Consumption of sorghum grains and its products is associated with the ability to improve intestinal function and decrease cholesterol levels, and this is due to the availability of non-starch polysaccharides [11,12]. Other health benefits of sorghum and its products include the ability to bind to steroids, a low glycemic index, cancer prevention, and anti-inflammatory characteristics [7,13].

Maize grain is traditionally processed and fermented spontaneously to obtain different types of products, such as *mahewu* [14]. Traditional technologies such as fermentation lead to a notable transformation in food and beverage, as they are a good basis for the development of safe food products [15]. Fermentation has been widely used over the years due to its many benefits, whether it prolongs shelf life, improves nutritional quality, improves digestibility, preserves and increases functional and physicochemical properties, aroma, and flavor [16–18]. Moreover, fermentation offers other benefits, such as destroying natural toxins and pathogenic microorganisms; therefore, it is inexpensive [19]. The traditional fermentation method falls under the spontaneous fermentation technique, in which no commercial starter culture, such as *Lactococcus* spp., is used. Today, other ingredients are added for flavor, nutritional value, and bioactive compound enhancement purposes, and microorganisms for traditional fermentation are believed to come from raw materials [20,21]. The dominant and unavoidable microorganisms in maize fermentation include lactic acid bacteria (LAB) and yeasts, which improve the nutritional value and shelf life of fermented maize products. The commonly consumed non-alcoholic beverage in southern Africa is *mahewu*, a product of maize [22].

Traditional *mahewu* is a spontaneously fermented non-alcoholic beverage with a pH ranging from 3.6 to 4.0 and is primarily produced from white maize or sorghum flour and LAB [23,24]. To produce *mahewu*, maize flour is cooked and gelatinized. The mixture is allowed to cool at room temperature with continuous stirring. Wheat flour is sometimes added to the mixture as an inoculum prior to natural fermentation for up to three days [22]. The microflora of maize/sorghum flour or water are inactivated during cooking of the maize or sorghum porridge. *Mahewu* is known by different names in southern Africa such as *amaheu* (South Africa and Eswatini), as well as *maxau* in Namibia [25,26]. The industrial production of *mahewu* with maize flour in South Africa is through environmentally controlled fermentation in which starter cultures such as *Lactobacillus bulgaricus* var *delbrueckii* and *Lactobacillus brevis* cultures are used [27]. On the other hand, the production of sorghum-based *mahewu* is not popular with consumers because of its brown-to-dark color compared to the creamy color of maize *mahewu*. It is consumed by people of various ages, including infants who consume it as a weaning food [28]. Nonetheless, maize and sorghum grains are low in protein content and deficient in essential amino acids such as lysine and tryptophan [12,29]. The availability of tannins (0.10–3.60%) in sorghum grains prevents the absorption and digestibility of proteins and other amino acids by forming complexes of tannin–protein or tannin–mineral [30]. The high content of tannins can also contribute to the bitter taste of *mahewu* if not removed during the processing of the sorghum grain.

The biggest challenge faced by traditional brewers is that they do not have a cold room for storage, most of them store *mahewu* at room temperature. This causes a continuation of the fermentation process and results in an over-souring of the product between two to four days of production [31]. Over-souring may also affect the physicochemical and microbiological properties of *mahewu*. Poor cleaning and sanitation harbor the growth of spoilage microorganisms in the production area, resulting in *mahewu* contamination [32]. Variations in the ingredients used, processing steps, and storage conditions have an impact on the quality and safety of *mahewu*. However, unexpected circumstances during *mahewu* production can contribute to changes in production practices, along with changes in consumer liking. In this context, Simatende et al. [33] identified LAB and determined selected biochemical characteristics in *emasi* (sour milk) and *emahewu* produced by households in Eswatini. The results showed that the strains found in *emahewu*, especially *Lactobacillus* spp., Weissella, and Enterococcus, were similar to those found in ting, a fermented product of South

African sorghum. However, the health-promoting compounds and physicochemical and microbiological properties of sorghum *mahewu* produced by different traditional brewers have not been studied. Therefore, the aim of this work was to investigate the health-promoting compounds, physicochemical and microbiological properties of sorghum-based *mahewu* produced by different traditional brewers in Thohoyandou, South Africa. This study is important in the upscaling and commercialization of traditional sorghum-based *mahewu*.

2. Materials and Methods

2.1. Sample Collection

A total of 18 freshly produced (within 24 h) traditional sorghum-based *mahewu* samples, each packed in a one-liter plastic bottle, were collected from six traditional brewers (3 samples per brewer) at different sites in Thohoyandou, Limpopo province, South Africa. Commercial sorghum-based *mahewu* (one liter) was purchased from a local shop in Thohoyandou and used as a control sample. All *mahewu* samples were kept below −1 °C until further analysis. The general production of traditional sorghum *mahewu* is like that of maize *mahewu*, and it is as follows: one part of the sorghum meal is added to 9 parts of boiling water. The suspension is cooked for 15 min at 90 °C with intermittent stirring, allowing it to cool to about 40 °C, and then transferred to a container for the fermentation process. Wheat flour (5% of the sorghum meal used) is added during cooking since it serves as a source of inoculums. The porridge is cooled and allowed to ferment at a controlled temperature of 37 ± 5 °C for 72 h [28]. The production process of *mahewu* may differ amongst the traditional brewers since most of them do not control the temperature during manufacturing and storage. In contrast, other brewers do not add wheat flour, which is used as inoculum, and the quality of the final product depends on the ingredients used and storage conditions.

