Rapid Macronutrient Profiling of Maize Varieties Grown in Kirinyaga County, Kenya Using Atr-Ftir Spectroscopy and Chemometric Techniques.

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ABSTRACT

Maize *(Zea mays*), a staple food in Kenya, is vital for food security and economic sustainability, contributing 12.65% to the agricultural GDP. Despite its centrality, limited studies have explored the nutritional composition of maize varieties grown in Kenya. This study bridges the gap by applying Attenuated Total Reflectance Fourier-Transform Infrared (ATR-FTIR) spectroscopy and chemometric analysis to evaluate the macronutrient content (starch, proteins, and lipids) of 15 maize varieties cultivated in Kirinyaga County: Makueni, Duma 43, Sungura 301, Pioneer 3253, Pioneer 3506, Pioneer 3218, Pioneer 3812, Pannar 3M05, Dekalb 777, Dekalb 8031, Dekalb 8033, Dekalb 9089, Aminica 505, Tsavo 3106, and Ken Agro 5117. Using spectral data in the 4000–500 cm^{-1} range, key absorption bands were identified for starch (997, 1076, 1149 cm^{-1}), proteins (1519, 1539, 1646 cm^{-1}), and lipids (1746, 2854, 2925 cm^{-1}). Principal Component Analysis (PCA) explained 95.53% of the data variance, grouping maize varieties into clusters that highlighted genotypic similarities and differences. Heatmaps visualized nutrient distribution, identifying Ken Agro 5117 as the most nutrient-dense variety, while Duma 43 exhibited lower levels of starch, proteins, and lipids. The results emphasize the predominance of genetic factors in determining nutritional profiles, though agronomic practices contribute to variability. This study demonstrates the efficacy of ATR-FTIR spectroscopy as a rapid, cost-effective tool for nutritional profiling, offering valuable insights into optimizing maize varieties for dietary and ecological suitability

Keywords: Maize, ATR-FTIR Spectroscopy, Principal Component Analysis, Proteins, Starch, Lipids

INTRODUCTION

Maize (*Zea mays)* is a staple food in Kenya. Maize was originally domesticated by early civilizations in southern Mexico, spreading through the Americas and later spreading to the rest of the world. (Goodman and Galinat, 1988) Maize is one of the most cultivated cereals in the world. Maize can be consumed directly or used to manufacture cooking oil, animal feeds, corn syrup, and other maize product (Orhun et al., 2013).Maize accounts for 40% of cultivated area, 2.4% of Kenya's GDP, and 12.65 % of agricultural GDP, with an average per capita intake of about 98 Kgs per annum (Muyanga et al.,2005) compared to 73 Kgs per annum,50 Kgs per annum and 42 Kgs per annum for Tanzania, Ethiopia, and Uganda respectively (Were,2021). Small-scale farmers produce more than 75 % of Kenya's maize yield. Maize in Kenya is ground into flour and consumed as stiff porridge by about 78% of Kenya's 50 million population. Its nutritional value directly impacts food security (Muyanga et al.,2005). In Kenya, maize cultivation spans diverse agroecological zones, resulting in various maize varieties with varying agronomic and genetic traits. However, limited attention has been given to understanding the nutritional composition of these maize varieties. Most studies have focused on yield improvement, disease resistance, pest control and other agronomic characteristics, while the nutritional aspects have often been overlooked (Naseem et al., 2018; De Groote, 2002; Khan, 2001; Omoyo, et al., 2015). FTIR has proved as an effective and cheap tool for rapid analysis of the nutritional composition different food substances such as sorghum (lin et al.,2021; Indrayanto and Rohman, 2020; Valand et al., 2020). Kirinyaga County represents a typical agricultural region in Kenya where maize farming is a crucial economic activity among small-scale farmers (Mbure et al., 2010). With a growing population and increasing demand for maize-based products, it is crucial to understand the nutritional content of different maize varieties cultivated in the country. The nutritional composition of maize is a crucial determinant of its dietary value and impact on human life.

