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The Effects of Storage on Nutrient Composition and Mycoflora of Stored Guinea Corn (*Sorghum bicolor*) Grains

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Authors' contributions

This work was carried out in collaboration between both authors. Author EDF designed the study, wrote the protocols, supervised the experiment and managed the literature searches. Author KOO did the laboratory work, performed the statistical analysis, managed the analyses of the study and wrote the first draft of the manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: This research work aims to determine the changes in nutritional (proximate, mineral) composition and mycoflora of *Sorghum bicolor* grains stored for 5 months in order to establish its fitness in meeting nutritional demand.
Study Design: Experimental study design was carried out
Place and Duration of Study: Department of Microbiology, Ekiti State University, Ado-Ekiti, from February 2017 to August 2018.
Methodology: The grain was procured from Usi market in Usi-Ekiti. It was further sundried for seven days and stored in an airtight container in the laboratory at room temperature. They were visually examined for external changes on the caryopsis and cultured to determine the spoilage fungi. Mineral and proximate analyses were routinely carried out to determine the changes in nutrient composition. These analyses were carried out monthly for five months to determine the changes in physicochemical properties and mycoflora associated with *Sorghum bicolor* on storage.
Results: During storage, spoilage such as external mouldiness, discoloration, musty odour and

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progressive depletion of external caryopsis were observed on the grain. Seven species of fungi namely *Penicillium glabrum, Aspergillus flavus*, *Penicillium* spp*., Fusarium* spp*., Alternaria* spp., *Aspergillus niger and Saccharomyces cerevisiae* were isolated using a combination of direct plating, dilution and washing method. The colony count of the mycoflora population increased from 6 to 16 spore-forming units per gram. The proximate component comprising ash, moisture, crude protein, fat and fiber content decreased but an increase in carbohydrate content was recorded. A negative Pearson correlation (*r* = - .990) between crude protein and carbohydrate content was recorded. This was attributed to the presence of resistant starch in *Sorghum bicolor* and the use of alternative source(s) of carbon for energy by the fungi. The entire mineral component decreased during storage. Copper was the barest mineral while magnesium was the most stable in the stored grain.

Conclusion: *Sorghum bicolor* grains contain vital minerals and nutrients. Prolonged storage of *Sorghum bicolor* increased the mycoflora population and consequently decreased the nutrient composition excluding the carbohydrate component. Some minerals and proximate components of the grain were relatively stable while others experienced pronounced depletion. Nutritionally deficient grains may lead to malnutrition especially in growing animals and human populations were adequate minerals and nutrients are required for rapid growth.

Keywords: Mycoflora; mineral; proximate; storage.

1. INTRODUCTION

Access to enough, safe and nutritious food is essential to sustaining life and maintaining good health status. Unsafe food harboring micro-organisms or toxic chemicals cause more than 200 diseases. About 600 million people fall ill due to the consumption of contaminated food and 420,000 die yearly leading to the loss of 33 million healthy populations [1]. Deterioration of nutrient and microbial spoilage of stored crops, malnutrition and food poisoning are significant threats to food security globally and specifically in developing countries because of the high rate of increasing population. About 155 million children suffer from stunted growth; 41 million are victims of obesity while 52 million are globally threatened by wasting [2]. During a shortage of food, people tend to shift to less healthy diets and consume unsafe foods whose chemical and microbial content may have the potential to cause harm [1].

Guinea corn is a prominent grain (cereal crop) with botanical name *Sorghum bicolor*. It belongs to the sub-kingdom Tracheobionta, the super division is Spermatophyta and the division is Magnoliophyta. It belongs to class Liliopsida, the subclass (Commelinidae), order (Cyperales), family (Poaceae or Grass), and genus Sorghum. There are various species such as *Sorghum bicolor*, *Sorghum almum* (Columbus grass), *Sorghum halepense* (Johnson grass), *Sorghum propinquum* (sorghum). *Sorghum bicolor* is also known as black amber, broomcorn, chicken corn, common wild sorghum, Drummond broomcorn, durra, Egyptian millet, feterita, forage sorghum , great millet, guinea corn, jowar, Kaffir-corn, Kaffircorn, milo, shallu, shattercane, Sudan grass, sweet sorghum, wild cane [3]. *Sorghum bicolor* is recognized as guinea corn in West Africa but locally called Okababa, Okili and Dawa in the three major languages in Nigeria [4]. Nigeria is the largest producer of the crop in Africa and second largest producer in the world after the United States with an annual production capacity of 6.5 million tons [5].

Fungi such as *Aspergillus, Penicillium, Fusarium, Alternaria, Phoma, Curvularia* and *Rhizopus* species are mycoflora associated with *Sorghum bicolor* and they release mycotoxins on the field and during storage of the grains [6]. Consumption of mouldy grains that contain carcinogenic substances such as mycotoxins at an unsafe level by man and animals has dire consequences on public health [7].