2.2. Preparation of Extracts

Phenolic extracts were prepared by refluxing 2 g of 20 mL of 1% HCl methanol sample at 60 °C ± 5 h. The mixtures were centrifuged (Rotina 380 R, Hettich, Tuttlingen, Germany) at 5000 rpm for 20 min, the extracts were filtered using cellulose paper, and the supernatant was used for further analysis.

2.3. Measurement of the Total Phenolic Content

The total phenolic content of the extract was measured using the Folin–Ciocalteu method, as explained by Singleton et al. [34]. The Folin–Ciocalteu reagent (1:10 v/v -dilution with water) (1.5 mL) was mixed with 0.5 mL of sample extract and kept for 5 min at room temperature. A 2 mL of 7.5% sodium carbonate was added after 5 min. The mixtures were incubated with occasional shaking in the dark for 60 min. Finally, absorbance values were measured at 725 nm using a spectrophotometer (Biowave II, 80–3003-75, Biochrom LTD, Cambridge, UK). The same spectrophotometer was used to measure the total flavonoid content, FRAP, and DPPH assays. Gallic acid was used to develop the standard curve; results were expressed as milligrams of gallic acid equivalents per gram of dry weight (GAE/g of DW).

2.4. Measurement of the Total Flavonoid Content

The method explained by Park et al. [35] was used to measure the total flavonoid content of *mahewu* samples. The extract of *mahewu* samples was mixed with 0.15 mL of $NaNO_2$ (0.5 M) in a test tube, and the mixture was allowed to rest for 5 min prior to the addition of 10% $AlCl_3$ (0.6 mL). Distilled water and 2 mL of 1 M NaOH were added after 6 min and vortexed. A spectrophotometer was used to measure the absorbance values at 510 nm. The standard quercetin solution (0 to 100 mg/L) was used to develop a standard curve. The results were expressed as milligrams of quercetin equivalents per gram of dry weight (mgCE/g).

2.5. Measurement of DPPH Radiant-Scavenging Activity

The method described by Souza et al. [36] was used to measure the DPPH assay of the *mahewu* sample with minor modifications. A 2 mL of 0.2 mM ethanolic DPPH solution was transferred to 2 mL of *mahewu* extract, which was prepared with different doses and kept for 10 min in the dark. Subsequently, the mixture was vigorously shaken, and the absorbance was measured at 517 nm using a spectrophotometer. The gallic acid solution was used to produce the standard curve, and the results were expressed as a percentage of the inhibition of the DPPH radical activity.

2.6. Measurement of Ferric Reducing Antioxidant Power (FRAP)

The method described by Thaipong et al. [37] was used to measure the FRAP assay of *mahewu* extract. Approximately 100 µL of acidified methanol extract was placed in a test tube, and with methanol, the volume was adjusted to 1 mL. The mixture was added to 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide and vortexed. The mixture was left in the water bath for 20 min at 50 °C. Subsequently, 2.5 mL of 1% trichloroacetic acid was added, and the mixture was centrifuged at 6000 rpm for 10 min. Furthermore, 2.5 mL of distilled water and 0.1% ferric chloride 0.5 mL were added to the mixture, and the absorbance was measured at 700 nm using a spectrophotometer. Results were measured in milligrams of gallic acid equivalents (GAE) per gram of dry weight (mg GAE/g DW).

2.7. Color Analysis

The *mahewu* color profile was analyzed using a Hunter Lab colorflex (Reston, VA, USA) with a D65 light source and calibrated with a white and black tile. L* shows lightness, 0–100, with 0 indicating black and 100 indicating white. Values L* (Lightness/darkness), a* (redness/green), and b* (yellowness/blue) were recorded. Hue, chroma, and total color difference were calculated using the equations below:

$$\text{Hue} = \tan^{-1}(b^*/a^*)$$

$$\text{Chroma} = (a^{*2} + b^{*2})^{½}$$

$$\Delta E = (\Delta L^2 + \Delta a^2 + \Delta b^2)^{½}$$

2.8. pH Measurement

A digital pH meter (Crison instrument, Midrand, South Africa) was used to determine the pH of *mahewu* samples as described by the AOAC [38] method No. 981.12. Twenty milliliters of the sample were transferred to a 50 mL beaker, and the pH was determined. Standard buffer solutions (pH 3.0, 7.0, and 9.0) were used to standardize the electrode of the pH meter.

2.9. Viscosity Measurement

The digital display viscometer (Brookfield, Model No. RVDVE230, Middleboro, MA, USA) was used to determine the viscosity of the samples. The temperature was maintained at 28 °C, while the shear rate of 60 rpm and spindle No. 04 were kept constant. A 150 mL beaker was used, and a 100 mL sample was added to bring the sample to immersion on the spindle shaft.

