

ORIGINAL RESEARCH ARTICLE

Effect of Pigeon Pea and Sorghum Flour Supplementation on Protein Content, In Vitro Protein Digestibility, and Selected Storage Quality Indicators of Millet Flour Blends

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ABSTRACT

Protein-energy malnutrition (PEM) remains a significant public health issue among children in many parts of Kenya. This study aimed to utilize locally available sorghum, pearl millet, and pigeon peas to develop a protein-rich complementary flour blend that could contribute to efforts to improve the nutritional quality of complementary foods for young children in Tharaka Nithi County, Kenya. The study employed a nested experimental design with pigeon pea level (0, 15, 30, 45, and 60% of the total flour blend) as the main factor and sorghum level (0, 20, and 40% of the remaining flour after pigeon pea addition) nested within each pigeon pea level to evaluate protein content and *in vitro* protein digestibility of flour blends. An additional factor, storage period (days), was included to assess selected storage quality indicators of the millet-based flour blends during storage. Formulated flour blends were then analysed for protein content, protein digestibility, yeast and mold counts, titratable acidity, and pH. The results revealed that the addition of pigeon peas significantly ($p < 0.001$) increased the protein content and the digestibility of the flour blends. The average relative humidity and temperature during storage were 55.5% and 25.2 °C, respectively. The addition of pigeon peas to the blends significantly ($p < 0.002$) increased the titratable acidity of the composite flour blends. The initial mean yeast and mold count was 2.439 ± 0.147 Log CFU/g, which was below the East African Community recommended safety limit for millet flour (4 Log CFU/g). The yeast and mold count slightly increased during storage in the first 16 days, followed by a significant decrease, reaching 1.052 Log CFU/g by day 45. These findings demonstrate the potential of pigeon peas, sorghum, and millet as locally available ingredients for the development of nutritionally improved complementary foods.

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1. INTRODUCTION

Cereal-based diets are a staple food source in many parts of the world, especially in sub-Saharan Africa and South Asia (Ramashia et al., 2021). In Kenya, they form part of the daily meal for weaning children as well as for school-going children (Temba et al., 2016). These cereals are often energy-dense and low in protein and micronutrient density. As a result, malnutrition remains a public health concern among children in Kenya (Okutse & Athiany, 2025). Thousands of children in Kenya are suffering from moderate acute malnutrition, some of whom are affected by severe acute malnutrition, with stunting rates at 18 per cent among children under five (Richardson & Teta, 2025; Richardson et al., 2025). Without appropriate interventions, these cases can be fatal, contributing to the deaths of thousands of children under five years of age in Kenya each year (Richardson & Teta, 2025). Children from socioeconomically disadvantaged households are particularly vulnerable and face a higher risk of stunting, underweight, and wasting (Okutse & Athiany, 2025).

According to Gudu et al. (2020), child feeding practices are one of the major causes of malnutrition among children under five years old in Kenya. In their research, they found that 84% of children who had age-inappropriate anthropometric measurements were undernourished. Tharaka Nithi County is classified as a semi-arid region. Although the prevalence of poor or borderline food consumption was reported as occurring between 5-10%, its classification of stunting prevalence by the WHO is still very high (30.0% - 39.9%). In addition, the rates of acute malnutrition were reported to be alarming, with the potential for deterioration (WFP & UNICEF, 2022).

In many parts of Kenya, including Tharaka Nithi County, dietary protein sources may be available but remain underutilized despite high malnutrition cases. For instance, pigeon pea production is relatively high in eastern Kenya, including Tharaka Nithi County, yet cases of stunting among children persist due to reliance on cereal-dominated diets (KNBS, 2024). Recent national nutrition indicators show that 21% of children under five years of age in Tharaka Nithi County are stunted, 3% are wasted, and 8% are underweight. This paradox is largely attributed to prevailing dietary patterns in which cereal-based foods dominate complementary feeding and meals for school-going children. Among these cereals, millet and sorghum are particularly important because of their adaptability to harsh climatic conditions, drought tolerance, and their contribution to food security in semi-arid regions (Anal et al., 2024; Ramashia et al., 2025). Sorghum is occasionally used together with millet in weaning diets in Tharaka Nithi County (Mucioki et al., 2016). Sorghum inclusion in millet-based products can help to diversify the amino acid profile of the final product. Additionally, sorghum is rich in phytochemicals such as phenolic compounds, which have antioxidant properties and play a role in extending the shelf life of food products by inhibiting lipid oxidation and microbial growth (Fernando et al., 2025). However, despite these advantages, cereal proteins are often deficient in certain essential amino acids, particularly lysine, tryptophan, and threonine, which limits their protein quality when consumed as the primary dietary protein source. Further, they may also contain anti-nutritional factors such as tannins, phytic acid, and phenols (Kaur et al., 2024; Ramashia et al., 2025).

To address the limitations of cereal proteins, fortification with legumes has been widely recommended (Parle et al., 2026) particularly utilizing underutilized grains such as pigeon peas (Ogunniran et al., 2024). Legumes are rich sources of protein and essential amino acids such as lysine, although they are typically limited in methionine, which is present in cereals. Because of this complementary amino acid profile, combining cereals and legumes can significantly improve overall protein quality in food formulations (Vilakazi et al., 2025).

Pigeon pea (*Cajanus cajan*) is one of the important legumes grown in eastern Kenya and has considerable potential for improving the nutritional quality of cereal-based foods. It contains high levels of protein (approximately 18–25%), dietary fiber, B-vitamins, and essential minerals such as iron and zinc (Haji et al., 2024). However, plant-based foods often contain anti-nutritional factors such as phytates, tannins, and protease inhibitors that may reduce protein digestibility and mineral bioavailability (Ogunniran et al., 2024). Thus, processing to enhance protein digestibility is required whenever these legumes are used in product development (Yu et al., 2020).

Therefore, this study aimed to improve the protein content and quality of millet flour by supplementing it with pigeon pea and sorghum flour and to investigate the effect of this supplementation on protein content, *in vitro* protein digestibility and selected storage quality indicators.

2. MATERIALS AND METHODS

2.1 Study site

This study was carried out at Chuka University, Department of Food Technology and the Department of Animal Science laboratories. The materials (millet, sorghum and pigeon peas) used in this study were sourced locally from Chuka town in Tharaka Nithi county, Kenya. All grains were sourced from the same supplier in a single purchase. The grains were mature, free from visible defects, insect damage, and mold, and were stored under dry ambient laboratory conditions before processing.

2.2 Experimental design

A nested experimental design was used to evaluate protein content and *in vitro* protein digestibility, with pigeon pea level (0, 15, 30, 45, and 60% of the total flour blend) as the main factor and sorghum level (0, 20, and 40% of the remaining flour after pigeon pea addition) nested within each pigeon pea level. The study involving selected storage quality indicators employed the same nested design, with storage period (days) included as an additional factor. All samples were analysed in triplicate.

