

# Correlation between Chemical Composition, Water Holding Capacity and Flavonoids Content of Maize Varieties Harvested at Buxedeni Village of KwaNongoma in KwaZulu Natal, South Africa

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**Abstract** Maize is the most important grain crop in South Africa, especially in the rural places of KwaZulu Natal. Protein, starch, fat, minerals, fibre, and flavonoids content was determined, together with water holding capacity in four different maize varieties obtained from rural village of KwaNongoma (KwaZulu Natal Province). Red maize variety had higher macro nutrients than other varieties however, all varieties showed low micro nutrients. The soaking time and soaking temperature showed to have an effect on water holding capacity of maize varieties. Red maize variety had higher capacity in all soaking temperatures which increased as the temperature increases with 1.69% at 25°C, 8.79% at 35°C and 11.89% at 45°C. Red and yellow variety showed phenolic content of more than 100 mg 100g<sup>-1</sup>. This study showed that all maize varieties yielded high nutritional value and flavonoid contents which makes maize produced from rural village of KwaNongoma suitable for human consumption.

**Keywords:** protein, starch, flavonoids and water holding capacity

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## 1. Introduction

Legumes and cereals are important agricultural protein sources; this makes them possible ingredients for a wide range of processed food. Maize (*Zea mays L*) is the third most consumed cereal grain following wheat and rice. Maize is the vital source of iron, minerals, carbohydrate, protein and vitamin B, which makes it a significant item in the diet of most South Africans. Iron improves transportation of oxygen to different parts of the body through red blood cells and its low levels may result in tiredness and fatigue. Minerals play a role in building strong bones and transmission of nerve impulse, its low levels may result in weak bones and fatigue. Carbohydrates provide the body with energy and its low levels may result in ketosis. Proteins play an important role as building block of bones, skin, muscles, cartilage, and blood in the body and its low levels may result in lean body mass muscle strength, and function. Vitamin B have a direct impact on the body energy levels, brain function, and cell metabolism and its low levels may result in body weakness and fatigue [1]. Hence, it is important that human body receives these nutrients.

It is important that food is preserved to improve its safety. Drying method is the preservation strategy in most agricultural material as it allows removal of moisture content and thus permit safe storage for a longer period. This represents a significant step in the food processing industry [2]. Grain hydration process is a food processing step that increases moisture content through soaking which improves physicochemical and nutritional quality [3]. This process is required to facilitate different operations such as canning and cooking processes, extraction, fermentation, germination and malting [4]. Soaking process is the primary reason to gelatinize the starch in maize grain which can be achieved either through conditioning below the gelatinization temperature and then cooking above the gelatinization temperature or through direct cooking above the gelatinization temperature [5]. Grain processing and quality of the final product is directed by the consideration of water absorption in grains during soaking. Consequently, in the processing industry, water absorption into maize grain is of theoretical and practical interest [6,7]. During soaking, extrinsic (temperature, soaking time, etc.) and intrinsic (chemical and physical) factors influence the absorption of water into grain [8,9]. Thus, studying processing variables effect is significant for processing industry to optimize

food-processing equipment and characterize soaking conditions and predict water uptake as a function of time and temperature [10,11]. Furthermore, food formulation is profoundly affected by the rate of water absorption. For example, addition order of dry ingredients into the mixture is influenced by the rate of water absorption and it determines whether hydration of dry ingredients is needed or not before addition to the mixture [8,9]. Restoration of raw material properties is achieved by rehydration process which eliminate wide variety of dehydration of food and the concerns for meeting quality specification and energy conservation [12,13].

In South Africa, maize quality and analysis is mostly done on those maize that are produced from big farms for commercial purposes. On the other hand, people from South African rural communities grow their own maize for human consumption and feeding their livestock. However, the food quality of agricultural products in rural communities is not monitored. As such, there are no scientific based studies conducted on the maize quality grown for grain processing in South African rural communities. Therefore, the objective of this study was the valorisation of maize varieties grown in rural household by evaluating the chemical composition, flavonoids and water holding capacity.

## 2. Methods and Materials

### 2.1. Maize Collection and Pre-treatment

Maize samples were harvested in a private household located in a small village of Buxedeni situated in a town of KwaNongoma in KwaZulu Natal, South Africa. The collected samples were identified based on their colours and pre-treated individually. Each sample was milled into whole grain flour and passed through 100 mm screen mesh. Analysis were conducted in triplicate.