2.10. Determination of the Total Sugar Content (°Brix)

The total sugar content was measured using a bench refractometer (Model MA871, Szeged, Hungary) according to the AOAC [38] method number 932.14. A sample was placed onto the screen of the refractometer (which was pretreated/cleaned with the correct procedures), and the total sugar content values in Brix were read off the scale of the refractometer.

2.11. Titratable Acids (% Lactic Acids)

The AOAC [38] method No. 942.12 was used to determine the titratable acidity (%lactic acids) of *mahewu* samples. Two grams *of mahewu* were transferred to a 100 mL conical flask, 8 mL of distilled water was added to the mark, and three drops of the Phenolphthalein indicator were added. The mixture was titrated against 0.1 N NaOH to a pink color.

The results were expressed as % lactic acids

$$\%\text{lactic acid} = \frac{N \times V \times M}{S \times 10}$$

where:
N = Normality of standard NaOH solution used in titration.
V = Volume of NaOH used for titration (mL).
M = Molecular weight of the predominant acid in the sample divided by the number of hydrogen ions in the acid molecule. The common values of M are as follows: acetic acid (vinegar) is 60; lactic acid is 90; citric acid is 64.
S = Sample size (mL).

2.12. Microbiological Characterization

A 10 mL sample was aseptically transferred to 90 mL buffered peptone water (BPW), and homogeneity was achieved by shaking vigorously for 2 min using a stomacher. To ensure appropriate 10-fold dilutions, 0.1 mL of each dilution was placed with the pour plate method.

2.12.1. Isolation of Yeasts and Molds

About 10 mL of *mahewu* sample was poured into the stomacher bag, and 90 mL of BPW was added to the same stomacher bag and homogenized for serial dilution of up to 10^{-5} and vortexed. Approximately 1 mL of the diluted *mahewu* sample was withdrawn from the stomacher bag and transferred into a 10^{-1} test tube containing 9 mL of distilled water. This process was continuously repeated to test tubes 10^{-5}, and then 1 mL from each test tube was transferred into an empty Petri dish, followed by the addition of potato dextrose agar (PDA). This was carried out in triplicates for each dilution [39]. The content was gently swirled and allowed to solidify before incubation for 5 days at room temperature (25 °C). The total numbers of colonies were estimated using a colony counter.

2.12.2. Isolation of Coliforms

The total coliform count of the *mahewu* sample was carried out according to the International Standard Organization [40]. One milliliter of each of the *mahewu* beverages was transferred to 9 mL of sterile distilled water and vigorously mixed, where 1 mL of aliquots were spread on potato dextrose agar. The set was incubated at 30 °C for 48 h. This was done in triplicates for each dilution. After incubation, different colonies were collected according to their colony shape and color, and the total number of colonies was estimated using a colony counter.

2.12.3. Isolation Total Plate Count

The total plate count of the *mahewu* beverage was performed according to the method of the International Standard Organization [41]. Ten milliliters of *mahewu* were transferred to the stomacher bag, and 90 mL of BPW was added to the same stomacher bag and homogenized for a serial dilution of up to 10^{-5} and vortexed. Approximately 1 mL of the diluted *mahewu* beverage was withdrawn from the stomacher bag into a 10^{-1} test tube with 9 mL of distilled water. This process was continuously repeated to test tubes 10^{-5}, and then 1 mL from each test tube was transferred to an empty Petri dish, followed by the addition of nutrient agar. This was carried out in triplicates for each dilution. The content was gently swirled and allowed to solidify before incubation for 48 h at 37 °C. The total number of colonies was estimated using colony counters.

2.13. Statistical Analysis

The data obtained were subjected to SPSS Software (Version 24, for Windows, IBM, Chicago, IL, USA). One-way analysis of variance was used to analyze the data and mean differences were determined by Duncan's multiple range tests at a significance level ($p < 0.05$). Results were expressed as the mean values ± the SD of three replicates. Moreover, Pearson's correlation analysis was implemented to investigate correlations between physicochemical, polyphenolic compounds, and microbiological properties.

3. Results and Discussion

3.1. Polyphenolic Compounds and Antioxidant Activity of Sorghum-Based Mahewu

Table 1 shows the polyphenol compounds and antioxidant activity of the *mahewu* samples. The results of the total phenolic content (TPC) ranged from 27.37 to 65.89 GAE/g, with commercial sorghum *mahewu* having a lower value and sorghum *mahewu* collected from traditional brewer 6 (TB6) having a higher value. However, no significant variation was observed between *mahewu* collected from traditional brewer 3 (TB3) and the commercial *mahewu* sample. The higher TPC in traditional *mahewu* sorghum samples could be due to the activities of microbial enzymes during fermentation that disintegrated the cell walls of the flour, leading to the alteration of insoluble bound polyphenols to their free or soluble form [42]. Furthermore, cooking could have increased phenolic acids in the soluble fraction due to increased solubilization of ferulic and p-coumaric acids [43]. Ferulic and p-coumaric acids in sorghum grain are distinctively ester-associated with the cell wall and are believed to influence cell wall digestibility [44,45]. Nevertheless, Ng et al. [46] observed that fermentation increases TPC. Burdette et al. [47] stated that the amount of TPC in fermented sorghum slurry/porridge ranges from 20.0 to 62.5 GAE /g, which is in line with the results of this study. The same author indicated that the variation in TPC was mainly due to the sorghum variety, the part of the grain used, and the extraction method.