2.3 Preparation of individual grain flour samples

The preparation of millet and sorghum flour was done as shown in **Figure 1**, while that of pigeon pea flour followed the steps outlined in **Figure 2**. The grains were sorted separately, followed by further cleaning by winnowing to remove broken kernels and foreign matter. The grains were then washed using potable water to remove dirt and dust, rinsed again using potable water followed by oven drying to a moisture content of

12%. Before drying, pigeon peas were soaked in potable water overnight (12 hours) to reduce the levels of antinutrients through dissolution and leaching, then rinsed with clean water. After soaking, the peas were boiled using a saucepan for 30 minutes, to cook them adequately but not mushy, for ease of handling during drying. Draining of the boiling water followed

by instant cooling using cold potable water, followed by oven drying at 70 °C for 48 hours was done. The dry peas were transferred into an airtight container and stored in a cool dry place awaiting milling. The dry grains were milled to fine flour with a laboratory hammer mill fitted with a 0.8 mm screen.

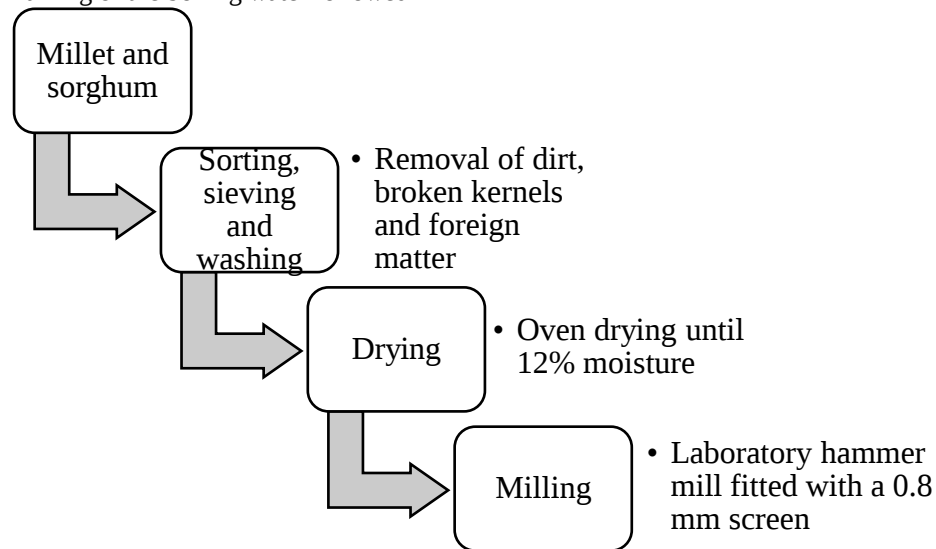


Fig. 1. Flow diagram illustrating the preparation of millet and sorghum flours used in the formulation of composite flour blends.

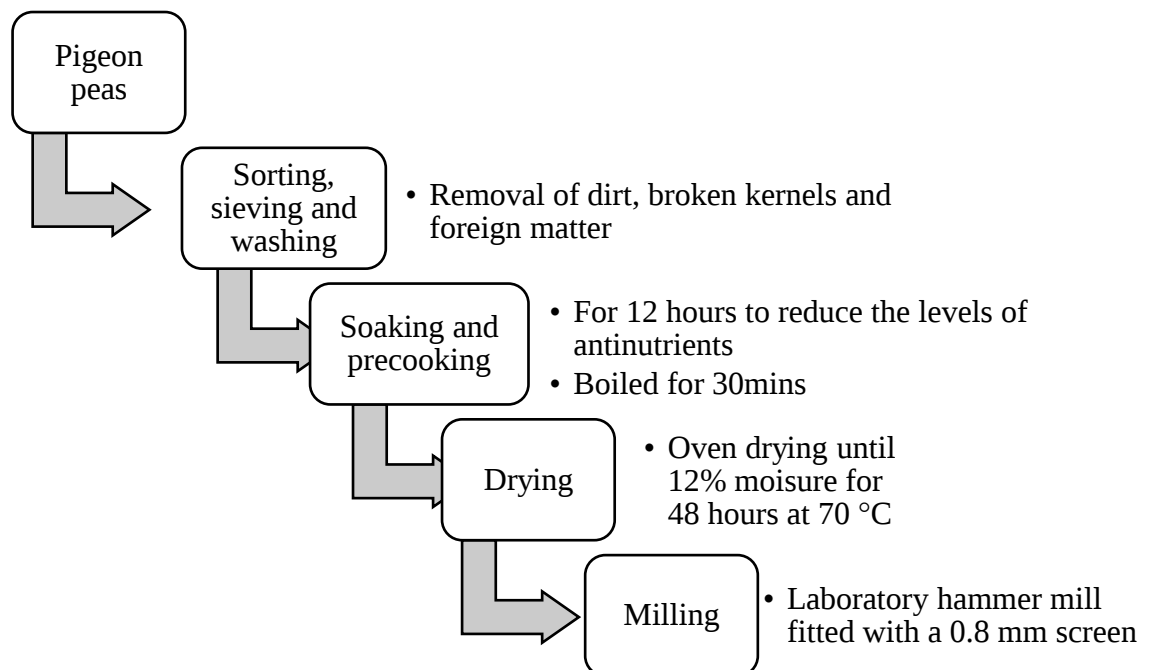


Fig. 2. Flow diagram illustrating the preparation of soaked and precooked pigeon pea flour used in the formulation of composite flour blends.

2.4 Preparation of flour blends

The ground flours were used immediately in sample formulation to get the different flour blends. The flours were mixed in ratios to yield 15 different flour blends as shown in **Table 1**. The soaked and precooked pigeon pea flour was varied from 0 to 60% of the total flour blend, while sorghum flour varied from 0-40% of the remaining proportion after the addition of pigeon pea flour. The flour blends were prepared by first adding pigeon pea flour at the specified level (0, 15, 30, 45

and 60%), then incorporating sorghum flour at 0, 20, or 40 percent of the remaining proportion after the addition of pigeon pea flour, and finally completing the mix with millet flour to make up to 100%. For example, blend number 5 in **Table 1**, was made such that 15% was purely pigeon pea flour, 20% of the remaining 85% of the blend was contributed by the sorghum (17% of the overall blend) and the remainder by millet (68%). The prepared blends were then stored in paper bags, each containing 250 grams of flour, and placed inside an airtight plastic container at room temperature awaiting further analysis.

Table 1. Formulation of millet–sorghum–pigeon pea composite flour blends showing ingredient proportions (%)

Blend	Millet (%)	Soaked and pre-cooked pigeon peas (%)	Sorghum (%)
1	100	0	0
2	80	0	20
3	60	0	40
4	85	15	0
5	68	15	17
6	51	15	34
7	70	30	0
8	56	30	14
9	42	30	28
10	55	45	0
11	44	45	11
12	33	45	22
13	40	60	0
14	32	60	8
15	24	60	16

Sorghum percentages at each pigeon pea level represents 0, 20, and 40% of the remaining flour fraction after pigeon pea incorporation.. All formulations total 100%.