### 2.2. Methods

#### 2.2.1. Nutrition Composition

##### Protein

The LECO Truspec Nitrogen Analyser (LECO Corporation, Michigan, USA) was employed to measure the content of protein in the samples using Official Analytical Chemists (AOAC) official method 990.03, [14]. Triplicate samples were measured and placed into a combustion chamber at 950°C with an autoloader and the percentage of protein was calculated using equation (1).

$$\% \text{ crude protein} = \%N \times 6.25 \quad (1)$$

##### Fat

The Soxhlet Fat extractor (Büchi, Flawil, Switzerland) was employed for the determination of the fat content in the samples with petroleum ether as the extract solvent. The analysis were conducted following the AOAC Official Method 920.39 [14] and the percentage of crude fat was determined using equation (2).

$$\% \text{ Crude fat} = \frac{\text{beaker} + \text{fat} - \text{beaker}}{\text{sample mass}} \times 100 \quad (2)$$

##### Fibre

The fibre was determined using the Van Soest method [15] as neutral detergent fibre (NDF). The 0.5 g sample was weighed into a scintered glass crucible. The marble/buffer beads and 50 mL of neutral detergent solution (NDS) were added to the crucible holder. The NDS was prepared with 45.3 g disodium tetraborate, 124 g ethylene diamine tetra-acetic acid, 67 mL 2-ethoxy ethanol, 30.4 g disodium hydrogen phosphate, and 200 g sodium lauryl sulphate. The sample glass crucible was placed into the crucible holder which was thereafter placed into a digestive block set at 110°C. A 1 mL of termamyl ( $\alpha$ -amylase) enzyme was then added into the sample crucible and allowed to boil for 70 minutes. Thereafter, the glass crucible was removed and placed on a draining rack. The filtration unit connected to the vacuum system used for suction and the sample was washed with boiling water until free from NDS. The sample was then rinsed with acetone and placed for 4 hours in a drying oven at 105°C. The sample were then cooled in a desiccator, the crucible was weighed and the NDF of the sample was calculated using equation (3).

$$\% \text{ NDF} = \frac{(\text{crucible} + \text{dry residue}) - (\text{crucible} + \text{ash})}{\text{sample mass}} \times 100 \quad (3)$$

##### Total mineral matter (ash)

Ash was determined using the AOAC 942.05 [14]. The samples were weighed and placed for 12 hours in a furnace at 550°C. After the volatilisation of the organic matter from the samples, the ash residues remained in the crucible and ash was calculated using equation (4).

$$\% \text{ ash} = \frac{\left[ \begin{array}{l} (\text{mass of the sample} \\ + \text{crucible after ashing}) \\ - (\text{mass of pre-dried crucible}) \end{array} \right]}{\left[ \begin{array}{l} (\text{mass of sample} + \text{crucible}) \\ - (\text{mass of pre-dried crucible}) \end{array} \right]} \times 100 \quad (4)$$

##### Starch

The starch content was determined by weighing 1 g of the sample into a test tube. Thereafter, 5 mL of 80% ethanol was added into the sample and vortexed then incubated at 80°C for 30 minutes. Test tube with the sample was placed back into the oven at 80°C until all the ethanol completely evaporated. A 10 mL of acetate buffer was added into the test tube followed by 200  $\mu$ L of Termamyl  $\alpha$  amylase enzyme. The mixture was vortexed and incubated for 30 min at 90°C, and allowed to cool. After cooling, 200  $\mu$ L of amyl glucosidase was added to the mixture in the test tube and gently shaken. The test tube with the mixture was incubated at 60°C for 8 hours. The sample was diluted in a 200 mL volumetric flask using deionized water and filtered through watmman filter paper no 541. A 5 mL of copper reagent was added into 3 mL of the filtrate in the test tube followed by addition of the arsenomolybdate reagent (5 mL). The test tube was shaken and allowed to stand for 90 minutes. The starch content of the sample was determined by UV absorption at 750 nm wavelength and the starch content was calculated using equation (5), [16].

$$\% \text{ Starch} = \frac{0.4555 \times \text{Absorbance of sample} \times 0.9}{\text{sample weight} \times \text{Absorbance of glucose standard}} \quad (5)$$

### Mineral element

The minerals element were analysed following the Agricultural Laboratory Association of Southern Africa (ALASA) Method [17]. Samples were ashed for 12 hours in a furnace at 550°C. The samples were digested in HCl followed by addition of HNO<sub>3</sub> and then analysed using an Inductive coupled plasma- Optical Emission Spectroscopy (ICP- OES).

