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Nutritional and Phytochemical Composition of *Mahewu* (a Southern African Fermented Food Product) Derived from White and Yellow Maize (*Zea mays*) with Different Inocula

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Abstract: *Mahweu* is an important indigenous beverage for many low-income and undernourished consumers in southern Africa. As a result, the nutritional and phytochemical profile of *mahewu* samples (obtained using optimized fermentation and boiling conditions from a previous study) as well as their related raw materials (white and yellow maize) were investigated. At these conditions, white and yellow maize *mahewu* (WM and YM) were prepared utilizing various inocula including sorghum malt, wheat, millet malt, or maize malt, and the pH, titratable acidity (TTA), total soluble solid (TSS), and proximate analysis were determined. The mineral content, amino acid composition, and phenolic compound profile were also investigated using inductive coupled plasma optical emission spectrometry (ICP-OES), high-performance liquid chromatography (HPLC), and ultra-high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UHPLC/Q-TOF-MS), respectively. Fermentation was observed to have influenced the proximate composition of obtained *mahewu* samples compared to the raw flour with significant ($p \leq 0.05$) improvement in protein from 8.59 to 9.7% (YM) and 8.78 to 9% (WM) as well as carbohydrate from 72.27 to 74.47% (YM) and 71.15 to 72.65% (WM). Sodium, magnesium, phosphorous, potassium, calcium, manganese, iron, copper, and zinc were the minerals detected in the *mahewu* samples, while potassium was the most abundant mineral, having values ranging from 3051.61 to 3283.38 mg/kg (YM) and 2882.11 to 3129.97 mg/kg (WM). Heavy metals detected in this study were all below the recommended tolerable levels by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Arginine and leucine with values ranging from 0.47 to 0.52 g/100 g (YM) and 0.48 to 0.53 g/100 g (WM) as well as 0.91 to 1.04 g/100 g (YM) and 0.95 to 1.01 g/100 g (WM), respectively, were the most abundant essential amino acids, whereas for non-essential amino acids, glutamic acid, aspartic acid, alanine, and proline were observed to be abundant. Based on the different inocula, the derived *mahewu* samples prepared using either white or yellow maize have varying nutritional and health beneficial components and the choice of inocula might still be determined by consumer preference.

Keywords: fermentation; proximate analysis; chromatography; maize; *mahewu*

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1. Introduction

Worldwide, the consumption of food that promotes health has compelled food manufacturers to meet the increasing demand of consumers for nutritional foods. Maize (*Zea mays*) is one of the most important food crops in the world and is processed into various types of products such as corn flour, cornmeal, and various types of breakfast cereal [1]. Several studies have shown the beneficial health effects of maize consumption including their protective role against obesity, diabetes, cardiovascular diseases, and cancers [2–4]. Maize is the basic ingredient in a fermented soft porridge known as *mahewu*. *Mahewu* is a non-alcoholic fermented beverage mostly consumed as a morning meal or refreshing drink

in southern Africa and several Arabian Gulf nations [5,6]. A mixture of maize flour, wheat flour, cassava, or grain malt (or other types of inocula) is typically used in the traditional preparation of *mahewu* [7,8].

A lack of access to advanced machinery and technologies in developing countries has contributed to the continued use of fermentation and malting as processing techniques [9]. These processing techniques have been used traditionally to produce indigenous foods with known health, sensory, and nutritional benefits [10]. Interestingly, science has proven that fermentation and malting improve the bio-accessibility of nutrients and lower antinutritional factors [9,11].

Fermented foods provide a low-cost supply of minerals and amino acids (AA) such as lysine, methionine, and tryptophan in significant amounts [12]. The importance of essential amino acids (EAA) is well-established, and because they cannot be synthesized in the human body, their intake through diet is vital. Non-essential amino acids (NEAA) are equally important, although these can be formed in the body. Minerals provide essential nutrients required by humans to sustain life. Phenolic acid and flavonoids are highly distributed phenolic compounds in maize [13]. Maize is also a significant source of carbohydrates, vitamins, fat, fiber, and minerals [14]. Studies have highlighted the potential of maize and derived products in mitigating health problems [15,16].

Different inocula are used during the preparation of *mahewu* such as sorghum malt, wheat, millet malt, or maize malt. Subsequent variations in these inoculum influences the final composition of the product. Therefore, this study investigated the nutritional and phytochemical profiles of *mahewu* samples obtained using different maize sources (yellow and white) and their varying inocula (sorghum malt, wheat, millet malt, or maize malt).

2. Materials and Methods

2.1. Sample Collection and Preparation

Raw materials of white maize (*Zea mays* L.) (RW), yellow maize (RY), finger millet (*Eleusine coracana*), wheat (*Triticum*), and sorghum (*Sorghum bicolor* L.) were sorted and cleaned. Afterward, the sorghum, finger millet, RW, and RY were malted and milled (Perten Laboratory Mill 3310 Instruments AB, Helsinki, Finland) into fine flour according to the procedure described in a previous study [17]. The sorghum malt (SM), wheat (W), millet malt (MM), RW malt, RY malt, RW, and RY flours were subsequently used in preparing the *mahewu* samples.

2.2. Preparation of Mahewu

Mahewu samples were prepared according to the optimized method described by Daji et al. [17]. Briefly, 1000 mL of distilled water was boiled at 90 °C in an aluminum pot. Maize flour (70 g) was poured into the boiling water with occasional stirring to avoid lump formation. The slurry was allowed to boil for 19 min for white maize *mahewu* with fermentation at 25 °C for 54 h, and a boiling time of 13 min for the yellow maize *mahewu* with fermentation at 29 °C for 72 h, and then the porridge was cooled to 25 ± 2 °C. Next, the eight varieties of *mahewu* products produced with either white maize flour (RW) or yellow maize flour (RY) were inoculated with 5% of either sorghum malt flour (SM), wheat flour (W), malted millet flour (MM), malted white maize flour (MWM), and malted yellow maize flour (MYM) to form a slurry and the eight varieties of *mahewu* included (i) WM *mahewu* with SM (WSG); (ii) WM *mahewu* with wheat (WW); (iii) WM *mahewu* with MM (WM); (iv) WM *mahewu* with MWM (WMW); (v) YM *mahewu* with SM (YMSG); (vi) YM *mahewu* with wheat (YW); (vii) YM *mahewu* with MM (YMM); and (viii) YM *mahewu* with MYM (YMY).

2.3. Measurement of pH, Titratable Acidity (TTA), and Total Soluble Solids (TSS) in Mahewu Samples

After fermentation, the pH, TTA, and TSS were analyzed upon the collection of each sample. The pH value for each derived *mahewu* product was recorded using a pH meter

(Hand-Held EcoSense pH10A pen Tester, Beijing, China). At the same time, TTA analysis was carried out following the method outlined by Qaku et al. [18]. Briefly, an aqueous extract of each *mahewu* sample (2 g) dissolved in distilled water (20 mL) was titrated against 0.1 N NaOH. Phenolphthalein was used as an indicator by continuously stirring the substance while waiting for the first noticeable change to a pink color. Results obtained were expressed in percentage (%). The TSS content was performed using a refractometer (Hanna H196801 Woonsocket, RI, USA), and the results were expressed as °Brix. Following this, samples were freeze-dried for 24 h at −55 °C under vacuum (Telstar LyoQuest, Labotec, Midrand, South Africa) and subsequently used for analysis.

2.4. Proximate Analysis

Determination of proximate compositions such as ash, crude fiber, fat, and moisture content of the freeze dried *mahewu* samples was carried out according to the Association of Official Analytical Chemist (AOAC) procedure [19] and the 99.30 method for crude protein [20]. Total carbohydrates were determined by subtracting ash, crude protein, crude fiber, fat, and moisture content from the total percentage.

2.5. Determination of Mineral Content Using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES/MS)

The mineral content of raw maize flours (RW and RY) and the obtained *mahewu* samples were determined using a modified method from Biata et al. [21]. Briefly, 1 g of each sample and related raw materials were weighted separately and 10 mL concentrated nitric acid was added in the samples in a digestion vessel to form a solution and heated in a fume cupboard until the desired color changes. Thereafter, the mixture was allowed to cool, filtered (using a 0.45 µm syringe filter), and made up to 100 mL with deionized water prior to analysis. The ICP-OES standard solution of sodium (Na), magnesium (Mn), phosphorous (P), potassium (K), calcium (Ca), chromium (Cr), manganese (Mn), iron (Fe), cobalt (Co), nickel (Ni), copper (Cu), Zinc (Zn), and molybdenum (Mo) were used as the working standard solutions. Samples were examined by ICP-OES (Spectro ARCOS, Spectro Analytical Instruments, Kleve, Germany) under the operation specifications of coolant gas flow = 12 L/min; purge gas flow = boost and nebulizer type = cross flow; auxiliary gas flow = 0.5 L/min; nebulizer gas flow = 0.75 L/min; and RF power (emission intensity) = 1150 W. All experiments were conducted in triplicate.