2.5 Packaging and storage conditions

After preparation, the composite flour blends (15) were immediately aseptically packaged into paper bags, which were breathable and most ideal for flour products. Each packet contained approximately 250 grams of composite flour. After packaging, samples were stored at room temperature (20-25 °C) for up to 65 days. The packs were stored in an airtight plastic container, which was only opened during sample collection and immediately closed to limit exposure to the surrounding environment. Samples were collected for analysis on days 0, 16, 34, 45 and 65. During packaging, 5 samples were prepared per blend in total (one per sampling day) and thus, once opened, samples were disposed. A humidity and

temperature data logger (ET-176) was used to monitor the relative humidity and temperature inside the storage container.

2.6 Determination of crude protein content

Crude protein content was determined through the Kjeldahl method (AOAC, 2000). Flour samples were digested at 420 °C with concentrated sulphuric acid, potassium sulphate and a metal catalyst (copper (II) sulphate), followed by distillation. Boric acid was used to trap ammonia gas. Titration was then done using 0.1N hydrochloric acid. Total Nitrogen (%) was calculated as shown in Equation 1. Total Crude protein (%) content was calculated by multiplying the total nitrogen (%) by a conversion factor of 6.25 as shown in Equation 2 (Fujihara et al., 2008).

Equation 1.

$$\text{Nitrogen (\%)} = \frac{(V_{\text{acid}} - V_{\text{blank}}) * N_{\text{acid}} * 1.4007}{W_{\text{sample}}}$$

Where, V_{acid} = volume of acid used for the sample (mL), V_{blank} = volume of acid used for the blank (mL), N_{acid} = normality of the acid, and W_{sample} = sample weight (g).

Equation 2.

$$\text{Crude Protein (\%)} = 6.25 * \text{Nitrogen (\%)}$$

Where 6.25 is the nitrogen-to-protein conversion factor,

2.7 In vitro protein digestibility

Protein digestibility was determined following the method described by Ouma et al. (2022). In the digestion tube, 0.5 g of composite flour sample was suspended in 9.5 ml of 0.1M HCl and mixed with 5mg pepsin (1:3000; activities 0.8 Anson units/mg, Mumbai, India) in 0.5 ml 0.1M HCl and covered. The mixture was incubated in a shaker water bath at 37°C for 2 hours. The solution was neutralized by adding 10 ml 1.0M phosphate buffer (pH 8.0) followed by the addition of trypsin (Pankreasprotease; activity min 200 FIPU/g, Merck Darmstadt,

Germany) (100:1 substrate-to-enzyme ratio, w/w). The digestion tubes were covered and incubated again in the water bath at 37°C for 2 hours. Enzyme activity was terminated by adding equal volumes (10 ml) of trichloroacetic acid (10% w/v). Samples were immediately transferred to a freezer (-25°C) for 20 min, after which samples were vortexed for 15 minutes and left for 1 hour to settle. The supernatant was decanted and the residue transferred into a moisture dish and dried at 105°C for 3 hours. About 0.2 g residue was weighed and the nitrogen content of the residue was determined by the Kjeldahl method as described in the section on determination of crude protein content. A reagent blank containing all

digestion reagents but no flour sample was subjected to the digestion process in the same manner as the test samples. Protein digestibility was calculated by getting the difference between the total amount of protein and the residual amount of protein after pepsin and trypsin digestion divided by the total Equation 3.

$$\text{Digestibility (\%)} = \frac{TAP - RAP}{TAP} \times 100$$

Where: TAP = total amount of protein before digestion, and RAP = blank-corrected residual amount of protein remaining after pepsin and trypsin digestion.

Determination of the Selected Storage Quality Indicators of the Composite Flour Containing Sorghum, Millet and Soaked and Precooked Pigeon Peas

2.8 Microbiological analysis

Determination of yeast and mold counts was done following the method outlined by Tournas et al. (2001) in the Food and Drug Administration's bacteriological analysis manual. Briefly, 10 g of flour was aseptically homogenized with 90 mL of sterile peptone water to obtain a 10^{-1} dilution. Serial ten-fold dilutions were prepared as required, and 1 mL aliquots of the appropriate dilutions were pour-plated in triplicate on chloramphenicol yeast glucose agar. The plates were incubated at room temperature (20–25°C) for 5 days. Visible colonies were counted and recorded and results expressed as colony-forming units per gram (CFU/g). The method detection limit was 10 CFU/g. Samples with microbial counts below the detection limit (<10 CFU/g) were assigned a value of half the detection limit (5 CFU/g) prior to logarithmic transformation. Microbial counts were then $\log_{10}$ -transformed before statistical analysis. Results are presented as mean $\pm$ standard error (SE) of the log-transformed data.

2.9 Analysis of titratable acidity and pH

Titratable acidity was determined following the method outlined by Acheampong et al. (2024) with some modifications. Briefly, 10 g of composite flour was accurately weighed into a 250 mL conical flask, and 100 mL of distilled water was added. Equation 4.

$$Y_{ijk} = \mu + P_i + S_{j(i)} + \varepsilon_{ijk}$$

where μ is the overall mean, P_i is the effect of the i th pigeon pea level (0, 15, 30, 45 and 60% of the total flour blend; $i = 1-5$), $S_{j(i)}$ is the effect of the j th sorghum level nested within the i th pigeon pea level ($j = 1-3$), and ε_{ijk} is the random error associated with the k th replicate ($k = 1-3$).

The storage quality indicators (pH, titratable acidity, and yeast and mold counts) were analyzed using the same nested structure with storage period included as an additional factor as shown in Equation 5.

Equation 5.

$$Y_{ijkl} = \mu + P_i + S_{j(i)} + D_k + \varepsilon_{ijkl}$$

where μ is the overall mean, P_i is the effect of the i th pigeon pea level (0, 15, 30, 45 and 60% of the total flour blend; $i = 1-5$), $S_{j(i)}$ is the effect of the j th sorghum level nested within the i th pigeon pea level ($j = 1-3$), D_k is the effect of the k th storage period (days 0, 16, 34, 45 and 65), and ε_{ijkl} is the random error associated with the l th replicate ($l = 1-3$).

Where a factor was significant, means were separated using Tukey's Honestly Significant Difference (HSD) test. All results are reported as mean $\pm$ standard error (SE). All analyses were carried out at $\alpha=0.05$ level of significance.

3. RESULTS AND DISCUSSION

3.1 Protein content and protein digestibility

The mean crude protein content of millet, sorghum, and pigeon pea used in the formulation of composite flour blends were

amount of protein and expressed as a percentage (Equation 3). The nitrogen content of the reagent blank was subtracted from the residual nitrogen content of each sample before calculating protein digestibility.