### 2.2.2. Study of Water Holding Capacity

Water holding capacity (WHC) of grains was determined by soaking 10 g of samples in 100 mL of distilled water. The effect of the soaking temperatures was studied using are 25, 35 and 45°C. The distilled water, beakers, and grains were equilibrated to the required temperatures before the start of the experiments, so that the initial temperature is almost equal to the temperature studied. The grains were removed from the water at 2 hours intervals, blotted dry to remove excess water and then weighed. The water content at each interval was calculated as the difference between the weight of the dry solids and the soaked grains. The amount of WHC was calculated using equation (6), [4,18,19]

$$\% \text{ WHC} = \frac{\left[ \begin{array}{l} \text{(mass of the soaked seeds + beaker)} \\ - \text{(mass of dry seeds + beaker)} \end{array} \right]}{\text{(mass of the beaker)}} \times 100 \quad (6)$$

### 2.2.3. Flavonoids Analysis

About 30 mg of samples was weighed and transferred into a 15 mL falcon tube and 400 µL distilled water was added. The samples were boiled for 30 minutes in a water bath at 100°C. The mixture was allowed to cool and extraction buffer (2 mL) was added to each sample. The 100 mL buffer was made by mixing 2 mL distilled water, 94.8 mL of 95% ethanol and 3.2 mL of 37% hydrochloric acid. The sample solutions were vortexed and left agitating overnight. Thereafter, the samples were centrifuged for 15 minutes at 13,000 rpm and the supernatants were collected. The 1 mL extraction buffer was added to each sample pellet, vortexed and left agitating for two hours. The samples were then centrifuged for 15 minutes at 13,000 rpm and the second supernatant was collected and mixed with the first one. The collected supernatant from each sample was centrifuged again for 30 minutes at 13,000 rpm before reading. The absorbance was measured using ultraviolet visible spectrophotometer (Cary 50, Germany) at 530 nm, 350 nm and 280 nm respectively for anthocyanins, flavonols and phenolic acids. Extraction buffer was used as blank. The phenolic acid content was calculated as ferulic acid equivalents [molar extinction coefficient (ε) 14700 Lm<sup>-1</sup> mol<sup>-1</sup>, MW 484.82], the amounts of flavonols and anthocyanin were calculated as quercetin 3-glucoside (ε 21877 Lm<sup>-1</sup> mol<sup>-1</sup>, M.W 464.38) and cyanidin 3-glucoside (ε 26900 Lm<sup>-1</sup> mol<sup>-1</sup>, MW 484.82) equivalents, [20].

### 2.2.4. Statistical Analysis

The Statistical Package for Social Science (SPSS version 25.0 SPSS Inc, Chicago, IL, USA) was used for the analysis of nutrition data. The mean values and standard deviations of the four maize samples were calculated for all replicate measurements. The significant differences in nutritional composition across maize varieties were determined using Kruskal Wallis non-parametric test. Upon the identification of the significant difference, The Mann-Whitney U test was employed to determine the specific differences. Significance was measured at the 5% level throughout.

## 3. Results and Discussion

### 3.1. Chemical Composition

#### 3.1.1. Macro-nutrients Analysis

The chemical compositional of maize varieties (white, yellow, red as well as white and red) is shown in Table 1. The red maize (8.95%) had the highest content of protein while, yellow maize (7.70%) had the lowest protein concentration, however, there was not significant difference (P > 0.05) in all the maize varieties. The high content of protein in maize could be due to the genotype as well as the cultural conditions. The lower protein content in yellow compared to white maize is in agreement with the results obtained by Cantaluppi et al. [21]. Govender et al. and Nkosi et al. [22,23] reported 10.22% and 10.10% protein on white maize, respectively which are higher than the values determined in the present study.