The goal of this study was to apply ATR-FTIR and chemometric analysis to identify the presence of select macronutrients (carbohydrates, starch, and proteins) and the potential similarity in their quantities among the different varieties grown in Kirinyaga County, Kenya, which would suggest potential genotypic similarities. Twenty-five maize samples were collected from demo farms in Kirinyaga County. The wave numbers associated with the different nutrients were identified to achieve the set goal. The absorbance intensities for those particular wave numbers were used to indicate the quantities of the nutrients in a given variety. It was hypothesized that a meaningful variation in the nutritional profiles of the different maize varieties was found.

MATERIALS AND METHODS

Fourier-transform infrared (FTIR) spectroscopy is a highly versatile analytical technique utilized for qualitative and quantitative analysis across various scientific domains, such as chemistry, pharmaceuticals, environmental science, and the food industry (Petibois and Desbat, 2010; Simonescu, 2012). FTIR spectroscopy passes infrared radiation through a sample and records the absorbed wavelengths. Each molecular bond within the sample absorbs specific frequencies of infrared light, resulting in a unique spectral fingerprint (Berthomieu and Hienerwadel 2009). Quantitative analysis can be achieved by correlating the intensity of absorption bands to the concentration of analytes, typically using the Beer-Lambert Law. Advanced computational techniques, such as multivariate calibration, refine the quantification process by accounting for complex sample matrices and overlapping spectral features.

The concept of Attenuated Total Reflectance (ATR) was first presented by Harrick in 1967. In ATR measurements, an infrared (IR) beam passes through a prism and is reflected internally at the back surface of the prism, which is in contact with the sample. For total internal reflection to occur, the following conditions must be met, $sin hi > n1/n2$, where hi represents the angle of incidence on the back of the prism, n1 is the refractive index of the prism material (diamond, in this case), and n2 is the sample's refractive index. Under these conditions, an evanescent wave is generated at the interface, penetrating a small distance into the sample, enabling it to interact with the sample and causing the absorption of part of the IR radiation. The extent of absorption depends primarily on how well the sample is in contact with the prism, as well as the penetration depth of the evanescent wave (Blum and John, 2012).

Harrick (1967) further noted that the penetration depth depends on the wavelength of the IR light; longer wavelengths result in deeper penetration. For example, polysaccharides such as starch absorb IR radiation in the 1200–800 cm^{-1} range (with wavelengths around 8 to 12 µm), leading to an average penetration depth of roughly two µm. This is one reason ATR is often considered a surface-sensitive technique, given the relatively shallow depth of wave penetration (Sevenou et al., 2002).

Kirinyaga County (0.6591° S, 37.3827° E) is located in central Kenya near the foothills of Mount Kenya, it covers 1,478.1 square kilometers and is bordered by Embu, Nyeri, and Murang'a counties. The county enjoys a tropical climate, with annual rainfall ranging from 1,000 mm to 2,500 mm and temperatures between 12°C and 26°C. Its agro-ecological zones range from highlands, suitable for tea and dairy farming, the midland zones support mixed farming, including crops like maize and beans, while the lowlands, particularly the Mwea region, are ideal for irrigated rice farming. Its strategic location within the Mount Kenya region makes it a vital agricultural hub in the country. Kirinyaga's fertile volcanic soils vary by altitude, andosols in the highlands for tea and coffee, vertisols in the lowlands for rice, and nitisols in mid-altitudes. Agriculture drives the economy, with key activities including rice farming, tea, coffee, maize, horticulture, and dairy farming. Tourism, trade, and fish farming also contribute to the local economy. Administratively, the county is divided into five sub-counties: Kirinyaga Central, Kirinyaga East, Kirinyaga West, Mwea, and Ndia, with its headquarters in Kerugoya. The county's climate, soils, and irrigation systems make it a significant agricultural hub in Kenya. Samples were collected largely in the maize growing mid laying region, effort was made to collect samples from the five sub

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counties.