The mixture was stirred thoroughly and allowed to stand for 30 min before filtration through Whatman No. 1 filter paper. A 10 mL aliquot of the filtrate was titrated with standardized 0.1 N sodium hydroxide (NaOH) solution using phenolphthalein as the indicator until a permanent pale pink endpoint was attained. Titratable acidity was expressed as the volume (mL) of 0.1 N NaOH required to neutralize the titratable acids extracted from 10 g of composite flour. Values were calculated by multiplying the titre obtained for the 10 mL filtrate aliquot by a factor of 10. The pH of the filtrate was determined using a calibrated pH meter (Adwa AD8000) according to AACC Approved Method 02-52.01 (AACC, 1999).

2.10 Statistical data analysis

Statistical analysis was done using Minitab software (version: 22.2.1). Yeast and mold counts were $\log_{10}$ -transformed prior to analysis, with values below the detection limit handled as described in Section 2.8. Since the sorghum levels (0, 20 and 40%) were expressed as proportions of the flour remaining after pigeon pea incorporation, the absolute sorghum content differed at each pigeon pea level. Sorghum was therefore treated as a factor nested within pigeon pea level and the data were analyzed by nested analysis of variance (ANOVA). Thus, crude protein content and *in vitro* protein digestibility data were analyzed using the model shown in Equation 4.

11.75 $\pm$ 0.00%, 12.23 $\pm$ 0.16%, and 18.89 $\pm$ 0.13%, respectively. The results of protein content and protein digestibility of the composite flour blends containing varying levels of sorghum, millet and pigeon pea flours are shown in Tables 2 and 3.

Table 2: Effect of pigeon pea incorporation level on crude protein content (%) and *in vitro* protein digestibility (%) of millet–sorghum–pigeon pea composite flour

	Crude Protein content (%)	Protein Digestibility (%)
Pigeon pea levels (%)		
0	11.845±0.030 ^e	34.950±3.810 ^c
15	12.888±0.024 ^d	38.207±0.945 ^{bc}
30	13.930±0.021 ^c	39.989±0.819 ^{abc}
45	14.973±0.021 ^b	45.250±1.010 ^{ab}
60	16.016±0.025 ^a	46.340±1.730 ^a
p-values	<0.001	<0.001

Values are presented as mean ± standard error (SE). Means within a column with different superscript letters differ significantly according to Tukey's HSD test ($p < 0.05$). $N = 9$.

The addition of pigeon peas significantly ($p < 0.001$) increased the protein content and digestibility of the different blends. Pigeon peas used in the study had an average of 18.89% protein content, which explains the increasing pattern in protein content of the blends. In addition, increasing pigeon peas resulted in an increase in the digestibility of the composite flour blends. The *in vitro* protein digestibility of the composite flours increased significantly with increasing pigeon pea inclusion, from 34.95% in blends without pigeon pea to 46.34% at 60% pigeon pea supplementation. Although the improvement was statistically significant, the digestibility values remained lower than those reported for processed pigeon pea flour alone. For example, Sun et al. (2020) reported an *in vitro* protein digestibility of 54.4% for untreated pigeon pea flour, which slightly increased to around 55.0% following soaking, thus soaking alone did not significantly improve digestibility.

Higher digestibility, reaching 80% was reported in *asha* pigeon peas variety alone (Anitha et al., 2020). The comparatively lower digestibility observed in the present study may be attributed to several factors. First, the composite flours contained substantial proportions of sorghum and pearl millet, whose storage proteins (kafirins and prolamins) are inherently less digestible than legume proteins because of their compact structure and interactions with starch and other macromolecules (Song et al., 2024). The soaking and precooking of pigeon peas before drying and milling may have improved protein digestibility by reducing heat-labile antinutritional factors and partially gelatinizing starch. In contrast, millet and sorghum were milled without precooking and therefore likely retained more of their native grain structure and antinutritional components, which may have contributed to the relatively lower digestibility of the composite flours.

Table 3: Effect of sorghum level nested within pigeon pea level on crude protein content (%) and *in vitro* protein digestibility (%) of millet–sorghum–pigeon pea composite flour

Pigeon pea level (%)	Sorghum level (%)	Crude protein content (%)	Protein digestibility (%)
0	0	11.750±0.000g	27.459±1.650a
	20	11.845±0.018fg	33.383±0.834a
	40	11.940±0.036f	44.007±10.000a
15	0	12.807±0.013e	37.849±1.910a
	17	12.888±0.007de	36.799±0.637a
	34	12.968±0.021d	39.972±2.010a
30	0	13.864±0.025c	40.737±0.786a
	14	13.929±0.014c	41.897±0.111a
	28	13.997±0.013c	37.333±1.340a
45	0	14.922±0.038b	47.353±2.750a
	11	14.973±0.029b	45.017±0.167a
	22	15.025±0.022b	43.381±0.847a
60	0	15.979±0.050a	50.215±3.990a
	8	16.015±0.043a	45.283±1.990a
	16	16.053±0.038a	43.510±1.970a
p-values		<0.001	0.078

Values are presented as mean ± SE ($N = 3$). Means within a column with different superscript letters differ significantly according to Tukey's HSD test ($p < 0.05$). Sorghum percentages at each pigeon pea level represents 0, 20, and 40% of the remaining flour fraction after pigeon pea incorporation.

Pretreatments of millet such as malting have been reported to have an improvement in digestibility of proteins in composite flour blends such as those reported by Syeunda et al. (2021) involving millet and cowpeas. Secondly, relatively low protein digestibility observed in the present study may also be attributed to the combined effects of residual antinutritional factors, including tannins, phytates, and protease inhibitors, as

well as the compact cereal protein matrix and interactions between proteins, starch, and dietary fibre, which reduce enzyme accessibility during digestion (Joye, 2019). Thirdly, thermal processing has been reported to alter protein structure and, under some conditions, reduce protein digestibility through the formation of protein aggregates (Gulati et al., 2017). In the present study, pigeon peas were dried at 70°C for

48 h prior to milling. However, the influence of this drying step on protein structure or digestibility was not evaluated and therefore cannot be determined.

Sorghum addition had no significant effect on the protein digestibility of the different blends (**Table 3**). However, the addition of sorghum significantly ($p < 0.001$) improved the protein content of the flour blends with the highest influence being found in the samples containing 40% sorghum. The lowest protein content (11.75 %) and digestibility was observed in pure millet samples. Although the protein content of sorghum was lower than that of pigeon peas, it was higher than that of millet which explains the increase in protein content with addition of sorghum at 40% level. The lack of influence on the digestibility of the protein is possibly due to the low quantities added in the flour blends as compared to the levels of millet and pigeon peas. This pattern indicates that increasing the percentage of pigeon peas in the composite flour blend significantly ($p < 0.001$) improved the mixture's total protein digestibility. When compared to certain other grains or legumes, peas are considered to contain a comparatively higher quality of protein and to be more digestible (Bessada et al., 2019). Higher pigeon pea incorporation likely improved protein digestibility, while precooking may have further enhanced digestibility by reducing antinutritional factors (Yu et al., 2020). This outcome is consistent with those obtained by Krause et al. (2022), who studied *in vitro* digestion of protein and starch in sponge cakes formulated with pea (*Pisum sativum*). In their work, pea sponge cakes exhibited greater protein digestibility than wheat and pure starch sponge cakes (Krause et al., 2022).