The major chemical component of the maize grain is carbohydrates. When comparing maize varieties, red maize had the highest content of starch (61.98%) while white maize had the lowest content (55.39%) however, there was no significant differences (P > 0.05) in the starch content observed in all maize varieties. Cantaluppi et al. [21] reported the same observation where lower starch content of 63.40% on white maize was obtained compared to 68.40% on yellow maize. The low starch content could result from the uncontrolled growing parameters that the maize varieties grew under resulting in maize not absorbing enough light energy to produce glucose, [24].

Furthermore, the differences in the starch content could be due to that starch composition in maize can be genetically controlled. Fibre content was the lowest in yellow maize (17.18%) and highest in red maize (26.01%), however, there was no significant difference in all the fibre content (P < 0.05) in all maize varieties. This indicates that yellow maize absorbed less glucose to make fibre resulting in the less fibre content which could be due to the structural component of the plant than other varieties. Nkosi et al. [23] reported 29.9% fibre on white maize, this finding agrees with results of this study. Govender et al. [22] reported 5.44% fibre on white maize which is lower than results of the present study. Fat content was comparable in all the maize varieties (4.32-5.51%). Similar finding, were reported by Cantaluppi et al., Nkosi et al. and Govender et al. [21,22,23].

Ash defined as the total mineral matter which, after combustion remain as in combustible residue of different maize varieties. Ash content for yellow maize (0.43%) was lowest while red, white, and white and red had comparable content (1.30-1.57%), however, all the ash content results had no significant difference ( $P < 0.05$ ). This indicated that yellow variety contain less proportion of non-endosperm material while, white, red, and white and red varieties contained great proportion of non-endosperm material [25]. This agrees with Govender et al. [22] findings on white variety.

### 3.1.2. Quality Assurance

Maize flour certified reference material (CRM) (FCNC21-AFE16) from Fera Science proficiency testing Ltd was analysed for quality assurance. The certified value was compared with the obtained value (Table 1). Certified value of fat (5.79%), protein (8.42%), starch (61.42) and NDF (25.72%) compared well with the measured values.

### 3.1.3. Micro-nutrients Analysis

Ca content was only detected in white maize (0.01%) and not in other varieties (Table 2). The absence of Ca content in yellow maize has been observed by Cantaluppi et al. and Govender et al. [21,22]. Mg content was present in all maize varieties with the highest amount in yellow maize (0.91%) and lowest in red maize (0.07%), however, statistical analysis indicated that there is no significant difference in Mg content from all maize varieties ( $P < 0.05$ ).

The Mg content obtained in this work is similar to those reported by Govender et al. and Cantaluppi et al. [21,22] on white maize which were 0.11% and 0.14%, respectively. However, the Mg content observed in yellow maize (0.91%) is higher than that reported by Cantaluppi et al. [21] which is 0.12%. The presence of K content was observed in all maize varieties with the highest content in white maize (0.40%) while red maize had lowest (0.24%) but all the K contents showed not to be statistically different in the maize varieties ( $P < 0.05$ ). Govender et al. [22] reported 0.31% K on white maize and Cantaluppi et al. [21] reported 0.33% K on white maize and 0.39% on yellow maize which are comparable to those obtained in this work. The Na content in all maize varieties was found to be less than 0.07% which were all not significantly different ( $P < 0.05$ ), however it was not found in white maize.

The absence of Na in white maize is in agreement with Cantaluppi et al. and Govender et al. [21,22] findings. Zn, Fe and Mn levels were not detected in all varieties which is in agreement with Cantaluppi et al. [21]. However, Govender et al. [22] reported 1.80% Zn, 2.05% Fe on white maize. The deficiency in the mineral composition may be due to genetic factors or environmental factors like soil composition, irrigation frequency and fertilizer used. However, a study conducted by Hussaini et al. [26], showed that up to 60 kg N ha<sup>-1</sup> of nitrogen fertilizer application can profoundly increase the concentrations of P, N, Ca and Mg in maize grain.