Food product such as “ogi” produced from the fermentation of sorghum, millet or maize has been implicated in causing kwashiorkor among infant [8]. Thus, it is pertinent to explore potential diets that are acceptable, broadly familiar, meet minimum nutritional requirements for light activity, fit for human consumption, easily digestible for all, maximize the use of available resources and are economical in terms of fuel requirement, preparation time and waste [9].

2. MATERIALS AND METHODS

2.1 Collection of Samples

Sorghum bicolor grains were purchased from the market at Usi-Ekiti in Ido-Osi local government area in Ekiti north senatorial district of Ekiti state,

Nigeria. The grains were identified at the Department of Plant Science, Ekiti State University, Ado-Ekiti. Debris, shafts and dust were removed by hand picking and winnowing. The grains were further sundried for seven days and stored in an airtight/insect free labeled container that was earlier disinfected with ethanol. They were stored for laboratory analysis at room temperature for 5 months [10].

2.2 Cultural Technique and Fungi Isolation

The grains were subjected to external examination. The methods of [10] were employed to isolate fungi species monthly from the stored grains. The methods include direct plating, dilution, and washing methods

2.2.1 Washing method

This was carried out by weighing one gram of the dried *Sorghum bicolor* grains into 10 ml of sterile distilled water in a beaker. The mixture was shaken thoroughly and drops of the aliquot were aseptically introduced into Petri dishes containing potato dextrose agar. The aliquot was evenly spread on the agar plate with the aid of a sterile glass spreader. The plates were incubated at 28 degree Celsius for 5 to 7 days and observed for visible fungal growth [10, 11].

2.2.2 Direct plating method

Ten grains of *Sorghum bicolor* were randomly selected and examined for external mouldiness. They were surface sterilized in 70% ethanol for 2 minutes and later washed in sterile distilled water. A sterile dissecting forceps was used to directly transfer the grains to potato dextrose agar (PDA) plate under aseptic conditions and incubated at 28 ^{0}C for 5 to 7 days [12]. The fungi colonies were sub-cultured on sterile plates containing PDA by successive hyphae tip transfer until pure colonies were obtained [10]. The cultures were examined under the microscope for fruiting bodies and hyphae to determine the common fungi present.

2.2.3 Dilution method

This method was also used to determine the type of fungi present in the stored *Sorghum bicolor* grains. About one gram of the grain sample was surface sterilized with ethanol for 2 minutes and washed in sterile distilled water. The sample was ground in 10 ml of sterile distilled water as diluents. The mixture was shaken thoroughly and 1 ml of suspension was transferred with a pipette into a sterile test tube containing 9 ml of distilled water. This was thoroughly mixed. The sample was serially diluted and 1 ml each of aliquots of 10^{-5} and 10^{-6} were inoculated into molten PDA plates. The plates were swirled gently, allowed to solidify and incubated at room temperature for 5 to 7 days. The culture was examined and fungal colonies were counted every 24 hours [12]. The fungi colonies were sub-cultured on sterile plates containing PDA by successive hyphae tip transfer until pure colonies were obtained. The cultures were examined under the microscope for fruiting bodies and hyphae to determine the common fungi present [10].

2.3 Identification of Fungal Isolates

The isolates were firstly examined under bright daylight for the colour characteristics of the culture. Furthermore, detailed morphological characteristics of the fungi such as hyphae, reproductive structures (sporangia/conidia) in chains or single and the type of spores were observed and recorded [13]. The needle mount preparation and modified slide culture technique were used to identify the isolated fungi [13]. Pure cultures of all fungal isolates were identified by the characterization of colonies on the basis of their cultural and morphological features [14].

2.3.1 Needle mount preparation method

The method as described by [13] was used to identify the colonial morphology of the fungi. A drop of lactophenol blue was placed on a clean slide. Hyphae tip of young mycelium taken midway between the centre and edge of colonies were carefully teased out with a sterile needle, emulsified on the slide and stained with the lactophenol blue. The stained hyphae was covered with a cover slip and examined under X10 and X40 objective lens of the microscope. Pure cultures of all fungal isolates were identified by characterization of colonies on the basis of their cultural and morphological features [14]. All fungal isolates were prepared and examined in this manner with a microscope.

2.3.2 Slide culture technique

A bent glass rod was placed in the sterile Petri plate side and a sterile glass slide was put on the glass rod. From a plate approximately 2 mm deep, a 1-by-1-cm block potato dextrose agar cut with a sterile scalpel was then transferred to the

glass slide. Using sterile wire needle, the fungus was inoculated from the culture plate to the four vertical sides of the agar block. A sterile cover slip was put over the block with slight pressure to ensure adherence. Approximately 2 ml of sterile water was added to the bottom of the Petri plate, and the plate cover was replaced. The plates were incubated at 30 ^{0} C until adequate growth was observed. After removing the medium with a scalpel, the fungus adhering to both cover slip and the slide was examined [13]. A drop of alcohol was added followed by a drop of lactophenol blue and the preparation was covered and examined under the low power objective of a microscope.