Digestibility as a protein quality test can be used to estimate or predict protein bioavailability when the food is consumed. In essence, it's a measurement of a protein's susceptibility to proteolysis. Since a high-digestibility protein would deliver more amino acids for absorption via proteolysis, it has a higher nutritional value than a low-digestibility protein. Increased protein digestibility would give the customer much-needed protein in mixed diets with marginal or low protein levels.

There are wide variations in the digestibility of proteins across different foods. These variations are caused by factors such as the type of protein, the food matrix, the molecular interactions between proteins and other food components, food formulation, processing and storage conditions, and other factors (Orlien et al., 2023).

3.2 Selected storage quality indicators

3.2.1 Temperature and humidity during storage

As shown in **Figure 3**, the storage temperature fluctuated between 23.3 °C and 27.0 °C. The average storage temperature was 25.2 °C. Mean daily relative humidity fluctuated between 54.6 and 57.1 %, with a mean relative humidity of 55.5%. This is partly due to the storage of flour samples in an airtight container. As a result, the relative humidity in this study was fairly constant showing minor fluctuations but remaining between 54–58%. Temperature showed fluctuation as affected by the ambient temperatures during storage.

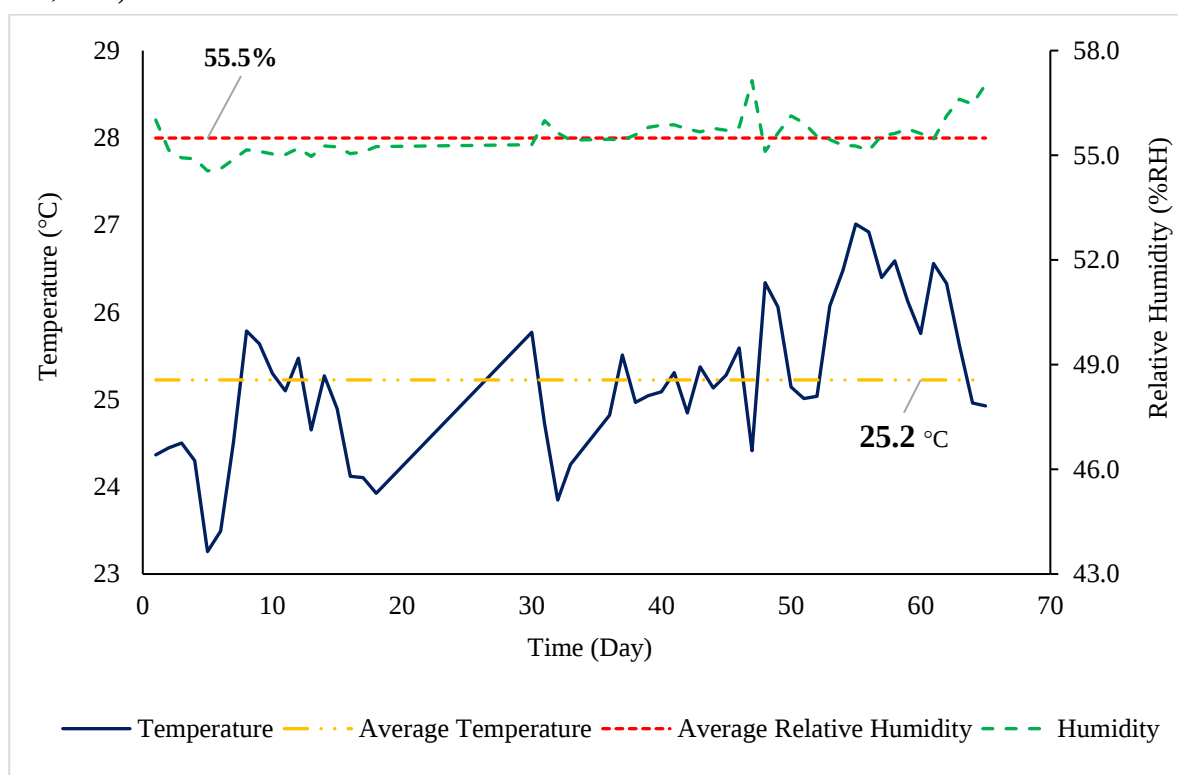


Fig. 3: Mean daily storage temperature (°C) and relative humidity (%RH) recorded during the 65-day storage period of millet–sorghum–pigeon pea composite flour blends.

The shelf life and storability of flour is influenced by multiple factors. These can either be extrinsic elements like temperature, package, gases, and vapours, or intrinsic factors like moisture content and composition (Forsido et al., 2021). Increased

humidity can cause moisture to be absorbed, which can hasten spoilage and encourage the growth of microorganisms. In addition, higher air temperature may increase the specific

humidity making water more available for chemical and microbial reactions (Lancelot et al., 2021).

The mix of materials and the exchange of moisture and oxygen within the package have an impact on the quality attributes of composite products when they are stored. A food product's stability is often determined by the methods used during manufacturing and the environment under which it is stored (Asadzadeh et al., 2014). Nonetheless, an increase in temperature accelerates the rate of deterioration reactions (chemical and microbiological) in foods (Forsido et al., 2021). Understanding the impact of storage temperature, relative humidity and packaging materials on the physico-chemical, microbiological, and sensory qualities of the stored flour is vital to maximizing its potential in food preparation, either alone or in combination with other components (Forsido et al., 2021).

The ability of different packing materials to withstand moisture and oxygen varies, thus selecting the right material is essential (Asadzadeh et al., 2014). The airtight plastic container was used to store flour samples packed in paper packaging material which explains the low humidity levels throughout the storage period. The recommended relative humidity (%RH) for storing flour, including millet, sorghum, and pigeon pea flour, typically falls within 50% to 60%. Higher relative humidity may increase the level of moisture in the flour whereas lower humidity might

cause moisture loss from the flour (Lancelot et al., 2021). In both cases, the quality of the product may be affected. For instance, higher moisture in flour has been linked to higher incidences of toxigenic molds and the presence of mycotoxins in flour.

3.2.2 Titratable acidity and pH

The addition of pigeon peas to the blends at different levels significantly ($p = 0.002$) increased the titratable acidity of the composite blends. The samples containing 60% pigeon pea flour required the greatest volume of sodium hydroxide for neutralization (3.267 mL NaOH per 10 g of flour), indicating the highest titratable acidity (**Figure 4**). In addition, this addition significantly ($p < 0.001$) reduced the pH of the blends (**Figure 4**), with the highest pH being observed in samples with no pigeon peas. Pigeon peas contain organic acids such as malic acid, which contribute to the overall acidity of the blends. As the proportion of pigeon peas in the blend increases, the levels of these organic acids also increase, leading to a significant rise in titratable acidity. Nonetheless, flour from pigeon peas is more prone to oxidative breakdown which also potentially produces acidic products which could have increased the levels of acidity in the flour with increasing pigeon pea level. This however requires further studies to establish.