Table 1. Polyphenolic compounds and the antioxidant activity of sorghum *mahewu*.

Mahewu Samples	TPC (GAE/g)	TFC (mg/g)	DPPH (%)	FRAP (GAE/g)
CM	27.37 ± 1.05 ^{a}	0.30 ± 0.013 ^{b}	49.00 ± 0.39 ^{c}	1.46 ± 0.01 ^{a}
TB 1	65.89 ± 2.42 ^{d}	0.19 ± 0.01 ^{a}	44.75 ± 0.23 ^{a}	2.36 ± 0.19 ^{c}
TB 2	53.02 ± 0.31 ^{c}	0.19 ± 0.06 ^{a}	45.02 ± 0.39 ^{a}	1.59 ± 0.01 ^{a}
TB 3	29.96 ± 1.12 ^{a}	0.20 ± 0.07 ^{a}	45.15 ± 0.23 ^{a}	1.57 ± 0.01 ^{a}
TB 4	41.17 ±1.74 ^{b}	0.19 ± 0.07 ^{a}	44.62 ± 0.01 ^{a}	1.52 ± 0.03 ^{a}
TB 5	64.13 ± 0.42 ^{d}	0.18 ± 0.01 ^{a}	45.15 ± 0.46 ^{a}	1.49 ± 0.17 ^{a}
TB 6	64.22 ± 0.29 ^{d}	0.23 ± 0.05 ^{a}	47.01 ± 0.69 ^{b}	2.18± 0.75 ^{b}

Values are shown as mean ± standard deviation of triplicates. Values with different superscript letters in a column are significantly different ($p < 0.05$). TPC = total phenolic content, TFC = total flavonoid content. CM = commercial mahewu, TB1: Traditional brewer 1, TB2: Traditional brewer 2, TB3: Traditional brewer 3, TB4: Traditional brewer 4, TB5: Traditional brewer 5, TB6: Traditional brewer 6.

In addition, the grain color might have contributed to the variation in the TPC of *mahewu* samples, as well as to the fermentation microorganisms and processing conditions [48]. The values obtained in this study are in line with those stated by Awika et al. [49] and Burdette et al. [47] on fermented sorghum slurry/porridge.

The total flavonoid content (TFC) in all traditional sorghum *mahewu* samples did not differ significantly but was lower compared to commercial sorghum *mahewu*. The TFC values ranged from 0.18 to 0.23 mg/g for traditional sorghum *mahewu* samples, and the commercial sample had a higher value of 0.30 mg/g. The high TFC value of the commercial *mahewu* sample might be attributed to higher acidic strength during fermentation, which releases and makes the binding flavonoid components bioavailable. Yaho et al. [50] observed that fermentation increases the overall flavonoid and nonflavonoid content. However, the low TFC values of traditional *mahewu* samples could be due to fractions of generated

polyphenols that could have migrated into endosperm-forming networks with macromolecules, making them less bioavailable [48,51]. Zaroug et al. [52] recorded a TFC of 0.82 mg/g after 24 h of fermentation of traditional Sudanese *kisra* prepared from two cultivars of sorghum.

The DPPH scavenging activity of the sorghum *mahewu* ranged from 44.62% to 49.00%, with commercial *mahewu* having the highest value and *mahewu* of brewer 4 (TB4) having the lowest value. Sorghum grains contain polyphenols, anthocyanins, and tannins and could be responsible for the high antioxidant activity of commercial sorghum *mahewu*. Furthermore, synergistic influences of other antioxidant components, such as melanoidins, free amino acids, and oligoproteins, might have increased the antioxidant activity of commercial *mahewu* [53]. The results of the FRAP assay showed statistical differences ($p < 0.05$) between values ranging from 1.47 to 2.36 mg GAE/g. The commercial sorghum *mahewu* sample had a lower value, while the *mahewu* of traditional brewer 1 (TB1) had a higher value. Kim et al. [54] indicated that thermal processes such as cooking increase antioxidant capacity, and the reduction of Fe^{+3}/Fe^{+2} ferric ions is increased. The synergism of polyphenols and melanoidins generated by the Maillard reaction during the cooking and fermentation process is attributed to this phenomenon. However, the higher amount of antioxidant activity observed in *mahewu* from traditional brewer 1 (TB1) could be the result of an increase in total phenolic compounds during fermentation. Sorghum grains and their products are rich sources of tannins, flavonoids, phenolic compounds, and antioxidants and, therefore, are classified as functional foods [54].