Maize Varieties

Twenty-five samples of fifteen maize varieties (Makueni, Duma 43, Sungura 301, Pioneer 3253, Pioneer 3506, Pioneer 3218, Pioneer 3812, Pannar 3M05, Dekalb 777, Dekalb 8031, Dekalb 8033, Dekalb 9089, Aminica 505, Tsavo 3106 and Ken Agro 5117) were collected directly from small-scale farmers' fields after the maize was mature and some drying had taken place. The study region and sampling sites is represented in the map in Figure 1:

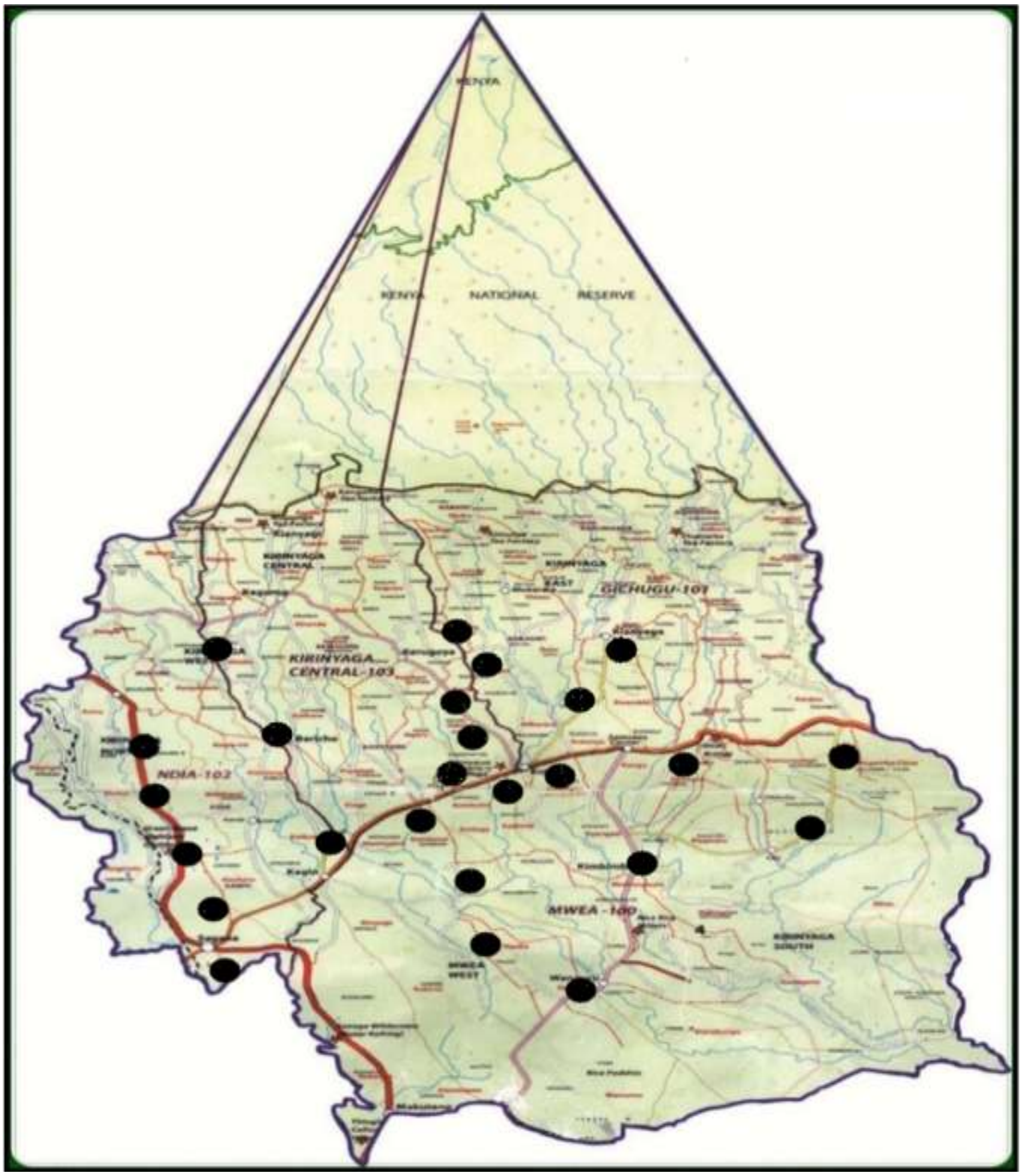

Figure 1: Map of Kirinyaga county showing sample collection points.

The farmers had received the planted seeds from the different seed producing companies as demonstration farms in the region.

Sample Preparation

Sun dried maize grain samples (500g dry weight) were milled and homogenized using a rotary miller and sieved using a 0.5 mm sieve. Each ground sample was stored in 500 g Ziplock sample collection bags, then stored at room temperature, away from moisture and direct sunlight prior to the infrared spectra acquisition.