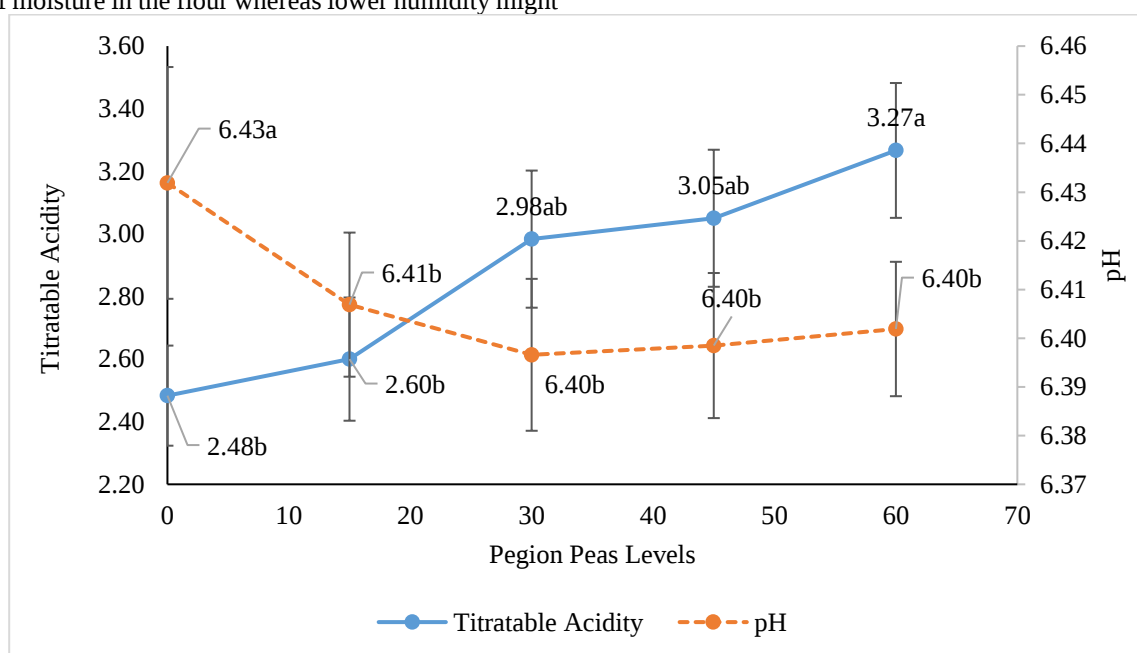


Fig. 4: The effect of pigeon pea addition on the mean titratable acidity (mL 0.1 N NaOH/10 g flour) and pH of flour blends over the 65 days of storage. Values are presented as mean $\pm$ standard error (SE). Error bars represent $\pm$ SE. Means sharing different letters in the same line graph differ significantly according to Tukey's HSD test ($p < 0.05$).

The titratable acidity, expressed as NaOH consumption, increased significantly ($p < 0.001$) during storage, from 1.667 mL NaOH per 10 g of composite flour on day 0 to 3.700 mL NaOH per 10 g of composite flour by day 65 (**Figure 5**). In addition, as shown in **Figure 5**, the pH of the different flour blends significantly ($p < 0.001$) reduced over time from day 0 (pH of 6.55) to the 65th day (pH of 6.32). The mechanisms contributing to the observed increase in titratable acidity in flour varies depending on factors such as storage conditions, flour type, and processing method. During storage, factors like temperature, moisture content, and exposure to oxygen can

promote microbial fermentation, leading to a gradual accumulation of acids. This explains the observed increase in titratable acidity during storage. The higher acidity over time suggested ongoing metabolic activity or slow fermentation processes that progressively increased the acid content of the flour blends. Previous studies have reported pH values of 6.19–6.93 for millet flour, indicating that cereal flours are naturally near neutral (Tortoe et al., 2019). Nonetheless, the change in pH recorded in this study may have minimal influence on the quality of the flour considering it was less than half a unit change from the initial starting value.

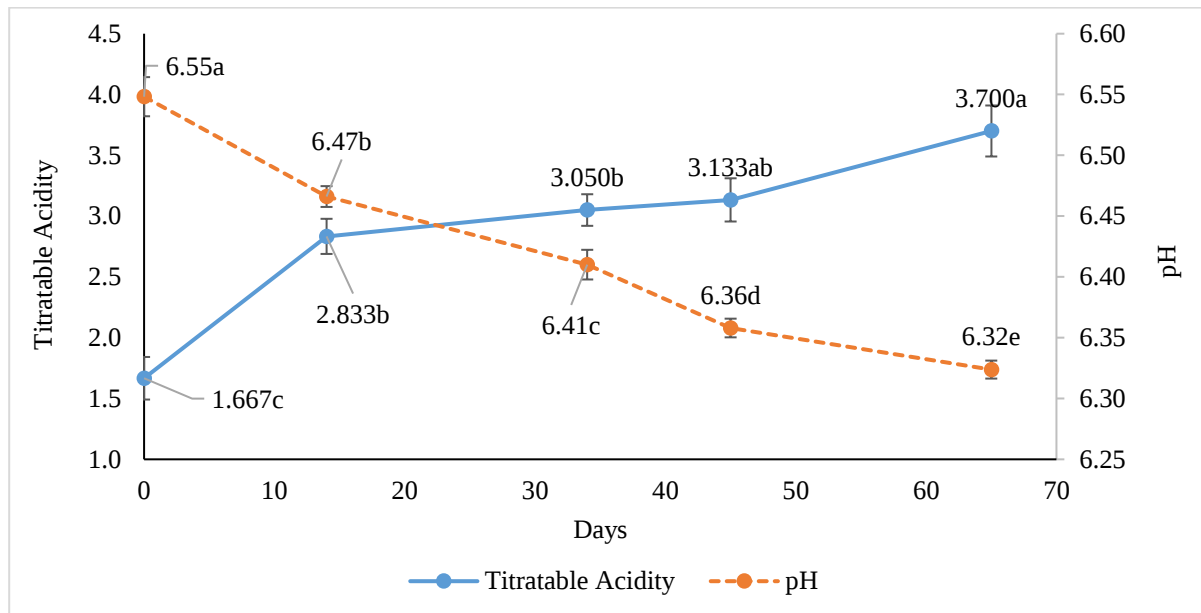


Fig. 5. Changes in mean titratable acidity (mL 0.1 N NaOH/10 g flour) and pH of millet-sorghum-pigeon pea composite flour during the 65-day storage period. Values are presented as mean $\pm$ standard error (SE). Error bars represent $\pm$ SE. Means with different letters in the same line graph differ significantly according to Tukey's HSD test ($p < 0.05$).