3.2. Physicochemical Characteristics of Sorghum Mahewu Samples

Table 2 shows the physicochemical characteristics of the sorghum *mahewu* samples. The pH ranged from 3.38 to 3.66, with *mahewu* from traditional brewer 1 (TB 1) having the lowest value and commercial *mahewu* having the highest value. The pH values of all *mahewu* samples were within the acceptable range of 3.4 to 4.0. The low pH values in sorghum based *mahewu* could be attributed to the presence of LAB, and the beverages were made of ingredients that have little or no possible fermentable sugars [55]. Lactic and acetic acids, glycerol, and ethanol have been identified as the main end products of *mahewu* fermentation, demonstrating that microorganism metabolism is primarily due to LAB and yeasts [23]. This makes the products sour, and the lower pH values critically act as a preservative, thus improving the shelf stability of *mahewu*. Moreover, low pH in all sorghum based *mahewu* samples is desirable, as spoilage and pathogenic microorganisms do not grow in low-pH environments [56]. Similar results were obtained for Ugandan *bushera*, a non-alcoholic beverage based on sorghum, which had a pH ranging from 3.7 to 4.5 [57].

Table 2. Physicochemical properties of sorghum *mahewu* sample.

***Mahewu* Samples**	**pH**	**Viscosity (cP) 60 RPM**	**TSS (°Brix)**	**TTA (% Lactic Acid)**
CM	3.56 ± 0.03^{c}	1297.0 ± 3.40^{e}	9.68 ± 0.12^{a}	0.86 ± 0.03^{bc}
TB 1	3.38 ± 0.01^{a}	730.0 ± 3.20^{c}	13.42 ± 0.03^{c}	1.17 ± 0.05^{e}
TB 2	3.46 ± 0.04^{a}	806.7 ± 3.51^{d}	15.47 ± 0.12^{d}	0.93 ± 0.05^{d}
TB 3	3.50 ± 0.03^{ab}	806.7 ± 3.52^{d}	12.97 ± 0.18^{b}	0.86 ± 0.01^{cd}
TB 4	3.66 ± 0.04^{d}	443.3 ± 3.49^{a}	9.92 ± 0.07^{a}	0.63 ± 0.05^{a}
TB 5	3.55 ± 0.02^{bc}	516.7 ± 3.50^{b}	17.49 ± 0.21^{e}	0.79 ± 0.04^{b}
TB 6	3.46 ± 0.01^{a}	806.7 ± 3.52^{d}	13.24 ± 0.29^{bc}	0.82 ± 0.01^{bc}

Values are shown as mean ± standard deviation of triplicates. Values with different superscript letters in a column are significantly different ($p < 0.05$). TSS = total soluble solids, TTA = total titratable acids. CM = Commercial *mahewu*, TB1: Traditional brewer 1, TB2: Traditional brewer 2, TB3: Traditional brewer 3, TB4: Traditional brewer 4, TB5: Traditional brewer 5, TB6: Traditional brewer 6.

Viscosity is a useful index for determining the amount of solids that have dissolved within liquids and also a good index of comparison between beverages produced following

indigenous knowledge and traditional methods [58]. The viscosity of the *mahewu* sorghum ranged from 444.33 to 1297.00 cP, and there were significant variations ($p < 0.05$) between samples. Commercial *mahewu* sample had the highest value, while traditional brewer 4 (TB4) *mahewu* had the lowest value.

The disparities in the viscosity of *mahewu* samples could be due to different temperatures and processing methods applied during *mahewu* production. The low viscosity observed in *mahewu* from traditional brewers 4 and 5 could be attributed to the severe boiling of the sorghum flour with water at the beginning of cooking. Initial boiling might have caused the starch granules to partially gelatinize, leading to the formation of sorghum *mahewu* with pre-gelatinized granules [59]. Thus, partially gelatinized starch granules might have decreased the viscosity of *mahewu* samples. In this context, low viscosity is advantageous since low viscous gruel is digested faster than thicker gruel and could be used as a weaning food, and a smaller amount of sorghum flour would be required to form a gel to efficiently improve nutrient density [60,61]. The results are in the range of those reported by Malleshi and Desikachar [62] of malted sorghum porridges (10% dry matter), with viscosities ranging from 310 to 1980 cP.

The results for total soluble solids (TSS) of the sorghum *mahewu* samples varied from 9.68 to 17.49 °Brix. Commercial *mahewu* had the lowest value, while traditional brewer *mahewu* (TB5) had a higher value. The accepted Brix value of non-alcoholic beverages such as *mahewu* is 8 °Brix and above [63]. Thus, the TSS of all sorghum *mahewu* samples was within the acceptable limit. Hydrolytic breakdown of complex sugars during fermentation releases simple sugars, increasing the TSS of most traditional *mahewu* samples [64]. Moreover, some traditional brewers may have added sugar during production, hence the reason for the high TSS in traditional *mahewu* samples. Sugar acts as a humectant and helps to prevent or slow the growth of bacteria, molds, and yeast. It helps improve the shelf stability of many fermented foods and is used extensively in home food fermentation due to its preserving characteristics. The results correspond to those found by Gassen [65] for *Sobia* samples (a traditional fermented beverage) in Saudi Arabia, which had a TSS value ranging from 10.50 to 15.10 °Brix.