Atr-Ftir Spectroscopy and Chemometrics

FTIR spectra were taken from Shimadzu IR Spirit with a QATR-S accessory. A background spectrum of the cleaned crystal was taken and subtracted from the sample spectra. About 0.1 g of the solid powdered samples were directly placed on the crystal, and the spectra obtained. The spectra were recorded in absorbance mode. The spectra were collected in triplicates for each sample and in total 75 spectra were collected.

The spectra were then averaged using Origin Pro, Version 2022. (OriginLab Corporation, Northampton, MA, USA.) the averaged spectra were baseline-corrected by drawing a straight line. IR absorbance values at 997, 1076, 1149 (starch), 2854, 2925, 1746, 1709 (lipids), and 1519, 1539, 1646 (proteins) were extracted from the baseline corrected values by recording the height of these absorbance peaks from the baseline (Kuhen et al., 2010; Achten et al., 2019; Ogbaga et al.,2017; Lin et al.,2020; Castillo-Guaca, 2023).

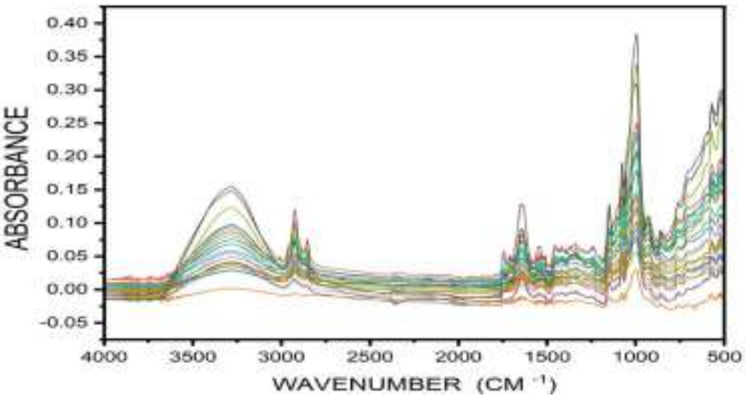

Figure 2 shows the FTIR spectra for the twenty-five ground maize samples. The majority of the absorption peaks were detected in the 3700 *to* 600 cm^{-1} spectral range.

The spectra in those regions showed minor differences which would make structural differences hard to detect however the absorbance peaks showed significant absorbance differences which indicates quantitative differences among the sample. Closer inspection of absorbance at different wavenumbers of interest (Figure 3), it is clear that the patterns are unique e.g. in Figure 3a the Ken-agro 5117 has the highest absorbance intensity at 1650 cm^{-1}, whereas in figure 3b, at 1000cm^{-1}, pioneer 3812 has the highest absorbance intensity.