2.4 Proximate Analysis

The proximate analysis of the samples for protein, moisture, ash, fiber, carbohydrate and fat were carried out according to the methods of [15]. The nitrogen content was determined by the micro-Kjeldahl method [16]. The percentage nitrogen was converted to crude protein by multiplying the nitrogen content with 6.25. All determinations were performed in triplicates and the mean proximate values were reported as g/100 g.

2.5 Mineral Analysis

The mineral content of the stored grain was analyzed at an interval of four weeks for six months by dry ashing the samples at 550°C to constant weight. The ash was dissolved in 500 ml volumetric flask using de-ionized water with a few drops of concentrated HCl. Sodium and potassium were determined by using a flame photometer with NaCl and KCl standards. Phosphorus was determined by the colorimetric method as described by [16] with KH_2PO_4 as standard. The concentration of other metals such as calcium, magnesium, zinc, iron, and copper were determined by atomic absorption spectrophotometry. All determinations were done in triplicates and the concentration of each mineral was measured in mg/100g. The optimum analytical range was 0.1 to 0.5 absorbance unit with a coefficient of variation of 0.87 to 2.20% [16].

2.6 Statistical Analysis

Statistical analyses were carried out using SPSS version 20.0 for the analysis of mean, standard deviation, and the Pearson correlation coefficient [17].

3. RESULTS AND DISCUSSION

Sorghum bicolor grains are renowned for supplying vital nutrients that bridge nutritional deficiency both in man and animals [18]. Harvested *Sorghum bicolor* grains are usually sundried, sorted, graded, packed, and preserved for their intended future use. These processes help to retain the grain viability and explore the economic prospects [19]. In Northern Nigeria, *Sorghum bicolor* is majorly stored as threshed grain while few others store it in the un-threshed form in a solid-walled container (rumbu), granary and bags. Materials such as cement, mud, zinc and grasses are employed in the construction of the storage facility [20].

Moulds can colonize grains on the field before harvesting or during storage [21]. Several factors lead to the loss of both viability and nutrients of *Sorghum bicolor* on storage. Globally, the major causes of loss are the degradations by pests, mould damage together with germination or sprouting [22]. Sorghum grain moulds constitute a significant challenge for its improvement and global production. Approximately annual economic losses in Asia and Africa due to grain mould infestation and damage are over 130 million dollars [19].

The external changes observed on the kernel of *sorghum bicolor* during storage are shown in Table 1. The degree of colonization during storage ranges from external mouldiness to discoloration and musty odour. The hard caryopsis comprising the testa and pericarp together with immediate anatomical parts were progressively degraded by a succession of mycoflora population over the storage time.

Fungi isolated from *Sorghum bicolor* grains during 5 months storage using direct plating method, washing method and dilution method are shown in tables 2, 3, and 4, respectively. In this study, seven species of fungi namely *Penicillium glabrum, Aspergillus flavus*, *Penicillium* spp*., Fusarium* spp*., Alternaria* spp., *Aspergillus niger and Saccharomyces* spp. that colonized the grains during storage at room temperature were isolated. This result is in agreement with findings of [23] and [24] that grains of *Sorghum bicolor* were infested and damaged by spoilage fungi such as *Aspergillus, Rhizopus, Penicillium, Fusarium* and *Alternaria* spp. on the field or during storage. The findings are also consistent with that of [25] that storage

fungi cause nutrient depletion, discoloration of grain, loss of viability, caking of grain, production of mycotoxins, increase in the temperature of stored grain, mouldy smell and taste. Some fungi are known to produce secondary metabolites called mycotoxins such as aflatoxins, trichothecenes, ochratoxins, citrinin, cyclopiazonic acid, deoxynivalenol, T-2 toxin, ergotamine, fumonisin, patulin, penitrem, sterigmatocystin, zearalenone, tenuazonic acid, verrucosidin and satratoxin [26]. The risk of fungal mycotoxins in stored grains can be increased on prolonged storage reaching levels beyond acceptable limits [27]. Mycotoxins can lead to debilitating clinical conditions such as hepatocellular cancer, congenital disabilities, convulsion, edema, neurotoxicity, aplastic anemia in humans and animals [28].