2.6. Amino Acid Composition

The amino acids of RW, RY, and differently prepared *mahewu* samples were separated by HPLC at the Agricultural Research Council (ARC)-Irene Analytical Research Laboratory, Pretoria, South Africa, using a similar approach to Adebiyi et al. [22]. α-Amino-β-guanidino propionic acid and 6 N HCl were used for the hydrolysis of the *mahewu* samples (7 g) at 1150 °C for 24 h, and the resulting solution was cooled to ambient temperature. Thereafter, the hydrolysate was centrifuged (3000 rpm) for 600 s and the supernatant was filtered (0.45 µm). The dried supernatant was derivatized with borate buffer and 9-flourenylmethyl chloroformate reagent (FMOC). The derivatized solution was then analyzed on the HPLC system equipped with a fluorescence detector (Perkin-Elmer LS-4, Shimadzu RF-530 (excitation and emission wavelengths of 260 and 313 nm, respectively) and Schoeffel FS 970); after this, the mixture was extracted with pentane. In the mobile phase, the HPLC grade was varied linearly to acetic acid:acetonitrile (50:50, v/v) for over 1 h. The eluents were acetonitrile, methanol (HPLC grade), and acetic acid (10:40:50, $v/v/v$). The initial temperature and gradient flow rate of the column was set for 180 min at 40 °C and 1.3 mL/min. The experimental conditions were adjusted to 2 mL/min for 30 s after the gradient. The calibration curves were used to obtain the concentration of AAs in RW, RY, and *mahewu* samples using amino acid standards purchased from Sigma Aldrich, (St Louis, MO, USA).

2.7. Identification of Phenolic Compounds in Raw Maize Flour (RW and RY) and Mahewu Samples Using UHPLC/Q-TOF-MS

Extraction and quantification of the phenolic compounds from the RW, RY, and *mahewu* samples were carried out at the Central Analytical Facility of Stellenbosch University, South Africa, according to the procedure of Adebiyi et al. [22] with slight modifications. For each sample, the raw materials and milled freeze-dried samples (2 g) were mixed with 20 mL of 1% formic acid in 80% methanol and shaken for 10 min using a cleansing ultrasonic bath (Integral system Ultrasonic Bath UMC 5). The homogenates were then centrifuged (Eppendorf 5702R Merck, Johannesburg, South Africa) at 3000 rpm for 10 min at 4 °C. Thereafter, the supernatant was dried to approximately 1 mL and further dried to completeness in a fume cabinet for 48 h at 25 °C. The desiccated extracts were recovered in dimethyl sulfoxide and methanol (50:50 v/v) filtered through a 0.2 µm syringe filter before untargeted analysis.

Subsequently, 2 µL of extract was injected into the Acquity ultra performance liquid chromatography (UPLC) system (Water, Milford, MA, USA) and separated within. The equipment was equipped with a Waters Synapt G2 Quadrupole time-of-flight (QTOF) mass spectrometer (MS) and utilized for UHPLC analysis. The following parameters were used: mobile phase consisting of a solution of 0.1% formic acid and acetonitrile containing 0.1% formic acid (v/v). The initial gradient was 100% formic acid and lasted for 1 min, but later changed to 28% acetonitrile containing 0.1% formic acid for over 20 min in a linear mode. The 100% acetonitrile containing formic acid was introduced at this point to change the chromatographic condition for 1 min, which then went to 40% of acetonitrile containing 0.1% formic acid for more than 45 s with a wash step of 1.5 min. Thereafter, a stability in column temperature at 55 °C and conditions of 4 min inflated at a constant run speed of 0.3 mL/min were maintained.

The operating conditions for the mass spectrometer were a temperature of 275 °C for the electron ionization (EI) in negative mode and desolvation gas at 650 L/h for a cone voltage of 15 V. The experimental data were achieved by scanning the resolution and MS^E mode from the mass to charge ratio (m/z) of 150–1500. One mass spectra data channel was carried out at a low pileup energy (4 V) and the other was conducted at a higher pileup energy of ramp (40–100 V) at the MS^E mode to generate the fragmentation data. Sodium formate was used to calibrate the instrument, and the separation process was attained on a water HSS T3, 2.1 × 100 mm, 1.7 µm column. The reference mass used for the precise determination was leucine enkephalin. The compounds were identified using UV spectral, pure standards, and mass spectra in negative ionization conditions in tandem (Chemspider, Metlin, scientific literature, and KNApSAcK databases).

2.8. Statistical Analysis

All experiments were performed in triplicate. Analysis of variance (ANOVA) software (version 22, SPSS statistical software (SPSS/IBM, Chicago, IL, USA) was used to determine the differences in mean values. The findings were presented as mean ± standard deviation, with $p \leq 0.05$ set as the statistical level of significance.

3. Results

3.1. The pH, Titratable Acidity (TTA), and Total Soluble Solids (TSS)

This study was carried out to determine how various preparations of *mahewu* using different inocula affected their nutritional value. The pH, TTA, and TSS (Table 1) were determined. Results derived from the processing of these eight fermented *mahewu* products using the optimized conditions [17] indicated that all samples showed a decrease in pH after fermentation (AF) from approximately 6.39 to 3.41 for WM and 6.41 to 3.44 for YM (Table 1). The decrease in pH values could be attributed to the presence of different microorganisms and the capabilities of these microorganisms to produce a high number of organic acids. The low pH values obtained in this study were a positive outcome, as most bacteria including pathogenic microorganisms, struggle to grow at low pH values and

this provides an extension of the shelf life for these *mahewu* products as well as microbial safety [23].

Table 1. Experimental values obtained for white and yellow maize *mahewu*.

WM	pH		TTA		TSS	
	BF	AF	BF	AF	BF	AF
SM	5.96 ^{bc} ± 0.01	3.54 ^{b} ± 0.01	0.20 ^{b} ± 0	0.60 ^{c} ± 0	5.20 ^{a} ± 0.21	4.67 ^{a} ± 0.21
W	6.39 ^{d} ± 0.01	4.51 ^{d} ± 0.01	0.15 ^{a} ± 0.03	0.47 ^{a} ± 0.06	5.90 ^{d} ± 0.31	5.17 ^{d} ± 0.31
MM	5.90 ^{a} ± 0.02	3.41 ^{a} ± 0.02	0.20 ^{b} ± 0	0.68 ^{d} ± 0.03	5.61 ^{c} ± 0.15	5.03 ^{c} ± 0.15
MWM	5.95 ^{b} ± 0.01	3.63 ^{c} ± 0.01	0.20 ^{b} ± 0	0.57 ^{b} ± 0.03	5.50 ^{b} ± 0.17	4.70 ^{b} ± 0.17
YM						
SM	5.97 ^{bc} ± 0.02	3.47 ^{b} ± 0.02	0.20 ^{b} ± 0	0.60 ^{c} ± 0	5.90 ^{d} ± 0.12	5.17 ^{c} ± 0.15
W	6.41 ^{d} ± 0.01	4.65 ^{c} ± 0.01	0.15 ^{a} ± 0.03	0.38 ^{a} ± 0.03	5.50 ^{b} ± 0.12	4.93 ^{b} ± 0.12
MM	5.96 ^{b} ± 0.01	3.49 ^{b} ± 0.01	0.20 ^{a} ± 0	0.57 ^{b} ± 0.03	5.61 ^{c} ± 0.01	5.53 ^{d} ± 0.12
MYM	5.91 ^{a} ± 0.01	3.44 ^{a} ± 0.01	0.20 ^{b} ± 0	0.62 ^{d} ± 0.03	5.20 ^{a} ± 0.15	4.80 ^{a} ± 0.36

WM—white *mahewu*, SM—sorghum malt, W—wheat, MM—millet malt, MWM—malted white maize, YM—yellow *mahewu*, MYM—malted yellow maize, TTA—titratable acidity, TSS—total soluble solid, BF—before fermentation, AF—after fermentation. Each value is a mean ± standard deviation of triplicate samples. Letters in superscripts within a column show a significant difference among the differently prepared *mahewu* samples.