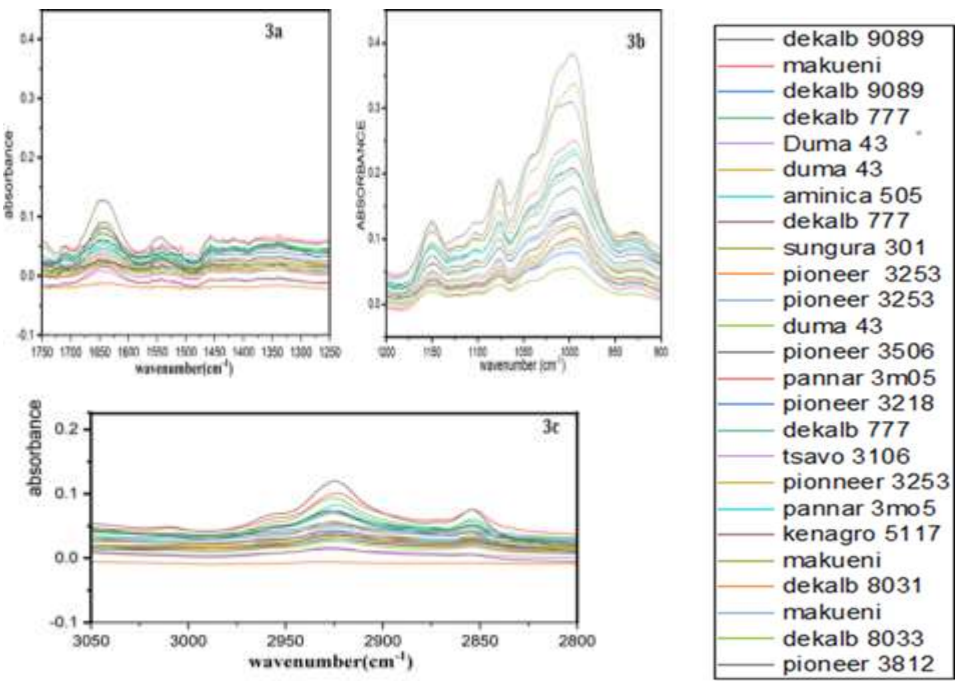

Figure 3: (3a) ATR-FTIR spectra 1250 *to* 1750 cm^{-1} ,((3 b) ATR-FTIR spectra 900 *to* 1200 cm^{-1} and (3 c) ATR-FTIR spectra 2800 *to* 3050 cm^{-1} of flour obtained from maize varieties collected in Kirinyaga, Kenya.

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Chemometric Analysis

PCA was then applied to allow for the extraction of more conclusive data from the extracted absorbance intensity peaks at 997 cm^{-1} ,1076 cm^{-1} ,1149 cm^{-1} (starch),2854 cm^{-1} ,2925 cm^{-1} , 1746 cm^{-1} *and* 1709 cm^{-1} (lipids) and 1519cm^{-1},1539cm^{-1},1646cm^{-1} (proteins). This was done using the multivariate data analysis software (OriginPro, Version 2022. OriginLab Corporation, Northampton, MA, USA.)