Total titratable acidity (TTA) ranged from 0.63 to 1.17% lactic acid (v/v). The TTA of *mahewu* from traditional brewer 1 (TB1) exhibited the highest value. The TTA values of all sorghum *mahewu* samples were within the acceptable limit. For example, the Ghana Standards Board [66] requires non-alcoholic beverages produced in Ghana to have a TTA ranging from 0.5 to 1.9% calculated as anhydrous citric acid. The high acidity in the *mahewu* sample from traditional brewers 1 and 2 compared to the commercial *mahewu* indicated the high amount of organic acids present. The higher TTA in these samples could be associated with traditional processing methods since they are not standardized with respect to the type of raw material used, the equipment, the quality of the final product, and the handling procedure [67]. Moreover, severe spontaneous fermentation can also be attributed to a higher TTA since it increases the amount of organic acids. The importance of TTA in beverages such as *mahewu* is related to its ability to alter the taste and flavor profile of such products [68]. Acidity and pH act as a predominant antimicrobial hurdle in beverages, and as such, the percentage of lactic acids becomes as crucial as the pH in the safety of traditional beverages. However, the quantity is so difficult to control and maintain [69]. The results are in line with those obtained by Lyumugabe [56], which show that the typical TTA of traditional beverages ranges from 0.5 to 2.0% depending on the storage conditions and the ingredients used to produce such beverages. Simango [70] also obtained similar results in which *mahewu* produced in Zimbabwe had a TTA of 0.9% after 48 h of fermentation.

3.3. Color Properties of Sorghum Mahewu Samples

Color is an essential quality characteristic that affects consumer judgment on the taste and perception of food and is one of the important factors dictating consumer preference for food. Color indices (L*, a*, b*, Chroma, Hue, and ΔE) showed significant differences between the sorghum *mahewu* samples, as shown in Table 3. The L* (lightness) values of the

mahewu samples, which are indicative of the lightness or darkness of the product, ranged between 53.19 and 63.92. *Mahewu* samples collected from traditional brewers 1, 2, 5, and 6 had lower lightness than commercial *mahewu*, indicating that they had darker colors. The boiling of the sorghum flour with water during cooking might have resulted in the generation of non-enzymatic browning products, thus decreasing the lightness of *mahewu* samples. However, the development of color in cooked products is due to the synergistic effect between the moisture uptake of flour and the gelatinization of starch that leads to the Maillard browning reaction [71,72]. A higher amount of reducing sugars greatly favors the formation of brown color in the Maillard reaction [73], and therefore, a higher °Brix resulted in greater color development. In this context, the *mahewu* sample from traditional brewer 5 (TB5) complied with this trend because it had a higher °Brix, manifested a darker color, and had the least values of L* (lightness). High-temperature treatment increases the brown color of cereal products due to Maillard reactions [74]. On the other hand, the higher value of L* (lightness) indicated a lighter color for *mahewu* from traditional brewer 3 (63.92), and this sample had a more appealing light-brown color. The high value of L* might be attributed to fermentation, which reduced the viscosity of the *mahewu*, making it lighter. Mashau et al. [31] obtained similar results of the *mahewu* measurements for the value of L* (lightness), which ranged from 54.91 to 61.14 on day 0 for *mahewu* added with aloe vera powder.

Table 3. Color profile of sorghum-based *mahewu*.

***Mahewu* Samples**	**L***	**a***	**b***	**Chroma**	**Hue**	**ΔE**
CM	59.35 ± 0.04^{e}	9.06 ± 0.02^{d}	15.29 ± 0.05^{d}	17.78 ± 0.05^{e}	59.36 ± 0.06^{d}	-
TB 1	55.46 ± 0.03^{c}	10.84 ± 0.07^{e}	16.35 ± 0.10^{e}	19.61 ± 0.05^{f}	56.46 ± 0.32^{a}	31.93 ± 0.06^{f}
TB 2	58.60 ± 0.01^{d}	7.90 ± 0.01^{c}	13.90 ± 0.04^{c}	15.99 ± 0.04^{d}	60.38 ± 0.02^{e}	30.55 ± 0.06^{e}
TB 3	63.92 ± 0.04^{g}	7.25 ± 0.01^{a}	13.04 ± 0.03^{b}	14.92 ± 0.02^{a}	60.92 ± 0.09^{f}	27.47 ± 0.05^{c}
TB 4	63.27 ± 0.07^{f}	7.52 ± 0.04^{b}	13.92 ± 0.02^{c}	15.82 ± 0.03^{c}	61.62 ± 0.14^{g}	26.97 ± 0.04^{b}
TB 5	53.19 ± 0.04^{a}	7.96 ± 0.03^{c}	12.70 ± 0.01^{a}	14.99 ± 0.02^{a}	57.97 ± 0.12^{b}	28.46 ± 0.01^{d}
TB 6	54.45 ± 0.20^{b}	7.91 ± 0.07^{c}	13.07 ± 0.06^{b}	15.28 ± 0.07^{b}	58.83 ± 0.12^{c}	25.812 ± 0.07^{a}

Values are shown as mean ± standard deviation of triplicates. Values with different superscript letters in a column are significantly different ($p < 0.05$). CM = Commercial *mahewu*, TM = Traditional *mahewu*, TB1: Traditional brewer 1, TB2: Traditional brewer 2, TB3: Traditional brewer 3, TB4: Traditional brewer 4, TB5: Traditional brewer 5, TB6: Traditional brewer 6.