Table 1. Macro-nutrients of maize samples

Maize samples	NDF (%)	Fat (%)	Ash (%)	Protein (%)	Starch (%)	References
White	24.27±0.34	4.32±0.53	1.57±0.44	8.82±0.24	55.39±0.19	This study
Yellow	17.17±0.16	4.95±0.47	0.42±0.16	7.70±0.49	59.44±0.38	This study
Red	26.01±0.26	5.51±0.34	1.39±0.02	8.95±0.16	61.98±0.58	This study
White and Red	24.53±0.04	5.11±0.11	1.30±0.15	8.56±0.35	60.17±0.16	This study
<b>P-value</b>	<b>0.025</b>	<b>0.041</b>	<b>0.020</b>	<b>0.014</b>	<b>0.017</b>	
White	5.44	3.89	1.21	10.22	NA	[22]
White	NA	4.06	NA	8.78	63.30	[21]
Yellow	NA	5.56	NA	7.44	68.40	[21]
White	29.9	4.60	NA	10.10	NA	[23]

NA – not analysed. \*mean±standard deviation. Kruskal Wallis test; Values in bold indicate  $P < 0.05$ .

Table 2. Micro-nutrients analysis of maize samples

Minerals	White (%)	Yellow (%)	White and Red (%)	Red (%)	Govender et al. [22] (White)	Cantaluppi et al. [21] (White)	Cantaluppi et al. [21] (Yellow)
Ca	0.01±0.12	0.00±0.00	0.00±0.00	0.00±0.00	0.00	0.00	0.00
Mg	0.15±0.04	0.91±0.15	0.09±0.10	0.07±0.28	0.11	0.14	0.12
K	0.40±0.28	0.28±0.28	0.27±0.61	0.24±0.13	0.31	0.33	0.39
Na	0.00±0.00	0.01±0.17	0.04±0.09	0.06±0.03	0.00	0.00	0.00
Zn	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	1.80	0.00	0.00
P	0.37±0.11	0.24±0.09	0.10±0.10	0.08±0.20	0.26	NA	NA
Fe	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	2.05	0.00	0.00
Mn	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	NA	NA	NA
<b>P-value</b>	<b>&lt;0.05</b>	<b>&lt;0.05</b>	<b>&lt;0.05</b>	<b>&lt;0.05</b>			

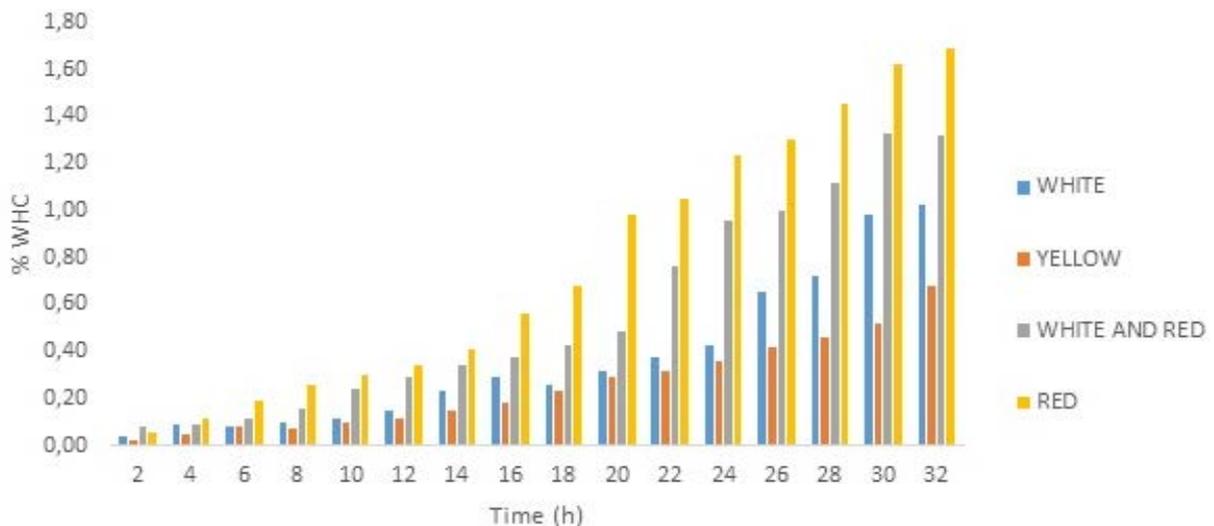
NA – not analysed. \*mean±standard deviation. Kruskal Wallis test; Values in bold indicate  $P < 0.05$ .