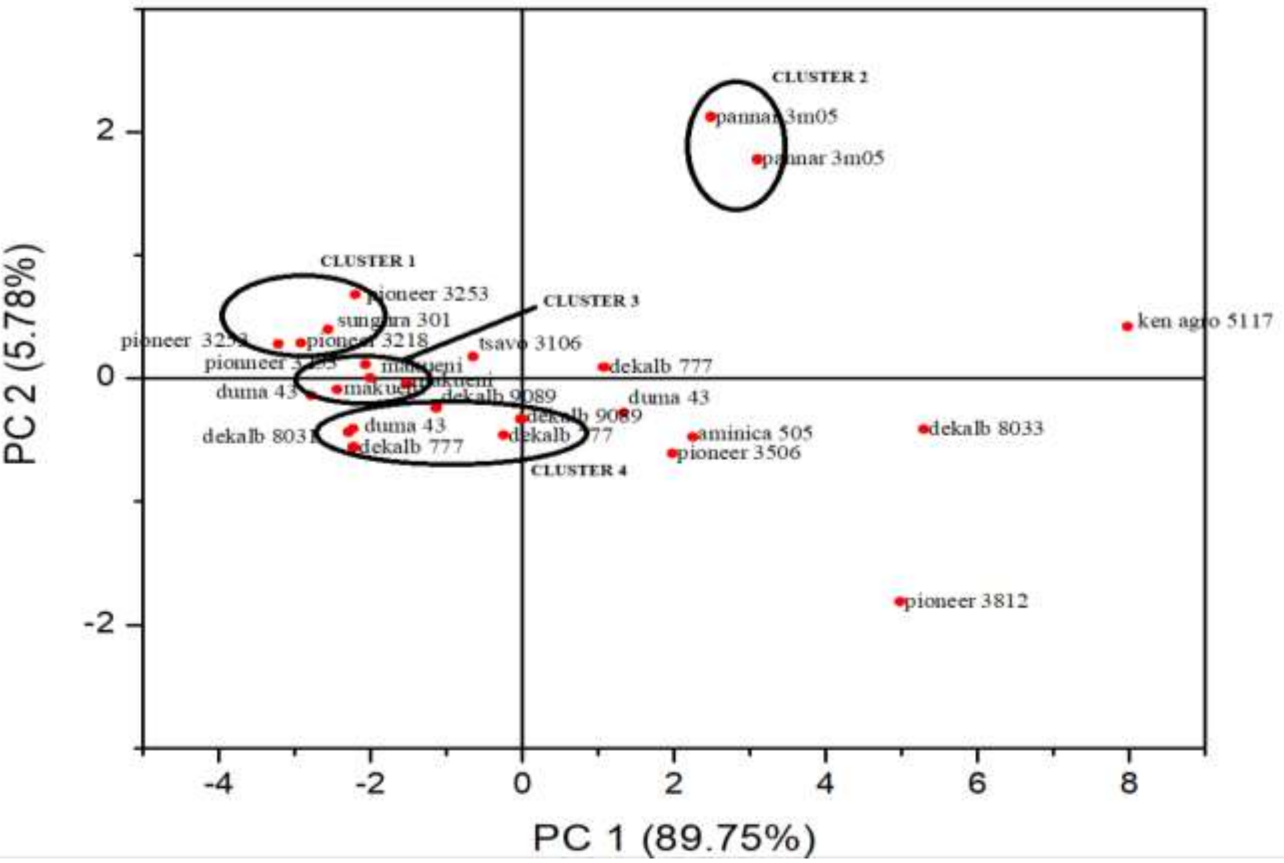

Figure 4: PCA scatter plot for the intensities in the wavenumbers 997cm^{-1},1076cm^{-1},1149cm^{-1},2854cm^{-1},2925cm^{-1},1746cm^{-1},1519cm^{-1},1539cm^{-1},1646cm^{-1} and 1709 cm^{-1}

PC1 and PC2 were responsible for 95.53% of the variability in the dataset. PC1 was responsible for 89.75% of the variability. The scatter plot was separated into four close but distinct clusters—cluster 1 contained Pioneer 3253, Pioneer 3218, and Sungura 301. The clustering implies that the three varieties have similar biochemical profiles. A second cluster, 2, was observed that had pannar 3M05 only. This cluster was separated from all the others, which implied that pannar 3M05 has a unique nutritional profile compared to the other varieties in this study. A third cluster, three, was located very close to cluster 1, comprised majorly of the Makueni variety (DLC 1). This variety was developed by the Kenya Agricultural and Livestock Research Organization (KARLO). The variety was bred to enhance food security in arid and semi-arid areas with a focus on drought resilience, early maturity, and improved yield in limited water supply. This can be thought of as indicating that the Makueni variety had its own unique nutritional composition.

Cluster 4 was the largest of the clusters contained Duma 43, DeKalb 777, DeKalb 9089 and, Dekalb 8031. This cluster comprised of three of the four DeKalb varieties which implies that the three varieties had similar nutritional profiles. This may arise from a similar genotypic make up since all the samples had been collected from different farmers in different locations therefore, any similarities that may arise would be largely due to similar genotypic makeup. The fourth DeKalb variety (8033) did not cluster in any of the clusters implying some uniqueness of its nutritional makeup. All DeKalb varieties have however, been separated by PC 1 with little separation by PC 2. Ken agro 5117 and Pioneer 3812 separated from the rest of the samples indicating unique profiles. Pioneer 3506 and Aminica 305 separated from the other clusters and shown similar nutritional properties. Both genetic and agronomic effects create some variation in the amount and quality of each of these

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constituents (Baye et al., 2006). However, as evidenced by this study despite agronomic effects producing some degree of variation on the nutritional composition within a variety the greatest determinant of the nutritional composition was mainly genotypic. This is evidenced by the fact that most of the varieties clustered together despite being grown in different areas and by different farmers.

The impact of farm practices on the final nutritional composition cannot be ignored since some varieties like Duma 43 which had three samples failed to cluster together. The variety showed little variance in terms of PC2 but were greatly separated by PC 1.

Heatmap.

To visualize the specific macro-nutrient uniqueness or differences of the maize varieties already highlighted in general terms by PCA, a heat map(green-yellow-red) of the spectral data set was plotted (Figure 5). From visual inspection it could be seen that both duma 43 and the Makueni varieties had generally low (red)