There was a significant increase in the TTA ($p < 0.05$) after fermentation for all *mahewu* products from approximately 0.15 to 0.68% for WM and 0.15 to 0.62% for YM (Table 1), resulting in the gradual decrease in pH. Titratable acidity was used as a guide to confirm how acidic the product would taste. The increases in TTA might have resulted from the ability of fermentative microorganism to produce acid, so that sugars the available within the substrates were broken down by lactic acid bacteria to produce organic acid. Organic acids produced during fermentation are known to affect the acidity, pH, and flavor of fermented foods [12]. Organic acids are essential for flavor development and maintaining the good quality of a product [12]. An early increase in TTA is important to avoid the multiplication of harmful microorganisms that result in bad fermentation. These results are consistent with those reported previously by other researchers working with *mahewu* or similar products [5–7,24–26]. A generally significant decrease in TSS ($p < 0.05$) in all *mahewu* products was observed (Table 1). This is due to the presence of fermenting microorganisms in the *mahewu* products, which translates to the rapid use of readily available solids [27].

3.2. Proximate Composition of Maize Flour (Yellow and White Maize) and Derived Mahewu Products

The macronutrients of the *mahewu* samples and the white raw maize and yellow maize flours are presented in Table 2. A reduction in the ash content of the *mahewu* samples compared to raw maize flours was observed. This observation is similar to the report by Fadahunsi et al. [5] regarding the production of *mahewu* and the findings of Adebiyi et al. [9] in fermented and malted pearl millet flours. This reduction in the ash content of the obtained *mahewu* samples compared to raw maize flours may be due to soluble inorganic salts being leached out during some processing phases such as steeping and boiling [18]. The protein content in the obtained *mahewu* samples was higher than in the raw maize flours, with the exception of white maize *mahewu* prepared with sorghum malt. The increase in the protein content during fermentation could be due to the utilization of maize starch for metabolic activity [28]. The observed reduction in protein content of white maize *mahewu* with sorghum malt could be linked to the metabolic activities of lactic acid bacteria (LAB) during fermentation [29,30].

Table 2. Proximate composition of *mahewu* prepared with white and yellow maize.

Nutrients	YMSG	YW	YMM	YMY	RY	WSG	WW	WM	WMW	RW
Moisture	$4.57^{cd} \pm 0.03$	$2.38^{a} \pm 0.35$	$3.66^{b} \pm 0.45$	$2.64^{a} \pm 0.14$	$6.68^{e} \pm 0.27$	$4.11^{bc} \pm 0.22$	$4.18^{bc} \pm 0.64$	$4.48^{cd} \pm 0.33$	$5.01^{d} \pm 0.06$	$8.65^{f} \pm 0.27$
Ash	$1.89^{a} \pm 0.19$	$1.86^{a} \pm 0.17$	$2.02^{a} \pm 0.37$	$2.0^{a} \pm 0$	$2.62^{b} \pm 0.65$	$2.0^{a} \pm 0$	$2.0^{a} \pm 0$	$2.14^{ab} \pm 0.02$	$2.15^{ab} \pm 0.01$	$4.43^{c} \pm 0.44$
Protein	$8.64^{bc} \pm 0.14$	$9.7^{e} \pm 0.09$	$8.68^{bc} \pm 0.10$	$8.59^{b} \pm 0.13$	$8.19^{a} \pm 0.08$	$8.75^{bc} \pm 0.05$	$8.78^{c} \pm 0.06$	$8.97^{d} \pm 0.10$	$9^{d} \pm 0.06$	$8.78^{c} \pm 0.11$
Fiber	$10.98^{abcd} \pm 0.28$	$10.69^{ab} \pm 0.10$	$10.77^{abc} \pm 0.35$	$10.64^{a} \pm 0$	$11.09^{cd} \pm 0.01$	$10.82^{abc} \pm 0.04$	$10.79^{abc} \pm 0.11$	$10.87^{abc} \pm 0.23$	$11.03^{bcd} \pm 0.01$	$11.30^{d} \pm 0.29$
Fat	$1.66^{a} \pm 0.01$	$1.65^{a} \pm 0.01$	$1.55^{a} \pm 0.19$	$1.66^{a} \pm 0.01$	$2.32^{b} \pm 0.01$	$1.66^{a} \pm 0.01$	$1.66^{a} \pm 0.01$	$1.52^{a} \pm 0.25$	$1.66^{a} \pm 0.01$	$2.20^{b} \pm 0.18$
Carbohydrate	$72.27^{d} \pm 0.06$	$73.95^{fg} \pm 0.23$	$73.31^{ef} \pm 0.90$	$74.47^{g} \pm 0.14$	$69.10^{b} \pm 0.72$	$72.65^{de} \pm 0.21$	$72.59^{de} \pm 0.80$	$72.02^{d} \pm 0.29$	$71.15^{c} \pm 0.09$	$64.64^{a} \pm 0.61$

YMSG—yellow maize *mahewu* with sorghum malt; YW—yellow maize *mahewu* with wheat; YMM—yellow maize *mahewu* with millet; YMY—yellow maize *mahewu* with malted yellow maize; RY—Raw yellow maize flour; WSG—white maize *mahewu* with sorghum malt; WW—white maize *mahewu* with wheat; WM—white maize *mahewu* with millet malt; WMW—white maize *mahewu* with malted white maize; RW—raw white maize flour. Values indicate mean and standard deviation. Each value is a mean ± standard deviation of triplicate samples (n = 3). Letters in superscripts within a column show a significant difference.

The fiber content in the *mahewu* samples was slightly lower compared to that of the raw maize flours. This concurs with the study of Fadahunsi et al. [5], who found a decrease in the fiber content of *mahewu* samples compared to that of raw maize. In addition, Tou et al. [31] reported a decrease in the fiber content of a fermented millet-based gruel called *ben-saalga*; likewise, Ogodo et al. [32] reported a similar decrease during the fermentation of maize. The decrease in fat content suggests the utilization of oxidized lipids by the fermenting microorganisms to generate energy for the growth and cellular activities [5]. A reduction in fat content was also observed by Assohoun et al. [28] in maize dough during *doklu* fermentation. Fat in food is known to positively improve mouth feel. However, due to lipid oxidation, products with high fat content are more likely to have a shorter shelf life [18]. The major chemical component of the *mahewu* samples and the maize flour is carbohydrate. When comparing the *mahewu* samples with the raw maize flours, the *mahewu* samples had the highest carbohydrate content (Table 2). Both the white and yellow maize *mahewu* samples had higher carbohydrate levels. The yellow maize *mahewu* prepared with malted yellow maize was the most abundant carbohydrate compared to the white maize *mahewu* prepared with either sorghum malt, wheat, or millet malt. Previous studies have similarly reported carbohydrate as the major nutrient in *mahewu* samples [5,33,34].

3.3. Mineral Composition of Raw Yellow and White Maize Flour as Well as Derived Mahewu Products

The mineral composition of the raw white, raw yellow maize, and the *mahewu* samples (Table 3) showed a significant ($p < 0.05$) increase in Na, Mg, P, K, Ca, Mn, Fe, Cu, and Zn when compared to the raw flour. Such an increase could be caused by the activity of microorganisms during fermentation [30,35]. In addition, antinutritional factors (that might limit the detection and availability of minerals by forming insoluble complexes within the food matrix) may have been degraded, leading to an increase in the available minerals [30,36]. In all of the fermented samples, Na, Mg, P, K, Ca, Fe, and Zn were abundant, while Mn and Cu were the most and least abundant micronutrients, respectively (Table 3). High levels of Na (148–167 mg/kg), Mg (1045–1113 mg/kg), P (1822–2251 mg/kg), K (2882–3283 mg/kg), Ca (213–386 mg/kg), Fe (17–39 mg/kg), and Zn (10–15 mg/kg) (Table 3) are important for nutrition, as these micronutrients are essential for the proper metabolic function, growth, and development of humans. Na is essential for physiological activities including muscle contraction and the control of fluid in the body [37]. Chelation can occur between Mg and vital intracellular anionic ligands (especially ATP) as well as compete with Ca for the binding sites of proteins and membranes [38]. P contributes to cell metabolism [39].