Sorghum level nested in pigeon pea levels significantly ($p = 0.002$) affected the titratable acidity levels of the blends with highest mean acidity values (4.150 ± 0.334 ml of NaOH/10g of composite flour) being recorded for samples containing 8% sorghum and 60% pigeon pea flour (Table 4). In addition, there was also a significant ($p < 0.001$) effect on the pH of the flour blends (Table 4). However, the effect did not show a specific pattern to conclude an increase or a decrease in acidity. Further investigation revealed that, within each level of sorghum addition, the acidity increased with increase in pigeon pea level further supporting the argument that pigeon peas were the key contributors to the changes in acidity of the flour blends, and not the amount of sorghum in the blends. Nonetheless, another key contributor to this outcome could have been microbial activity. Microorganisms present in a product like bacteria and fungi, upon metabolizing carbohydrates in the flour, can

produce organic acids such as lactic acid and acetic acid, which can increase the titratable acidity. Factors such as temperature and humidity during storage influence microbial growth and enzymatic activity, thereby affecting the rate of acid production in flour. Similarly, natural aging processes during storage, which can lead to enzymatic and chemical changes could also be the reason for this outcome. For instance, amylases and proteases can break down starches and proteins into simpler compounds, including sugars and amino acids. Subsequent microbial action on these compounds leads to the production of organic acids, contributing to increased acidity. Enzymes present in flour, particularly during milling, catalyse reactions that lead to the production of organic acids. For example, lipases can break down lipids into fatty acids, which can further undergo oxidation to produce organic acids.

Table 4: Effect of sorghum level nested within pigeon pea level on titratable acidity (mL 0.1 N NaOH/10 g flour) and pH of millet-sorghum-pigeon pea composite flour during storage

Pigeon pea level (%)	Sorghum level (%)	Blend label	Titratable Acidity	pH
0	0	s0p0	2.500 ± 0.224^b	6.383 ± 0.056^{bcd}
15	0	s0p15	2.250 ± 0.291^b	6.379 ± 0.026^{cd}
30	0	s0p30	2.850 ± 0.460^b	6.366 ± 0.027^d
45	0	s0p45	2.650 ± 0.334^b	6.395 ± 0.029^{bcd}
60	0	s0p60	2.650 ± 0.422^b	6.398 ± 0.027^{bcd}
0	20	s20p0	2.300 ± 0.335^b	6.436 ± 0.030^{abc}
15	17	s20p15	3.200 ± 0.416^{ab}	6.396 ± 0.021^{bcd}
30	14	s20p30	3.000 ± 0.415^{ab}	6.408 ± 0.028^{bcd}
45	11	s20p45	3.300 ± 0.423^{ab}	6.398 ± 0.028^{bcd}
60	8	s20p60	4.150 ± 0.334^a	6.401 ± 0.024^{bcd}
0	40	s40p0	2.650 ± 0.279^b	6.477 ± 0.030^a
15	34	s40p15	2.350 ± 0.236^b	6.446 ± 0.026^{ab}

Pigeon pea level (%)	Sorghum level (%)	Blend label	Titrateable Acidity	pH
30	28	s40p30	3.100±0.277 ^{ab}	6.415±0.026 ^{bcd}
45	22	s40p45	3.200±0.382 ^{ab}	6.403±0.023 ^{bcd}
60	16	s40p60	3.000±0.149 ^{ab}	6.406±0.024 ^{bcd}
p-value			0.002	<0.001

Values are mean ± SE. Means within a column with different superscript letters differ significantly according to Tukey's HSD test ($p < 0.05$).

3.2.3 Yeast and mould counts

The results of microbiological analysis for yeast and mold count both, during storage and as affected by the addition of sorghum and soaked and precooked pigeon peas are shown in **Table 5** and **Table 6**. The initial average contamination levels at the beginning were 2.119 and 1.908 Log CFU/g for yeasts and mold counts, respectively. These were below the limits established for yeast and molds counts in millet flour (4 Log CFU/g) according to the East African Community (2011) standards. This suggests that the microbial quality of raw materials as well as the processing steps followed for the product development were adequate in ensuring good microbiological quality established in standards for these flours were met. Other researchers have reported lower contamination

of yeasts and mold in flour formulations containing pigeon peas (Anaemene, 2020; Mohamed et al., 2022). In their study on complementary foods containing roasted and fermented pigeon peas, Anaemene et al. (2023) reported no growth of molds until the fourth week only in fermented pigeon peas containing product. The level of initial contamination is dependent on the quality of the ingredients and the processing techniques applied. This may explain the initial contamination level reported in this study and why it was different from the study by Anaemene et al. (2023). The precooking done on pigeon peas may have reduced the initial microbial load in the flour blends. This outcome gives the flour a good potential for application in real life as it is within safe limits for human consumption. Poor microbial quality products could create more problems such as food-borne diseases, while trying to address another problem of malnutrition.

Table 5: Effect of storage time and pigeon pea incorporation level on yeast and mold counts (log CFU/g) of millet–sorghum–pigeon pea composite flour.

	Category	Yeasts (log CFU/g)	Molds (log CFU/g)	Yeast and mold (log CFU/g)
Storage time (Days)	0	2.119 ± 0.141 ^a	1.908 ± 0.134 ^a	2.439 ± 0.147 ^{ab}
	16	2.400 ± 0.195 ^a	1.810 ± 0.182 ^a	2.799 ± 0.186 ^a
	34	1.502 ± 0.154 ^b	1.459 ± 0.156 ^a	1.893 ± 0.177 ^b
	45	0.843 ± 0.086 ^c	0.908 ± 0.085 ^b	1.052 ± 0.115 ^c
	65	1.000 ± 0.102 ^{bc}	0.928 ± 0.084 ^b	1.125 ± 0.120 ^c
p-value		< 0.001	0.003	< 0.001
Pigeon pea level (%)	0	1.469 ± 0.158 ^a	1.372 ± 0.138 ^a	1.766 ± 0.172 ^a
	15	1.612 ± 0.166 ^a	1.649 ± 0.157 ^a	2.017 ± 0.180 ^a
	30	1.742 ± 0.177 ^a	1.431 ± 0.139 ^a	1.949 ± 0.183 ^a
	45	1.534 ± 0.164 ^a	1.245 ± 0.140 ^a	1.751 ± 0.183 ^a
	60	1.679 ± 0.169 ^a	1.474 ± 0.162 ^a	2.006 ± 0.189 ^a
p-value		0.621	0.734	0.587

Values are mean ± SE. Means within a column with different superscript letters differ significantly according to Tukey's HSD test ($p < 0.05$).

Adding pigeon peas or sorghum did not significantly affect yeast and mold counts (**Table 5** and **Table 6**). However, yeast and mold count reduced significantly ($p < 0.001$) during the storage of composite flour blends. In the beginning days 1-16 days, there was an observed increase, even though this was not statistically significant. However, beyond day 16, the

contamination levels significantly ($p < 0.001$) reduced until day 45. Beyond this point, a slight but non-significant increase was observed in both yeast and mold counts. Although there was an initial increase in yeasts and mold counts, in both studies, further storage resulted in a decrease in contamination levels.