### 3.2. Water Holding Capacity

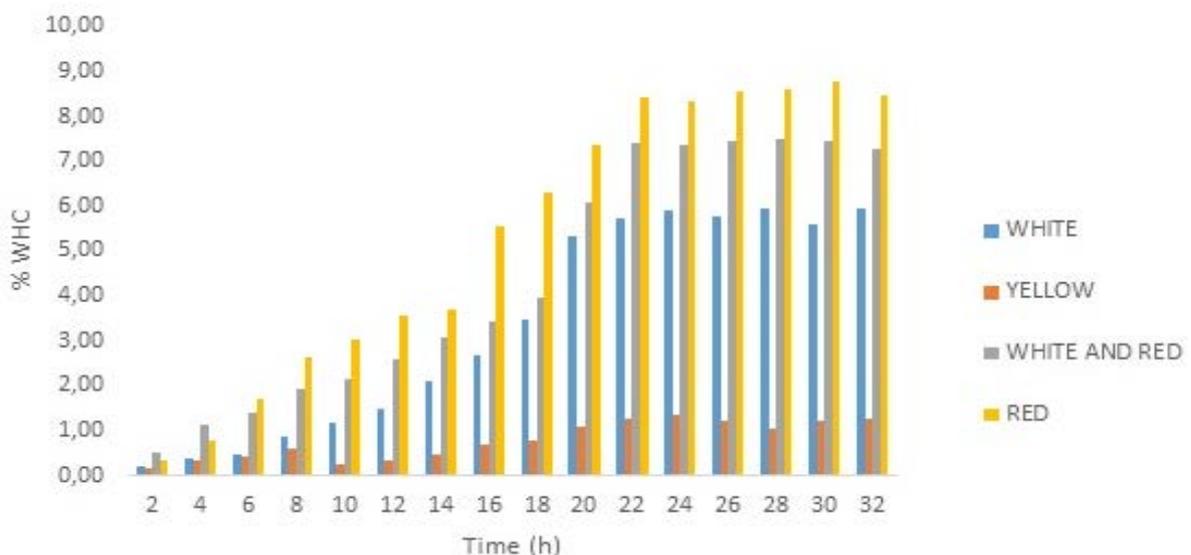
Water uptake characteristics of white, yellow, white and yellow and red maize grains are presented in Figure 1 - Figure 3, at different time and soaking temperature. Increase in soaking time showed significant effect on WHC which was observed to be initially rapid and slowed down as equilibrium was approached. This observation agrees with [27]. Similar trend was observed in all maize varieties studied in this work. This indicates that WHC increased as the grains hydration proceeds and consequently decreasing the driving force and the holding rate as the grains reaches equilibrium water content (EWC), [28]. Red maize had higher protein content and starch content showed high water holding capacity at all soaking times. This could be due to that in grains, protein is major component in absorbing water, while other components such as starch, cellulose, pectic and mucilages components also contribute to the phenomenon [6,11,29,30]. Also, WHC is influenced by carbohydrates, protein content and difference

between the water content at saturation and at a given time [11,31].

In this study with all grains investigated, high WHC was observed at high soaking temperature as compared to low soaking temperature. This finding is linked to high diffusion rate at high temperature than in low temperature [5,18,32]. The effect on WHC rate from these figures is very visible. Red maize yielded high absorption followed by white and yellow, white and yellow maize with the least absorption at all temperatures. Differences in chemical composition of maize grains and some physical properties, particularly percentage seed coat and porosity attributes to these differences. At 45°C the EWC for all varieties was reached after 18 hours (Figure 3) of soaking while at 35°C it was reached after 24 hours of soaking (Figure 2). It was noted that at 25°C the EWC was not reached after 32 hours of soaking which could be due to slow rate of diffusion at room temperature. These assessments are significant as they give an idea on how different varieties behave during rehydration, which is very vital in process and quality control.



**Figure 1.** Water content absorbed by white, red, yellow and white and red maize grains at different time intervals at 25°C.



**Figure 2.** Water content absorbed by white, red, yellow and white and red maize grains at different time intervals at 35°C.