Fe is found in hemoglobin in circulating erythrocytes, and their balance in the body is necessary for the synthesis of DNA as well as to transfer electrons and oxygen [40]. Zn plays a role in balancing oxidative stress for the prevention of mutagenesis, neurodegeneration, immune-logic disorders, and cancer [41]. K is abundant in the muscle and viscera and its functions include maintenance of the resting membrane potential of the cells and the intracellular osmolarity [42]. Potentially toxic elements including Cr, Co, Ni, Pb, V, and Mo were detected in significantly low amounts. In this study, the highest level of potentially toxic elements was observed for Ni (Table 3). An excessive concentration of Ni consumed in food may impair the brain and central nervous system function, affect the blood composition as well as the kidneys, lungs, and liver, and lower energy levels [43]. However, each heavy metal detection in this study was below the recommended levels of the Joint FAO/WHO Expert Committee on Food Additives (JECFA) [44–46]. This confirms that these products are safe and beneficial to potential consumers. Interestingly, the wide consumption of this product has been reported in the literature [47,48].

Table 3. Mineral profile (mg/kg) of the raw maize and *mahewu* prepared with yellow and white maize.

Minerals	YMSG	YW	YMM	YMY	RY	WSG	WW	WM	WMW	RW
Na	155.85 ^{g} ± 0.03	167.14 ^{h} ± 0.02	150.07 ^{de} ± 0.02	149.26 ^{cd} ± 0.02	1.12 ^{a} ± 0	155.20 ^{g} ± 0.01	153.06 ^{f} ± 0.03	148.89 ^{c} ± 0.03	153.58 ^{f} ± 0.02	1.77 ^{b} ± 0
Mg	1102.82 ^{g} ± 0.01	1112.71 ^{i} ± 0.01	1057.39 ^{d} ± 0.02	1069.07 ^{e} ± 0.02	971.42 ^{b} ± 0	1094.29 ^{f} ± 0.02	1109.29 ^{h} ± 0.04	1045.90 ^{c} ± 0.03	1113.51 ^{ij} ± 0.02	900.79 ^{a} ± 0
p	2224.36 ^{i} ± 0.03	2251.45 ^{j} ± 0.03	2050.47 ^{e} ± 0.04	2174.18 ^{h} ± 0.03	2102.97 ^{g} ± 0	1822.93 ^{b} ± 0.02	1945.62 ^{bc} ± 0.02	2001.51 ^{d} ± 0.04	2062.39 ^{f} ± 0.04	1707.79 ^{a} ± 0
k	3163.83 ^{gh} ± 0.03	3283.38 ^{hi} ± 0.02	3051.61 ^{f} ± 0.02	3076.38 ^{g} ± 0.02	2984.93 ^{d} ± 0	2882.11 ^{b} ± 0.02	3039.42 ^{e} ± 0.02	2945.72 ^{c} ± 0.01	3129.97 ^{gh} ± 0.02	2751.96 ^{a} ± 0
Ca	270.94 ^{f} ± 0.81	268.92 ^{e} ± 0.81	237.30 ^{d} ± 0.64	299.45 ^{g} ± 0.36	0	217.67 ^{c} ± 5.20	213.47 ^{b} ± 2.00	367.39 ^{h} ± 0.51	386.98 ^{I} ± 0.29	126.01 ^{a} ± 0.11
V	0.03 ^{ab} ± 0.42	0.03 ^{ab} ± 0.32	0.02 ^{a} ± 0.23	0.03 ^{ab} ± 0.31	0	0.03 ^{ab} ± 0.17	0.02 ^{a} ± 0.65	0.05 ^{c} ± 0.10	0.08 ^{d} ± 0.17	0.02 ^{a} ± 0
Cr	0.54 ^{e} ± 0.26	0.01 ^{a} ± 0.13	0.22 ^{c} ± 0.08	0	0.14 ^{b} ± 0.01	1.39 ^{d} ± 0.06	0	0.35 ^{d} ± 0.12	0	0
Mn	5.70 ^{d} ± 0.04	7.67 ^{i} ± 0.04	5.12 ^{c} ± 0.02	4.93 ^{b} ± 0.04	4.84 ^{a} ± 0	7.04 ^{f} ± 0.03	8.78 ^{j} ± 0.04	7.61 ^{h} ± 0.03	7.42 ^{g} ± 0.05	6.48 ^{e} ± 0
Fe	26.55 ^{f} ± 0.03	29.79 ^{h} ± 0.03	39.00 ^{i} ± 0.02	20.30 ^{c} ± 0.03	22.89 ^{d} ± 0	27.87 ^{fg} ± 0.02	23.43 ^{de} ± 0.02	29.29 ^{h} ± 0.02	17.09 ^{b} ± 0.02	13.99 ^{a} ± 0
Co	0.05 ^{cd} ± 0.28	0.04 ^{c} ± 0.31	0.03 ^{ab} ± 0.52	0.03 ^{ab} ± 0.34	0.02 ^{a} ± 0	0.06 ^{de} ± 0.25	0.04 ^{c} ± 0.32	0.11 ^{fg} ± 0.14	0.10 ^{f} ± 0.12	0.10 ^{f} ± 0
Ni	1.11 ^{j} ± 0.08	0.58 ^{e} ± 0.11	0.70 ^{h} ± 0.13	0.66 ^{g} ± 0.07	0.45 ^{d} ± 0	0.83 ^{i} ± 0.06	0.28 ^{a} ± 0.16	0.62 ^{f} ± 0.16	0.37 ^{c} ± 0.09	0.30 ^{b} ± 0
Cu	3.70 ^{f} ± 0.02	2.92 ^{d} ± 0.01	2.82 ^{c} ± 0.03	13.22 ^{j} ± 0.02	1.41 ^{a} ± 0	7.57 ^{h} ± 0.03	3.25 ^{e} ± 0.02	9.79 ^{i} ± 0.01	3.92 ^{g} ± 0.02	2.07 ^{b} ± 0
Zn	14.57 ^{de} ± 0.05	13.84 ^{cd} ± 0.04	13.15 ^{cd} ± 0.04	13.04 ^{cd} ± 0.05	12.47 ^{bc} ± 0	14.38 ^{de} ± 0.05	15.04 ^{ef} ± 0.04	10.97 ^{a} ± 0.05	11.15 ^{ab} ± 0.02	10.31 ^{a} ± 0
Mo	0.31 ^{i} ± 0.16	0.22 ^{gh} ± 0.18	0.18 ^{de} ± 0.18	0.19 ^{ef} ± 0.14	0.17 ^{d} ± 0	0.18 ^{bc} ± 0.16	0.02 ^{a} ± 0.66	0.13 ^{c} ± 0.11	0.10 ^{b} ± 0.15	0.21 ^{g} ± 0
Pb	0.02 ^{ab} ± 0.17	0.04 ^{cd} ± 0.15	0.02 ^{ab} ± 0.13	2.93 ^{g} ± 0.25	0.01 ^{a} ± 0	0.03 ^{bc} ± 0.14	0.01 ^{a} ± 0.16	0.05 ^{ef} ± 0.13	0.03 ^{bc} ± 0.12	0.04 ^{cd} ± 0

Na—sodium; Mg—magnesium; P—phosphorous; K—potassium; Ca—calcium; V—vanadium; Cr—chromium; Mn—manganese; Fe—iron; Co—cobalt; Ni—nickel; Cu—copper; Zn—zinc; Mo—molybdenum; Pb—lead. YMSG—yellow maize *mahewu* with sorghum malt; YW—yellow maize *mahewu* with wheat; YMM—yellow maize *mahewu* with millet malt; YMY—yellow maize *mahewu* with malted yellow maize; RY—raw yellow maize flour; WSG—white maize *mahewu* with sorghum malt; WW—white maize *mahewu* with wheat; WM—white maize *mahewu* with millet malt; WMW—white maize *mahewu* with malted white maize; RW—raw white maize flour. Values indicate mean and standard deviation. Each value is a mean ± standard deviation of triplicate samples (n = 3), Letters in superscripts within a column show a significant difference.