Table 6: Effect of sorghum level nested within pigeon pea level on yeast counts, mold counts, and total yeast and mold counts (log CFU/g) of millet–sorghum–pigeon pea composite flour

Pigeon pea level (%)	Sorghum level (%)	Yeasts (log CFU/g)	Molds (log CFU/g)	Yeast and Mold (log CFU/g)
0	0	1.285 ± 0.239 ^a	1.133 ± 0.167 ^a	1.502 ± 0.249 ^a
	20	1.746 ± 0.306 ^a	1.501 ± 0.279 ^a	2.206 ± 0.325 ^a
	40	1.374 ± 0.274 ^a	1.482 ± 0.259 ^a	1.591 ± 0.300 ^a
15	0	1.680 ± 0.300 ^a	1.543 ± 0.271 ^a	2.155 ± 0.313 ^a
	17	1.546 ± 0.259 ^a	1.763 ± 0.269 ^a	2.070 ± 0.295 ^a
	34	1.611 ± 0.317 ^a	1.642 ± 0.291 ^a	1.826 ± 0.342 ^a
30	0	1.629 ± 0.320 ^a	1.533 ± 0.269 ^a	2.043 ± 0.336 ^a
	14	1.793 ± 0.288 ^a	1.234 ± 0.207 ^a	1.953 ± 0.289 ^a
	28	1.803 ± 0.326 ^a	1.528 ± 0.251 ^a	1.850 ± 0.340 ^a
45	0	1.817 ± 0.339 ^a	1.243 ± 0.270 ^a	2.060 ± 0.368 ^a
	11	1.110 ± 0.225 ^a	1.135 ± 0.201 ^a	1.278 ± 0.261 ^a
	22	1.674 ± 0.262 ^a	1.357 ± 0.260 ^a	1.914 ± 0.298 ^a
60	0	1.963 ± 0.300 ^a	1.347 ± 0.260 ^a	2.210 ± 0.312 ^a
	8	1.518 ± 0.294 ^a	1.374 ± 0.275 ^a	1.817 ± 0.342 ^a
	16	1.556 ± 0.290 ^a	1.701 ± 0.314 ^a	1.990 ± 0.343 ^a
p-value		0.772	0.944	0.632

Values are expressed as mean ± standard error (SE). Means within a column with different superscript letters differ significantly according to Tukey's HSD test ($p < 0.05$).

The observed decline in microbial counts after day 16 was somewhat unexpected because microbial populations in stored flours often remain stable or increase when environmental conditions favour growth. Although the flour samples were stored in airtight containers under relatively stable ambient conditions (**Figure 3**: approximately 25.2°C and 55.5% relative humidity), this study did not measure flour moisture content, water activity, oxygen concentration, or carbon dioxide concentration during storage. Therefore, the mechanisms underlying the decline cannot be confirmed. One possible explanation is that prolonged storage may have progressively limited conditions required for microbial survival and proliferation, including reduced water availability or changes in the gaseous environment within the containers. The storage of the flour samples in an airtight container at nearly constant low humidity may have resulted in a reduction in the moisture level of the grain as well as oxygen levels which may have negatively impacted the growth of yeasts and molds. Uchekwu-Agua et al. (2015) reported similar trends while studying the effects of storage conditions on the microbial quality of cassava flour. However, these explanations remain speculative and require confirmation through studies incorporating measurements of these storage parameters. Further studies should therefore include measurements of flour water activity, moisture content, oxygen concentration, and carbon dioxide concentration among other storage parameters, to better explain microbial dynamics during flour storage.

The slight increase in yeast and mold counts observed after 45 days of storage may have resulted from changes in the storage environment associated with repeated opening of the containers during sample collection. As shown in **Figure 3**, the average

relative humidity during the latter stages of the 65-day storage period was higher than at the beginning of storage. Although the samples were stored in airtight containers, periodic opening during sampling may have allowed moisture exchange with the surrounding environment, resulting in a temporary increase in the relative humidity within the containers. Such changes could have created conditions that were more favourable for the survival and limited proliferation of yeasts and molds, thereby explaining the modest increase in microbial counts after 45 days.

According to the East African Community (2011) standard, the maximum permissible yeast and mold count for millet flour is 4 log CFU/g. The millet–sorghum–pigeon pea composite flour samples recorded yeast and mold counts (**Tables 5 and 6**), that were well below this limit. All samples remained below this maximum throughout the storage period. These findings indicate that the composite flour complied with the East African Community specification for yeast and mold counts over the storage period.

4. STUDY LIMITATIONS

Despite providing important insights into the nutritional and storage characteristics of millet–sorghum–pigeon pea composite flours, this study had several limitations that should be acknowledged. First, protein quality was assessed using *in vitro* protein digestibility, which provides a useful estimate of protein utilization but does not fully represent *in vivo* digestibility or amino acid bioavailability in humans. Second, storage quality evaluation was limited to pH, titratable acidity, and yeast and mold counts. Other important indicators of flour

stability, including moisture content, water activity, lipid oxidation, peroxide value, free fatty acids, colour changes, sensory acceptability, and mycotoxin contamination, were not determined. Third, although temperature and relative humidity were monitored during storage, changes in oxygen concentration, carbon dioxide concentration, and moisture content within the storage containers were not measured. Finally, the flour blends were evaluated under controlled laboratory storage conditions using a single packaging system, and therefore the findings may not fully reflect storage performance under commercial or household conditions where environmental conditions and packaging materials vary. Future studies should incorporate these additional quality parameters, evaluate different packaging systems and storage environments, and validate the nutritional benefits through animal or human feeding studies.

5. CONCLUSION

This study evaluated the effects of pigeon pea incorporation on the protein content, *in vitro* protein digestibility, and selected storage quality attributes of millet–sorghum–pigeon pea composite flours. Increasing pigeon pea incorporation significantly improved both protein content and *in vitro* protein digestibility, demonstrating its potential to enhance the nutritional quality of cereal-based composite flours. During storage, titratable acidity increased, indicating progressive physicochemical changes, while yeast and mold counts remained below the recommended limit throughout the storage period, suggesting microbiological stability with respect to this parameter. However, additional analyses, including lipid oxidation, peroxide value, free fatty acid content, sensory evaluation, and mycotoxin assessment, are required to comprehensively evaluate storage stability and overall product safety. These findings demonstrate the potential of pigeon peas as a locally available ingredient for enhancing the protein quality of cereal-based composite flours intended for complementary food formulation.

AUTHOR CONTRIBUTIONS

Conceptualization contributed by **CNM**; methodology contributed by **CNM**; formal analysis and investigation contributed by **CNM**; writing—original draft preparation contributed by **CNM**, writing—review and editing contributed by **CNM**, **JNN**, **KW**, and **JM**; resources contributed by **CNM**.

CONFLICT OF INTEREST

Authors declare that there are no conflicts of interest related to this research.

DATA AVAILABILITY

The data that support the findings of this study are available from the authors upon reasonable request.

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