Figure 5 Heat map plot for the absorbance intensities at wavelengths 997cm^{-1},1076cm^{-1},1149cm^{-1},2854cm^{-1},2925cm^{-1},1746cm^{-1},1519cm^{-1},1539cm^{-1},1646cm^{-1} and 1709 cm^{-1}

absorbance intensities in the wavelengths corresponding to different nutrients. This indicates a lower nutrient (starch, protein, and lipid) content than the other varieties. This could indicate that they are less nutrient-dense. The varieties are likely not ideal for nutrient-rich crops but may serve other purposes due to their resilience towards adverse conditions or for growth in specific ecological conditions. The DeKalb varieties (8033,777,8031 and 9089) showed generally medium (yellow) to high(green) levels of carbohydrates and proteins. As for lipids; these varieties showed medium levels providing a consistent source of fatty acids without being exceptionally high when compared to other varieties in this study. Dekalb 8033 had the highest levels and most balanced nutritional profile of all the DeKalb varieties. DeKalb 8031, on the other hand, had the lowest nutritional content of the varieties, with a moderate level of starch but low levels of lipids and carbohydrates.

Pioneer 3253 and 3218 showed low nutritional content across the three nutrients; however, the levels of lipids in Pioneer 3253 ranged from moderate to low. Pioneer 3812 and 3506 showed well-balanced nutritional profiles, with their nutritional contents ranging from moderate to high compared to the other varieties in the study. It is also worth noting that Pioneer 3812 had notably high levels of starch compared to all the varieties in the study. This could indicate its suitability as a carbohydrate-rich crop with a balanced nutrient source. Pannar 3M05 showed a balanced nutritional profile with moderately high levels of starch, proteins, and lipids. It was also noted that the variety indicated a very high absorbance intensity at 1746, which was particularly unique for all the samples in the study, this indicates its higher oil content. Aminica 505 and Tsavo 3106 have balanced nutritional profiles, with their levels of nutrients being moderate; however, it was noted that Tsavo had a lower level of proteins in comparison. Sungura 301 was noted to have a very similar profile to Pioneer 3253, which could indicate a similarity in their nutritional genetic makeup. Ken agro 5117 was the most nutritious of all the varieties in this study. It had high levels of all three nutrients that were measured, which could indicate its suitability as a balanced food source or its suitability to Kirinyaga's ecological profile.

CONCLUSION AND RECOMMENDATIONS

The nutritional profiling of maize varieties revealed substantial differences in macronutrient composition, which has important implications for both dietary planning and agricultural policy. Varieties such as Ken agro 5117, Pioneer 3812, and Pannar 3M05 demonstrated the highest and most balanced levels of starch, proteins, and lipids, making them ideal for nutrition-sensitive agriculture, household food security, and wider food security initiatives. In contrast, Duma 43 and Makueni exhibited consistently low nutrient levels, which limits their suitability as nutrient-rich food sources but highlights their potential role in marginal or drought-prone areas where resilience and ecological adaptability are more critical. Moderately nutritious varieties, including Aminica 505, Tsavo 3106, and DeKalb 8033, provide flexible options for general consumption, while Pioneer 3812 stands out as a carbohydrate-rich variety suitable for starch-based industries.

Based on these findings, farmers are encouraged to prioritize nutrient-dense varieties such as Ken agro 5117, Pioneer 3812, and Pannar 3M05 for household consumption, while cultivating resilient but less nutrient-dense varieties like Duma 43 and Makueni in high-risk environments where food security is threatened by climatic stress. Policymakers should integrate nutritional value into seed distribution and agricultural support programs, ensuring that high-nutrient varieties are promoted in areas with high nutritional vulnerability. Nutritionists, on the other hand, should emphasize dietary diversification in households that depend heavily on low-nutrient maize varieties, encouraging the inclusion of protein-rich legumes, vegetables, and animal-source foods to improve overall dietary quality. Importantly, fortification strategies should be adopted to enhance the nutritional value of low-density varieties. This may include blending maize with protein-rich legumes, promoting bio-fortified varieties, or applying post-harvest fortification with essential micronutrients. Finally, the agro-industry is encouraged to target specific varieties for specialized uses, such as employing Pioneer 3812 for starch-based products or Pannar 3M05 for oil-rich applications, thereby aligning nutritional strengths with market and consumer needs.

FTIR, PCA, and heat-maps proved to be useful in the study of diversity among maize varieties grown in Kirinyaga county, Kenya. The tools allowed for a cheap, fast, and relatively simple method of discriminating the varieties based on their composition while heat-maps provided an easy way for visualization and comparison of the nutritional profiles of the different varieties.

Conflict Of Interest

The authors of this paper declared no conflict of interest.

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