3.4. Amino Acid Profile of Yellow Maize Flour, White Maize Flour, and Mahewu Products

The amino acid contents of the RW, RY, and *mahewu* samples are presented in Table 4. Amino acids are vital biological components and building blocks of proteins that are essential for neurotransmission, biosynthesis as well as other metabolic actions [49]. They give cells their structure, repair tissues, and contribute to the formation of muscles, glands, and arteries as well as wound healing, storing, and transporting of nutrients. As observed in Table 4, fermentation improved the quantity of some of the EAA in *mahewu* compared to the RW and RY flour samples, except for arginine (YMSG, YMM, and WSG), threonine (YMSG, YMM, WSG, and WM), valine (YMSG, YMM, YMY, and WSG), phenylalanine (YMSG, WSG, WW, and WM), isoleucine (YMSG, YMY, and WSG), leucine (YMSG, YMM, WSG, WW, WM, and WMW), and lysine (WSG). For the non-essential amino acids, serine in YMSG, YMM, WSG, and WM, aspartic acid in YMSG, YMM, WSG, and WM, glutamic acid in YMSG, YMM, WSG, WM, and WMW, glycine in YMM and WSG, alanine in YMSG, YMM, and WSG, tyrosine in WSG, WW, WM, and WMW, proline in YMSG, YMM, WSG, WW, WM, and WMW, HO-proline yellow maize *mahewu* with wheat (YW) and YMM, and methionine in WSG, WM, and WMW were observed to decrease ($p \leq 0.05$).

Qaku et al. [18] noted a substantially low amino acid composition in *mahewu* compared to raw flour, which corresponded to the report of Adeyeye [50], who observed a reduction in amino acids during the fermentation of pearl millet compared to that of raw pearl millet, and Osman [51], who also observed low amino acids in steeped guinea corn compared to the raw product. Their findings do not concur with the report of Awadalkareem et al. [52] and Mohammed et al. [53], who reported an increase in the amino acid content of sorghum flour, soybean, and *injera* (a fermented food product), respectively. The high levels of amino acids obtained from the *mahewu* samples in Table 4, compared to the yellow raw maize and white raw maize, might be due to the synthesis of amino acids by bacteria as well as transamination during fermentation [30,54]. In addition, the observed decrease in certain amino acids suggests the potential use of certain amino acids by the fermenting organisms. Other studies have also found the potential of fermenting microorganisms to use up amino acids as nitrogen sources for nutrients and anabolic requirements [22,30].

Arginine and leucine were the most pronounced EAA, whereas among the NEAA glutamic acids, aspartic acid, alanine, and proline were found to be abundant. Interestingly, both aspartic and glutamic acids contribute to spicy foods [55]. Alanine is also known to confer a pleasant flavor to foods [56]. A high level of glutamic acid in *mahewu* samples was observed, and this is beneficial as it can translate into an advanced level of gamma-aminobutyric acid (GABA), with favorable physiological roles [57]. Alanine aids in glucose and organic acid metabolism [22]. Proline is incorporated into collagen, the most abundant protein in the body, which is known to be protective against oxidative stress in various diseases such as heart failure, stroke, hypertensive heart disease, and coronary heart disease as well as supporting digestive health, preventing joint pain, healing wounds, and repairing skin [58]. Leucine is essential in the synthesis of protein in the skeletal muscle and adipose tissue. It promotes mitochondrial biogenesis and fatty acid oxidation and improves energy balance by activating the mammalian target of the rapamycin signaling pathway [59]. Leucine provides skeletal muscles with an increased flux of lipids, supplying energy substrates to support protein synthesis [60]. Arginine maintains equilibrium and the elimination of excess nitrogen [61]. The availability of amino acids in *mahewu* is thus essential from a nutritional standpoint. In terms of nutrition, maize is deficient in lysine and methionine, which explains the low quantities observed in this study (Table 4). In addition, the basic side chains of histidine and lysine, which contain nitrogen, could have caused them to react differently during fermentation, which could explain the values obtained in the raw and processed samples.

Table 4. Amino acid composition (g/100 g) of the maize flour (yellow and white) and derived *mahewu* products.

Amino Acid (g/100 g)	YMSG	YW	YMM	YMY	RY	WSG	WW	WM	WMW	RW
Essential										
Arginine	0.47 ^{ab} ± 0.03	0.52 ^{fg} ± 0.09	0.46 ^{a} ± 0.09	0.52 ^{fg} ± 0.09	0.52 ^{fg} ± 0.02	0.48 ^{bc} ± 0.07	0.53 ^{gh} ± 0.05	0.5 ^{de} ± 0.08	0.51 ^{ef} ± 0.10	0.49 ^{cd} ± 0.02
Threonine	0.32 ^{b} ± 0.04	0.37 ^{ef} ± 0.07	0.31 ^{a} ± 0.02	0.36 ^{de} ± 0.05	0.34 ^{c} ± 0.03	0.34 ^{c} ± 0.06	0.39 ^{g} ± 0.04	0.35 ^{cd} ± 0.09	0.36 ^{de} ± 0.10	0.36 ^{de} ± 0.09
Valine	0.43 ^{ab} ± 0.12	0.49 ^{gh} ± 0.06	0.44 ^{bc} ± 0.08	0.42 ^{a} ± 0.09	0.45 ^{cd} ± 0.05	0.46 ^{de} ± 0.14	0.48 ^{fg} ± 0.04	0.48 ^{fg} ± 0.06	0.49 ^{gh} ± 0.10	0.47 ^{ef} ± 0.13
Phenylalanine	0.39 ^{a} ± 0.09	0.46 ^{e} ± 0.08	0.41 ^{bc} ± 0.06	0.41 ^{bc} ± 0.10	0.41 ^{bc} ± 0.06	0.39 ^{a} ± 0.09	0.41 ^{bc} ± 0.04	0.4 ^{b} ± 0.05	0.42 ^{cd} ± 0.07	0.42 ^{cd} ± 0.11
Isoleucine	0.32 ^{a} ± 0.07	0.39 ^{e} ± 0.09	0.34 ^{bc} ± 0.03	0.32 ^{a} ± 0.11	0.33 ^{ab} ± 0.09	0.33 ^{ab} ± 0.06	0.34 ^{bc} ± 0.08	0.34 ^{bc} ± 0.07	0.35 ^{cd} ± 0.07	0.34 ^{bc} ± 0.13
Leucine	0.91 ^{a} ± 0.03	1.04 ^{gh} ± 0.02	0.95 ^{b} ± 0.06	0.97 ^{cd} ± 0.08	0.96 ^{bc} ± 0.10	0.95 ^{b} ± 0.08	0.98 ^{de} ± 0.07	0.98 ^{de} ± 0.07	1.01 ^{f} ± 0.09	1.03 ^{g} ± 0.05
Histidine	0.35 ^{g} ± 0.11	0.39 ^{h} ± 0.04	0.22 ^{d} ± 0.09	0.24 ^{e} ± 0.05	0.13 ^{a} ± 0.08	0.29 ^{f} ± 0.10	0.19 ^{c} ± 0.07	0.17 ^{b} ± 0.09	0.19 ^{c} ± 0.06	0.29 ^{f} ± 0.10
Lysine	0.25 ^{ab} ± 0.09	0.29 ^{ef} ± 0.06	0.29 ^{ef} ± 0.10	0.24 ^{a} ± 0.09	0.24 ^{a} ± 0.08	0.25 ^{ab} ± 0.07	0.28 ^{e} ± 0.11	0.26 ^{bc} ± 0.05	0.27 ^{cd} ± 0.06	0.26 ^{bc} ± 0.17
Non-essential										
Serine	0.42 ^{ab} ± 0.09	0.46 ^{de} ± 0.02	0.43 ^{bc} ± 0.11	0.48 ^{fg} ± 0.04	0.45 ^{d} ± 0.06	0.41 ^{a} ± 0.05	0.48 ^{fg} ± 0.03	0.45 ^{d} ± 0.04	0.48 ^{fg} ± 0.10	0.47 ^{ef} ± 0.09
Aspartic acid	0.54 ^{b} ± 0.08	0.6 ^{ef} ± 0.10	0.42 ^{a} ± 0.01	0.59 ^{e} ± 0.03	0.59 ^{e} ± 0.02	0.54 ^{b} ± 0.04	0.6 ^{ef} ± 0.08	0.56 ^{c} ± 0.05	0.57 ^{cd} ± 0.09	0.57 ^{cd} ± 0.06
Glutamic acid	1.59 ^{b} ± 0.06	1.77 ^{fg} ± 0.08	1.46 ^{a} ± 0.03	1.75 ^{e} ± 0.13	1.73 ^{d} ± 0.08	1.66 ^{c} ± 0.09	1.87 ^{i} ± 0.11	1.76 ^{ef} ± 0.07	1.75 ^{e} ± 0.15	1.78 ^{gh} ± 0.04
Glycine	0.35 ^{b} ± 0.01	0.38 ^{de} ± 0.10	0.33 ^{a} ± 0.03	0.39 ^{ef} ± 0.03	0.35 ^{b} ± 0.05	0.35 ^{b} ± 0.09	0.37 ^{cd} ± 0.06	0.37 ^{cd} ± 0.08	0.39 ^{ef} ± 0.07	0.36 ^{bc} ± 0.01
Alanine	0.6 ^{a} ± 0.04	0.64 ^{de} ± 0.05	0.6 ^{a} ± 0.02	0.65 ^{ef} ± 0.01	0.61 ^{ab} ± 0.05	0.6 ^{a} ± 0.05	0.63 ^{cd} ± 0.11	0.64 ^{de} ± 0.08	0.64 ^{de} ± 0.04	0.62 ^{bc} ± 0.08
Tyrosine	0.21 ^{cd} ± 0.08	0.24 ^{f} ± 0.03	0.18 ^{a} ± 0.09	0.19 ^{ab} ± 0.10	0.18 ^{a} ± 0.07	0.21 ^{cd} ± 0.08	0.21 ^{cd} ± 0.12	0.21 ^{cd} ± 0.07	0.2 ^{bc} ± 0.05	0.23 ^{e} ± 0.09
Proline	0.67 ^{b} ± 0.10	0.77 ^{fg} ± 0.08	0.65 ^{a} ± 0.04	0.76 ^{ef} ± 0.05	0.7 ^{c} ± 0.11	0.74 ^{d} ± 0.07	0.75 ^{de} ± 0.08	0.78 ^{gh} ± 0.03	0.76 ^{ef} ± 0.13	0.81 ^{i} ± 0.10
HO-Proline	0.02 ^{ab} ± 0	0.01 ^{a} ± 0	0.01 ^{a} ± 0.01	0.02 ^{ab} ± 0.01	0.02 ^{ab} ± 0	0.01 ^{a} ± 0	0.01 ^{a} ± 0.01	0.02 ^{ab} ± 0	0.01 ^{a} ± 0	0.01 ^{a} ± 0.01
Methionine	0.11 ^{cd} ± 0.05	0.1 ^{bc} ± 0.04	0.1 ^{bc} ± 0.01	0.17 ^{f} ± 0.08	0.08 ^{a} ± 0.01	0.09 ^{ab} ± 0.03	0.14 ^{e} ± 0.02	0.11 ^{cd} ± 0.03	0.11 ^{cd} ± 0.06	0.14 ^{e} ± 0.09