The redness (a* value) ranged from 7.3 to 10.8. *Mahewu* from traditional brewer 3 (TB3) exhibited a lower value, whereas *mahewu* from traditional brewer 1 (TB1) showed a higher value. The high redness value of TB1 and commercial *mahewu* in comparison to other samples might be attributed to differences in heat processing, as some pigments might have leached out into the endosperm during cooking. Duodu et al. [75] indicated that this phenomenon occurs in cereal grains containing pigmented testa. The yellowness (b* value) ranged from 12.71 to 16.35. The higher value of b* (yellowness) (16.35) was observed in the *mahewu* of traditional brewer 1 (TB1). The higher b* value was due to the disintegration of carbohydrates during fermentation because the predominant carbohydrate in fermented *mahewu* is starch. This gives *mahewu* a bright color because of the brownish nature of sorghum flour, which allows other hues to emerge, resulting in a higher level of yellowness. Furthermore, the availability of oxidized polyphenols from enzymatic oxidation by polyphenol oxidase in the flour was probably the reason for the high value of b* in the TB1 sample. Similar data was obtained by Onyango et al. [76], where *uji* produced from fermented maize–finger millet had a higher yellowness.

Chroma values varied from 14.92 to 19.61, with *mahewu* from traditional brewer 1 (TB1) having the highest value and *mahewu* from traditional brewer 3 (TB3) having the lowest value. Sample TB1 generally showed a much more yellow hue because it had a higher value of b* compared to the other *mahewu* samples due to the intrinsic brown color of sorghum flour. The hue angle (h°) of the sorghum *mahewu* samples ranged between 56.46° and 61.62°. The total color difference (ΔE) showed significant differences among all

traditional *mahewu* samples ($p < 0.05$), with values ranging from 56.46 to 61.62. The samples were different in their visual appearance due to the variations in ΔE among them. Variation in ΔE usually occurs during the fermentation process, when the sugar available in the gruel is converted to carbon dioxide and ethanol by yeast. This results in the production of leaving capacity that contributes to the yellowness and redness of the gruel [64].

3.4. Microbiological Properties of Sorghum Mahewu

Table 4 shows the results of the microbiological properties of the sorghum *mahewu* samples. The coliform counts ranged from 3.68 to 5.96 $\log_{10}$ (cfu/mL), with *mahewu* from traditional brewer 5 (TB5) having the highest count. The presence of coliforms in traditional *mahewu* samples is related to poor hygiene practices since they were produced at home, usually in an unhygienic environment. These products may have been contaminated by microorganisms in raw materials and utensils [77]. In this context, the microflora of these *mahewu* samples depended on how the raw material and ingredients were prepared, processed, handled, and stored. Coliforms were used as indicator organisms of basic hygiene during *mahewu* processing, as well as packaging materials handling [32].

Table 4. Microbiological composition of sorghum *mahewu* samples.

***Mahewu* Samples**	**Total Coliform $\log_{10}$ (cfu/mL)**	**Yeast and Mould $\log_{10}$ (cfu/mL)**	**Total Plate Count $\log_{10}$ (cfu/mL)**
CM	ND	ND	8.95 ± 0.25^{bcd}
TB 1	5.96 ± 0.60^{c}	8.97 ± 0.65^{b}	8.96 ± 0.55^{cd}
TB 2	3.68 ± 0.20^{a}	7.99 ± 0.25^{a}	8.97 ± 0.45^{d}
TB 3	ND	7.94 ± 0.50^{a}	8.97 ± 0.40^{cd}
TB 4	ND	8.98 ± 0.40^{b}	7.91 ± 0.30^{a}
TB 5	ND	8.95 ± 0.25^{b}	8.92 ± 0.35^{ab}
TB 6	3.93 ± 0.60^{b}	8.95 ± 0.40^{b}	8.94 ± 0.30^{abc}

Values are shown as $\log_{10}$ (cfu/mL) ± standard deviation of triplicates. Values with different superscript letters in a column are significantly different ($p < 0.05$). ND = not detected. CM = Commercial *mahewu*, TB1: Traditional brewer 1, TB2: Traditional brewer 2, TB3: Traditional brewer 3, TB4: Traditional brewer 4, TB5: Traditional brewer 5, TB6: Traditional brewer 6.