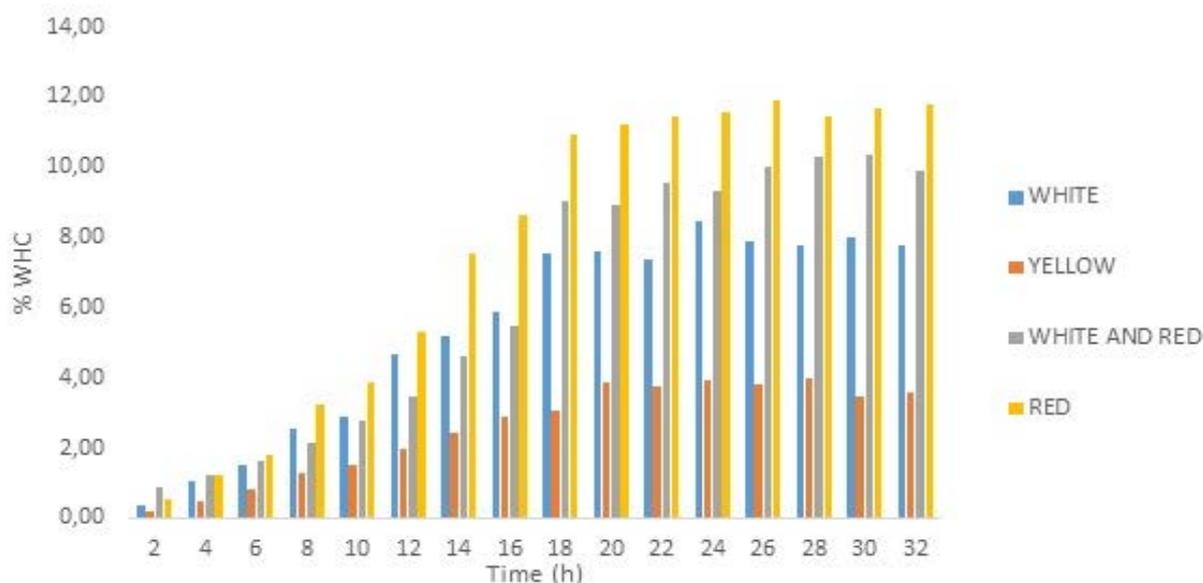


Figure 3. Water content absorbed by white, red, yellow and white and red maize grains at different time intervals at 45°C.

Table 3. Quantification of flavonoids in maize varieties

Parameter	White and Red	Yellow	White	Red
Anthocyanin	8.30±1.42	8.79±4.12	7.65±2.43	16.90±1.34
Flavonol	44.44±3.45	48.50±5.45	43.50±6.43	81.90±3.45
Phenolic acids	98.70±6.40	108.80±9.42	99.38±7.43	147.48±4.67

### 3.3. Flavonoids Quantification

The phenolic acids, flavonols, and anthocyanin were quantified as mg ferulic acid equivalents, quercetin 3-glucoside equivalents and cyanidin-3-glucoside equivalents per 100 g of maize sample, respectively. White variety showed low anthocyanin (Table 3) compared to other varieties in this study however, it was higher than white maize (3 mg 100g<sup>-1</sup>) a colourless standard reported by Lago et al. [20]. This is due to low pigmentation in white variety compared to other varieties. Phenolic acids showed high content in all varieties with the highest value of 147.48 ± 4.67 mg 100g<sup>-1</sup> for the red variety. Ferulic acid as one of phenolic compounds is advantageous to humans for cancer prevention [33]. Red and yellow variety showed phenolic content of more than 100 mg 100g<sup>-1</sup> which could be due to high pigmentation of these varieties. The high antioxidant content is often related to the biosynthetic pigments [20]. These results are in agreement with Cantaluppi et al. [21] findings.

## 4. Conclusion

Variability observed in fat, protein, ash, carbohydrates, and fiber content is both environmental and genetic which greatly influence the individual chemical composition and weight distribution of the hull and endosperm of the kernels, thus influence WHC of maize grains. These results will be beneficial to understand the nutritional properties of maize varieties planted in rural communities and guide in designing strategies that maximize maize germplasm conservation. Red maize variety was more nutritious and of good quality compared to other varieties.

Information generated indicates how each maize variety behaves during soaking process at different temperature, hence necessitating different process and quality control. This study showed that varieties maize grown in rural area of KwaZulu Natal under no controlled environment are of good quality, nutritious and contains medicinal properties.

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