G—gram; HO—hydroxy; YMSG—yellow maize *mahewu* with sorghum malt; YW—yellow maize *mahewu* with wheat; YMM—yellow maize *mahewu* with millet malt; YMY—yellow maize *mahewu* with malted yellow maize; RY—raw yellow maize flour; WSG—white maize *mahewu* with sorghum malt; WW—white maize *mahewu* with wheat; WM—white maize *mahewu* with millet malt; WMW—white maize *mahewu* with malted white maize; RW—raw white maize flour. Each value is a mean ± standard deviation of triplicate samples (n = 3). Units = (g/100 g), Letters in superscripts within a column show a significant difference.

3.5. Identification of Phenolic Compounds in Raw Maize Flours (RW and RY) and Mahewu Samples

Maize contains different bioactive compounds including phenolics. Phenolics are plant-derived metabolites formed mainly as a defense mechanism by the plant and are important in preventing disease and promoting health [62]. According to the literature, there is a dearth of knowledge on the profiling of phenolic compounds in *mahewu* samples and their raw materials with the use of UHPLC/Q-TOF-MS such as those reported in Table 5. A total of 26 compounds were identified in all of the samples investigated. At a retention time of 3.78 min, Compound 1 had a molecular ion of 503 m/z, with fragments of m/z = 252, 179, and 117, characteristic of raffinose. Raffinose oligosaccharides contribute to food flavor, quality, and physicochemical characteristics, and are an excellent source of prebiotics and fiber [63]. Compound 2, which had a molecular ion of 205 m/z, was identified as scoparone with fragments of m/z = 179, 173, and 133; this is an important type of coumarin which shows favorable efficacies for all types of liver disease [64].

Compound 3, at a retention time 7.60 min, with a molecular ion of 353 m/z and fragments of m/z = 190, 174, and 130, indicates chlorogenic acid, resulting from the change in quinic acid and derivatives to an ester. Chlorogenic acid exhibits antibacterial, antioxidant, and antiviral properties in food products [65]. Compound 4 was identified as blumealactone C at a retention time of 7.71 min, with a molecular ion of 323 m/z, and fragments of m/z = 192, 146, and 125. Blumealactone C was identified as the major terpene lactone in the analyzed samples. These are important bioactive constituents that contribute to neuro protective effects and the inhibition of platelet-activating factors and atherosclerosis [66]. Compound 5, with a m/z of 315 at retention time 7.82 min and fragments of m/z = 259, 183, and 151, was identified as gibberellin A9. Gibberellins are a large group of tetracyclic diterpenoid carboxylic acids that function as important hormones for plant growth [67]. Compound 6 was detected as (+)-Streptazolin, a phenolic compound from the oxazolidinones group. This was present in all of the analyzed samples, with a precursor ion of m/z of 206 at retention time 7.90 min, and fragments of m/z = 204, 198, and 175. The oxazolidinones are a new class of antimicrobial agent [68].

Compound 7 had a molecular ion of 353 m/z and fragments of m/z = 191, 173, and 135, and at a retention time of 8.12 min was identified as (+)-Sesamin, a phenolic lignin that is present in sesame seed and sesame oil [69]. Lignans are plant secondary metabolites amongst the diphenolic compound class. They are significant due to their potential health benefits, having anti-inflammatory, anticancer, antiestrogenic, and antioxidant properties [69]. Compound 8 was categorized as gardenin B, a naturally occurring phenolic compound from the methoxyflavonoid group, with a molecular ion of 357 m/z, at a retention time of 8.20 min and fragments of m/z = 276, 214, and 178. Gardenin B is a potential anticancer agent [70]. Compound 9 was identified as 4-hydroxymethyl-2-methoxyphenyl-1-O-beta-D-apiofuranosyl-(1- > 6)-O-beta-D-glucopyranoside from a group of phenolic glycosides, with a precursor m/z of 447, at a retention time of 8.4 min and fragments of m/z = 288, 175, and 107. They occur naturally in food as O-glycosides, and have been shown to possess anti-infective, neurological, and anti-obesity activities [71]. Compound 10 had a molecular ion of m/z 436, produced fragments of m/z = 433, 313, and 223 at a retention time of 8.7 min, and was identified in all of the analyzed samples as lunarine from the macrolactams group. Macrolactams are an important class of compounds that exhibit exceptional activity in the treatment of atopic dermatitis and other inflammatory dermatoses [72].

Table 5. Identification and concentration of phenolic compounds in the raw maize flour (RW and RY) and *mahewu* samples.