The yeast and mold count of *mahewu* sorghum was within the specifications (that is, plates should have between 20 and 120 visible colonies formed at counting), ranging from 7.95 $\log_{10}$ (cfu/mL) to 8.99 $\log_{10}$ (cfu/mL). *Mahewu* of traditional brewer 2 (TB2) had the highest value, while *mahewu* of traditional brewer 3 (TB3) had the lowest value. There was no isolation of yeast and molds in commercial *mahewu* samples. The most common yeast that is the possible strain detected from fermented products is *Saccharomyces cerevisiae*, which is one of the most studied natural fermenting agents for alcoholic and non-alcoholic beverages [78]. *Saccharomyces cerevisiae* is described as the main fermenting microorganisms in foods such as *ogi* [78], *kisra* [79], and *hussuwa* [80]. The results are in line with those found by Banwo et al. [81] on the fermentation of sorghum-*ogi*, which had an average of 8.0 $\log_{10}$ cfu/mL enumerated in all samples after 72 h of fermentation.

Total plate counts of sorghum *mahewu* samples varied from 7.914 to 8.978 $\log_{10}$ (cfu/mL). According to the requirements specified by the Uganda Standard, fermented (non-alcoholic) cereal beverages must conform to maximum limits of 100 (cfu/mL) for total bacterial counts. The results show that *mahewu* is safe for human consumption. *The mahewu* sample from traditional brewer 2 (TB2) had the highest value of total plate count, and this could be attributed to the natural microflora of spontaneous fermentations being disorderly, uncertain, and unsuitable [82]. On the other hand, the low total plate count for *mahewu* from traditional brewer 4 (TB4) could be attributed to the acidic nature of fermented sorghum *mahewu*. The acidic nature was not favorable for the rapid multiplication of spoilage microorganisms [83]. The value obtained in this study corresponds to the results of the total plate count in *motoho*, a traditional fermented non-alcoholic beverage produced by the Sotho people. The total plate count varied from 7.9 to 8.3 $\log_{10}$ cfu/mL and also in

the range of 6.36 and 8.20 $\log_{10}$ cfu/mL of *oshikundu,* which is below the limit of not safe for drinking [84].

3.5. Pearson Correlation Analysis

The results of the Pearson correlations are presented in Table 5. Viscosity and total phenolic content had the highest correlation (r = 0.789; $p < 0.01$), while viscosity and yeast had the lowest correlation (r = −0.841; $p < 0.01$). All correlations among the response variables were significant at $p < 0.01$. pH had a strong negative correlation with coliforms (r = −0.630; $p < 0.01$), TTA (r = −0.641; $p < 0.01$), and had a weak correlation with viscosity (r = −0.276; $p < 0.01$) and yeast (r = −0.178; $p < 0.01$) but had a positive correlation with total flavonoids content (r = 0.129; $p < 0.01$). However, TTA had a strong positive correlation with coliforms (r = 0.0637; $p < 0.01$) but a negative correlation with pH (r = −0.641; $p < 0.01$). This means that acidity increases throughout fermentation, thus decreasing the pH values. This is in line with the literature, which explains that a rise in TTA decreases the pH value since high acids have a pH lower than 4.6.

Table 5. Pearson linear correlation among the physicochemical, polyphenols compounds, and microbiological properties.

	pH	TTA	Brix	Viscosity	TFC	TPC	Coliform	Yeast	Plate Count
pH	1	−0.641	0.483	−0.276	**0.129**	−0.387	−0.659	−0.178	−0.630
TTA	−0.641	1	**0.253**	0.280	0.009	0.339	**0.637**	0.049	**0.582**
Brix	−0.483	0.253	1	−0.391	−0.492	−0.485	0.196	0.528	0.126
Viscosity	−0.276	0.280	−0.391	1	0.789	0.659	0.010	−0.841	0.406
TFC	**0.129**	0.009	−0.492	**0.789**	1	−0.450	−0.126	−0.805	0.072
TPC	−0.387	0.339	**0.659**	−0.485	−0.450	1	0.695	**0.573**	−0.125
Coliform	−0.659	**0.637**	0.196	0.010	−0.126	**0.695**	1	0.336	0.262
Yeast	−0.178	0.049	0.528	−0.841	−0.805	0.573	0.336	1	0.031
Plate count	−0.630	0.582	0.126	0.406	0.072	−0.125	0.262	0.031	1

TTA = total titratable acid, TFC = total flavonoid content, TPC = total phenol content. Bolded values show strong correlation.

It could be seen that TTA and coliforms were profoundly affected by the changes that occurred at pH because of the strong negative correlations with pH. There was a positive correlation between TTA and Brix (r = 0.253; $p < 0.01$). This was indirectly influenced by titratable acids.

4. Conclusions

The results show that traditional *mahewu* samples are a rich source of health-promoting compounds such as phenolic compounds, although they have a low flavonoid content compared to the commercial *mahewu* sample. They are also rich in antioxidant activity, such as FRAP, while they are low in DPPH. The low pH observed in traditional *mahewu* samples is advantageous because they will be expected to have a longer shelf life during storage. The high total soluble solids in traditional *mahewu* make the product taste better than commercial *mahewu*. Nevertheless, the traditional *mahewu* samples had a darker color than the commercial ones. The results of the microbiological properties of traditional *mahewu* were within the acceptable limits expected for a non-alcoholic beverage. In conclusion, the findings of this study demonstrated that traditional brewers are meeting the requirements for the commercialization of their products.

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