Cmpd. No.	t_R (min)	*m/z*	F	Cmpd	Polyphenol Class	Concentration (μg/g)									
						YMSG	YW	YMM	YMY	RY	WSG	WW	WM	WMW	RW
1	3.78	503.18	252,179,117	Raffinose	Oligosaccharides	ND	ND	ND	$2.5^{a} \pm 0.09$	ND	ND	ND	$260.6^{c} \pm 1.39$	$5.4^{b} \pm 1.10$	ND
2	7.19	205.06	179,173,133	Scoparone	Coumarins and derivatives	ND	$0.5^{ab} \pm 0.03$	ND	ND	$0.4^{a} \pm 0.02$	ND	ND	ND	ND	$1.1^{c} \pm 0.09$
3	7.60	353.10	190,174,130	Chlorogenic acid	Quinic acids and derivatives	$0.9^{c} \pm 0.06$	ND	ND	ND	$2.6^{f} \pm 0.04$	$1.3^{e} \pm 0.08$	$0.5^{a} \pm 0.06$	$1.1^{d} \pm 0.02$	ND	$0.7^{b} \pm 0.05$
4	7.71	323.14	192,146,125	Blumealactone C	Terpene lactones	$1.8^{de} \pm 0.9$	ND	$0.1^{a} \pm 0.03$	$1.7^{d} \pm 0.10$	ND	ND	ND	ND	$1.1^{b} \pm 0.06$	$1.4^{c} \pm 0.09$
5	7.82	315.13	259,183,151	Gibberellin A9	C19-gibberellin 6-carboxylic acids	$1.4^{b} \pm 0.11$	ND	$2.1^{c} \pm 0.09$	ND	ND	ND	$0.9^{a} \pm 0.04$	ND	ND	ND
6	7.90	206.08	204,198,175	(+)-Streptazolin	Oxazolidinones	$65.1^{h} \pm 0.26$	$174.5^{j} \pm 0.42$	$0.5^{a} \pm 0.08$	$70.8^{I} \pm 0.18$	$34.8^{f} \pm 0.11$	$2.1^{d} \pm 0.09$	$10.1^{e} \pm 0.16$	$1.1^{c} \pm 0.03$	$0.9^{b} \pm 0.07$	$48.1^{g} \pm 0.19$
7	8.12	353.11	191,173,135	(+)-Sesamin	Furanoid lignans	ND	$1.7^{c} \pm 0.04$	ND	ND	ND	$0.5^{a} \pm 0.04$	ND	ND	$0.5^{a} \pm 0.09$	$1.1^{b} \pm 0.06$
8	8.20	357.13	276,214,178	Gardenin B	8-O-methylated flavonoids	$1.2^{b} \pm 0.02$	ND	$0.8^{a} \pm 0.11$	ND	ND	ND	$1.8^{c} \pm 0.07$	ND	ND	ND
9	8.40	447.16	288,175,107	4-Hydroxymethyl-2-methoxyphenyl-1-O-beta-D-apiofuranosyl-(1->6)-O-beta-D-glucopyranoside	Phenolic glycosides	$0.8^{b} \pm 0.05$	ND	ND	ND	ND	$0.5^{a} \pm 0.03$	ND	ND	ND	ND
10	8.75	436.24	433,313,223	Lunarine	Macrolactams	$74.1^{I} \pm 0.14$	$69.3^{h} \pm 0.11$	$76.1^{j} \pm 0.19$	$64.2^{f} \pm 0.21$	$11.9^{b} \pm 0.16$	$19.0^{d} \pm 0.25$	$67.1^{g} \pm 0.33$	$15.3^{c} \pm 0.29$	$26.4^{e} \pm 0.33$	$9.4^{a} \pm 0.29$
11	8.78	263.17	229,192,132	Subaphylline	Hydroxycinnamic acids and derivatives	ND	ND	ND	ND	ND	$3.4^{c} \pm 0.10$	ND	$2.0^{a} \pm 0.09$	$2.1^{ab} \pm 0.09$	ND
12	9.30	324.13	315,283,164	Monocrotaline	Pyrrolizines	$1.2^{d} \pm 0.04$	$1.8^{f} \pm 0.06$	$0.7^{b} \pm 0.05$	$1.3^{de} \pm 0.05$	$0.8^{bc} \pm 0.07$	$0.8^{bc} \pm 0.09$	$0.3^{a} \pm 0.08$	ND	ND	$1.3^{de} \pm 0.05$
13	9.52	165.08	1,659,944	Perillic acid	Menthane monoterpenoids	$32.4^{h} \pm 0.16$	$81.9^{j} \pm 0.11$	$63.5^{i} \pm 0.11$	$27.9^{g} \pm 0.10$	$0.5^{a} \pm 0.07$	$7.9^{e} \pm 0.08$	$14.8^{f} \pm 0.09$	$1.6^{c} \pm 0.07$	$2.2^{d} \pm 0.09$	$0.8^{b} \pm 0.05$
14	9.98	261.16	259,248,187	[1R-(1alpha,4abeta,6alpha,8aalpha)]-1,2,4a,5,6,8a-Hexahydro-6-hydroxy-4,7-dimethyl-a-methylene-1-naphthaleneacetic acid methyl ester	Sesquiterpenoids	$1.2^{e} \pm 0.05$	$4.3^{h} \pm 0.09$	ND	$2.4^{g} \pm 0.07$	$1.1^{d} \pm 0.07$	ND	$1.3^{ef} \pm 0.06$	$0.5^{a} \pm 0.06$	$0.9^{bc} \pm 0.08$	$0.8^{b} \pm 0.03$
15	10.48	187.13	184,169,127	Dimethyltryptamine	Tryptamines and derivatives	$27.9^{j} \pm 0.18$	$13.6^{h} \pm 0.13$	$16.2^{i} \pm 0.14$	$8.8^{g} \pm 0.09$	$6.2^{e} \pm 0.11$	$3.4^{c} \pm 0.13$	$2.8^{a} \pm 0.08$	$4.9^{d} \pm 0.08$	$2.9^{ab} \pm 0.16$	$7.1^{f} \pm 0.13$
16	10.63	366.14	357,301,253	Isatidine	Alkaloids and derivatives	$2.9^{e} \pm 0.09$	$1.4^{d} \pm 0.07$	$0.5^{a} \pm 0.08$	$1.1^{b} \pm 0.10$	ND	ND	$1.2^{bc} \pm 0.05$	$1.2^{bc} \pm 0.13$	$1.2^{bc} \pm 0.08$	ND
17	10.76	159.10	156,146,129	Tryptamine	Tryptamines and derivatives	$106.9^{g} \pm 0.14$	$379.9^{h} \pm 0.13$	$1.1^{b} \pm 0.05$	$2.6^{d} \pm 0.10$	ND	$1.7^{c} \pm 0.09$	$76.7^{f} \pm 0.11$	$0.1^{a} \pm 0.05$	$4.2^{e} \pm 0.10$	ND
18	10.95	366.14	359,356,221	Casuarine 6-alpha-D-glucoside	O-glycosyl compounds	$0.8^{b} \pm 0.03$	$1.3^{d} \pm 0.06$	$1.1^{c} \pm 0.03$	$1.1^{c} \pm 0.05$	ND	ND	$0.5^{a} \pm 0.07$	ND	ND	$0.4^{a} \pm 0.02$
19	11.12	409.20	366,278,174	Heliocide H1	Hydroxyanthraquinones	$72.2^{g} \pm 0.15$	$100.7^{i} \pm 0.18$	$76.4^{h} \pm 0.15$	$66.0^{f} \pm 0.17$	$111.8^{j} \pm 0.12$	$32.6^{e} \pm 0.13$	$3.8^{a} \pm 0.10$	$10.0^{c} \pm 0.10$	$13.4^{d} \pm 0.11$	$5.9^{b} \pm 0.13$
20	11.23	439.19	431,343,227	10-Deacetyl-2-debenzoylbaccatin III	Taxanes and derivatives	$0.8^{a} \pm 0.09$	$2.6^{b} \pm 0.06$	$15.6^{d} \pm 0.10$	$4.0^{c} \pm 0.10$	$140.5^{j} \pm 0.10$	$40.6^{e} \pm 0.08$	$72.6^{g} \pm 0.12$	$69.7^{f} \pm 0.11$	$84.2^{h} \pm 0.14$	$93.8^{i} \pm 0.11$
21	12.92	329.25	297,203,171	9,12,13-TriHOME	Long-chain fatty acids	$343.8^{e} \pm 0.20$	$241.7^{c} \pm 0.21$	$364.6^{f} \pm 0.11$	$347.1^{g} \pm 0.24$	$116.4^{b} \pm 0.19$	$318.6^{d} \pm 0.22$	$446.2^{j} \pm 0.14$	$399.8^{h} \pm 0.21$	$407.8^{i} \pm 0.25$	$102.8^{a} \pm 0.21$
22	13,11	331.27	318,187,126	Floionolic acid	Long-chain fatty acids	$1.1^{e} \pm 0.08$	$204.7^{f} \pm 0.09$	$0.7^{b} \pm 0.05$	$0.7^{b} \pm 0.04$	$0.8^{bc} \pm 0.05$	$1.1^{e} \pm 0.08$	$0.9^{cd} \pm 0.04$	$0.7^{b} \pm 0.05$	$0.5^{a} \pm 0.04$	$0.7^{b} \pm 0.06$
23	13.61	564.34	353,314,143	JSTX 3	Resorcinols	$19.2^{a} \pm 0.10$	$147.2^{e} \pm 0.15$	$177.4^{f} \pm 0.11$	$108.9^{d} \pm 0.10$	$231.7^{i} \pm 0.18$	$204.0^{j} \pm 0.21$	$195.9^{h} \pm 0.16$	$184.4^{g} \pm 0.19$	$62.2^{b} \pm 0.20$	$69.9^{c} \pm 0.16$
24	13.94	313.26	311,287,216	Dronabinol	2,2-dimethyl-1-benzopyrans	$227.2^{g} \pm 0.10$	$113.8^{c} \pm 0.11$	$227.6^{g} \pm 0.12$	$222.6^{f} \pm 0.18$	$93.5^{b} \pm 0.14$	$154.1^{e} \pm 0.15$	$147.4^{d} \pm 0.18$	$358.0^{i} \pm 0.18$	$295.4^{h} \pm 0.16$	$74.5^{a} \pm 0.20$
25	14.12	311.24	309,297,214	9(S)-HPODE	Lineolic acids and derivatives	$112.8^{d} \pm 0.17$	$42.3^{b} \pm 0.19$	$126.8^{f} \pm 0.19$	$666.2^{h} \pm 0.14$	$6.3^{a} \pm 0.10$	$294.7^{f} \pm 0.20$	$113.1^{de} \pm 0.10$	$80.9^{c} \pm 0.22$	$612.0^{g} \pm 0.11$	$6.3^{a} \pm 0.09$
26	14.35	295.24	265,221,131	Alpha-dimorphecolic acid	Lineolic acids and derivatives	$188.4^{d} \pm 0.19$	$182.3^{c} \pm 0.28$	$200.5^{g} \pm 0.26$	$207.3^{h} \pm 0.13$	$119.8^{a} \pm 0.22$	$189.5^{de} \pm 0.21$	$257.2^{i} \pm 0.13$	$285.4^{j} \pm 0.17$	$195.3^{f} \pm 0.16$	$136.6^{b} \pm 0.19$

Cmpd—compound; t_R—retention time; *m/z*—mass-to-charge ratio; F—fragments; MF—molecular formula; YMSG—yellow maize *mahewu* with sorghum malt; YW—yellow maize *mahewu* with wheat; YMM—yellow maize *mahewu* with millet malt; YMY—yellow maize *mahewu* with malted yellow maize; RY—raw yellow maize flour; WSG—white maize *mahewu* with sorghum malt; WW—white maize *mahewu* with wheat; WM—white maize *mahewu* with millet malt; WMW—white maize *mahewu* with malted white maize; RW—raw white maize flour, ND—not determined. Each value is a mean ± standard deviation of triplicate samples (n = 3), Letters in superscripts within a column show a significant difference.

Compound 11 was identified as subaphylline, with an identified molecular ion of 263 at a retention time of 8.78 min. This compound produced fragments of m/z = 229, 192, and 132, with characteristics of hydroxycinnamic acid derivatives, which are known to display antimicrobial, antioxidant, and anti-inflammatory properties [73]. For compound 12 at a retention time of 9.30 min, a molecular ion with 324 m/z was produced, with fragments of m/z = 315, 283, and 164. This was identified as monocrotaline from the pyrrolizine group, with secondary allelochemicals associated with plant defense against herbivores, with biological activities that include neurotoxicity, cytotoxicity, hepatotoxicity, and tumorigenicity [74]. Compound 13 was identified as perillic acid from a group of menthane monoterpenoids and was present at a retention time of 9.52 min and produced a molecular ion of 165 m/z, with fragments of m/z = 165, 99, and 44. According to Fukumoto et al. [75], monoterpenes reach the brain in the form of perillic acid and have a potent stress alleviating effect [75]. Compound 14, with a retention time of 9.98 min, produced a molecular ion at 261 m/z, with fragments of m/z = 259, 248, and 187; this was identified as a [1R-(1alpha,4abeta,6alpha,8aalpha)]-1,2,4a,5,6,8a-hexahydro-6-hydroxy-4,7-dimethyl-a-methylene-1-naphthaleneacetic acid methyl ester from a group of sesquiterpenoids. This flavonoid exhibits antibacterial, anti-inflammatory, and antiviral properties [76].

Compounds 15, 16, 17, and 18 were classified as monoamine alkaloids (Table 5). Compound 15, at a retention time of 10.48 min, produced a molecular ion at 187 m/z, with fragmentation of m/z = 184, 169, and 127, characteristic of dimethyltryptamine from the group of tryptamines and derivatives. Compound 16 had a precursor ion at 366 m/z and was identified as isatidine (Table 5). It produced fragments of m/z = 357, 301, and 253 at a retention time of 10.63. Compound 17 was identified as tryptamine with a molecular ion of 159 m/z, and fragmentation of m/z = 156, 146, and 129 at a retention time of 10.76 min. Compound 18 was identified as casuarine 6-alpha-D-glucoside from the O-glycosyl compounds, with a molecular ion at 366 m/z and fragments of m/z = 359, 356, and 221 at a retention time of 10.95. Chemically, alkaloids serve as defense compounds against competitive plants, microbes, and herbivores [74]. Plants store these chemical substances in locations such as seeds, leaves, fruits, and flowers to serve as strategic defensive mechanisms [65]. Compound 19 had a molecular ion of 409 m/z, with fragments of m/z = 366, 278, and 174, and was identified as heliocide H1 from a group of hydroxyanthraquinones. These are naturally occurring compounds that have been used as dyes and are derivatives of an anthraquinone through the replacement of one hydrogen atom by a hydroxyl group. Reported biological properties of this compound include antiviral, antimalarial, antitumor, antibacterial, and antifungal [77].

Compound 20, with a molecular ion of 439 m/z, at a retention time of 11.23 min and fragments of m/z = 431, 343, and 227, was identified as 10-deacetyl-2-debenzoylbaccatin III from a large group of taxanes and their derivatives. Taxanes are plant secondary metabolites known for their anticancer properties in humans [78]. Compound 21 was identified as 9,12,13-TriHOME, with a molecular ion of 329 m/z at a retention time of 12.92 min and fragments of m/z = 297, 203, and 171, while Compound 22 was identified as floionolic acid with a molecular ion of 331 m/z at a retention time of 13.11 min and fragments of m/z = 318, 187, and 126. Both compounds are from the long-chain fatty acid group, which is the main component of triglycerides, phospholipids, and cholesterol esters. This group contains acidic monocarboxylic linear chains of variable length known to be a major source of physiologic energy [79]. Compound 23, with a molecular ion of 564 m/z and fragments of m/z = 353, 314, and 143, was identified as JSTX 3. This is a toxin isolated from spider venom, which could have resulted from an insect infestation on the maize. This compound is from a group of resorcinols, a phenolic chemical that is used for the treatment of skin disorders and infections such as eczema, warts, dermatitis, acne, and seborrheic dermatitis [80].

Compound 24, at a retention time of 13.94 min, had a molecular ion of 313 m/z with fragments of m/z = 311, 287, and 216, and was identified as dronabinol, which belongs to the class of organic compounds known as 2, 2-dimethyl-1-benzopyrans. Dronabinols are used for the treatment of nausea and vomiting in cancer chemotherapy patients as well as

for the treatment of anorexia and weight loss in HIV patients [81]. Compounds 25 and 26 were identified as 9(S)-HPODE and alpha-dimorphecolic acid, with molecular ions of 311 and 295 m/z, respectively. These are from the group of lineolic acids and their derivatives (Table 5). Lineolic acid is an essential fatty acid found mostly in plant oils and used in the biosynthesis of cell membranes and prostaglandins [82]. According to Marangoni et al. [82] increased intake of lineolic acid is associated with reduced incidence of cardiovascular disease.

The enzymatic actions of esterases, hydrolyases, phytases, and oxidases are responsible for the breaking down of phenolic compounds during fermentation [83]. This would explain the observed reduction in some of the discovered phenolic compounds. We also noted the variations in phenolic content, which might be a result of the structural configuration, phenolic complexity, and enzyme specificity of these compounds [84]. The high levels of phenolic compounds identified could be ascribed to a significant number of phytochemicals existing in whole grain maize [85]. This finding validates previous observations of high phenolic and flavonoid content derived from *mahewu* products [17].

4. Conclusions

The findings of this study show that fermentation affected an increase or decrease in the nutritional composition of *mahewu* samples. The observed drop in TSS is attributable to the presence of bacteria in the *mahewu* samples, which corresponds to faster use of the available solids, hence the increased carbohydrate content in *mahewu* samples. The enhanced carbohydrate and protein levels of improved *mahewu* products support claims that it may be used as a meal substitute for individuals who cannot afford appropriate meals for their daily activities. These findings also demonstrate that the *mahewu* samples had heavy metal concentrations within the acceptable limits established by the JECFA (FAO/WHO 2002). Additionally, the inclusion of important minerals and amino acids in these products may help to treat micronutrient shortages. Our results further revealed that the produced products and raw samples are rich in a variety of phenolic compounds required for development and good health. This finding confirms that the differences in inocula influence the overall composition of *mahewu*, which would be of further use in the production of high-quality *mahewu* for consumption.

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