Table 1. External changes observed on the kernel of *Sorghum bicolor* grains during storage

Time of storage (weeks)	Macroscopic changes observed
0	Dry, reddish-brown kernel
4	No visible change
8	External mouldiness
12	External mouldiness, discoloration, musty odour
16	External mouldiness, discoloration, and musty odour
20	External mouldiness, discoloration, musty odour, and dusty grain

Table 2. Summary of the fungi isolated from *Sorghum bicolor* grains during five months of storage using a direct plating method

Time of storage (weeks)	Fungal isolates	Colony count
0	A,B, E	4
4	A,B,C,E	6
8	A,B,C,D	6
12	A,B,C,D,F	8
16	A,B,C,D,F	8
20	A,B,C,D,F	10

Keys: A- Penicillium glabrum, B- Aspergillus flavus, C- Penicillium spp. D- Fusarium spp. E- Alternaria spp. F- Aspergillus niger

Table 3. Summary of the fungi isolated from *Sorghum bicolor* grains during five months of storage using the washing method

Time of storage (weeks)	Fungal isolates	Colony count
0	B,C,D,F	6
4	B,C,D,E	6
8	A,B,C,F,G	7
12	A,B,C,D,E,F	9
16	A,B,C,D,E	9
20	A,B,C,D,F	10

Keys: A- Penicillium glabrum B- Aspergillus flavus, C- Penicillium spp. D- Fusarium spp., E- Alternaria spp., F- Aspergillus niger, G- Saccharomyces spp

Table 4. Summary of the fungi isolated from *Sorghum bicolor* grains during five months of storage using the dilution method

Time of storage (weeks)	Fungal isolates	Colony count (sfu/g)
0	A,B,C,F	6
4	A,B,C,D,E	6
8	A,B,C,D,E,F	9
12	A,B,D,E,F	11
16	A,B,C,D,E,F,G	13
20	A,B,C,D,E,F,G	16

Keys: A- Penicillium glabrum B- Aspergillus flavus, C- Penicillium spp. D- Fusarium spp., E- Alternaria spp., F- Aspergillus niger, G- Saccharomyces spp , sfu/g- spore-forming unit per gram

Table 5. Summary of the mean proximate composition of *Sorghum bicolor* grains during 5 months of storage

Proximate composition (g/100g dry weight)						
Time of storage	**Ash**	**MC**	**CP**	**Fat**	**Fibre**	**CHO**
0	2.65	11.72	14.98	3.21	2.92	64.53
4	2.66	11.77	14.98	3.21	2.98	64.51
8	2.42	10.68	13.53	2.73	2.74	67.92
2	2.16	11.05	11.65	2.51	2.71	69.89
16	1.79	11.28	10.08	2.42	2.67	71.78
20	1.54	11.36	9.67	2.33	2.52	72.60
Mean	2.20	11.31	12.48	2.74	2.76	68.58
SD	0.46	0.41	2,36	0.39	0.17	3.51
CV	0.21	0.17	5.59	0.15	0.23	12.30
PC	41.89	3.07	35.45	21.50	13.70	12.51

Key: S.D - Standard deviation, MC- Moisture content, CP-Crude protein, CHO - Carbohydrate, CV- Coefficient of variation, PC- Percent of changes

Table 6. Summary of the mean mineral composition of *Sorghum bicolor* grains during five months of storage

(Mineral composition in mg/100g)										
Time of storage (weeks)	**Na**	**K**	**Ca**	**Mg**	**Zn**	**Cu**	**Fe**	**Mn**	**Pb**	**P**
0	19.99	595.02	10.32	230.62	7.16	1.45	13.75	5.09	Nd	69.94
4	19.84	590.05	9.70	230.32	7.12	1.48	12.97	4.99	Nd	70.24
8	18.70	496.12	9.34	229.17	6.55	1.42	10.56	4.75	Nd	75.10
12	18.61	488.66	7.44	221.55	6.33	0.42	8.32	3.19	Nd	71.22
16	17.93	484.24	6.23	217.29	5.57	0.38	6.52	2.15	Nd	65.12
20	16.89	465.75	5.35	200.74	3.67	0.33	5.32	1.94	Nd	60.13
Mean	18.67	519.97	8.06	221.62	6.07	0.91	9.58	3.69	Nd	68.63
SD	1.17	57.11	2.03	11.56	1.31	0.59	3.43	1.45	Nd	5.24
CV	1.37	3621.86	4.10	133.69	1.72	0.35	11.76	2.09	Nd	27.49
PC	15.51	21.73	48.16	12.96	48.74	77.24	61.31	61.89	Nd	14.03

Key: Nd- Not determined, CV- Coefficient of variation, S.D - Standard deviation, PC- Percent of change

Table 7. Morphological and microscopic features of fungi isolated from *Sorghum bicolor* during 5 months storage

Name of fungi	Colonial morphology	Microscopy
Alternaria spp	Wooly, compact, dark-brown colony	Short, septate, branched mycellium, brown hyphae, spore-forming
Aspergillus niger	Powdery-black colony	Long, un-branched, non-septate erect, hyphae, spore forming
Aspergillus flavus	Powdery, greenish-yellow colony.	Long un-branched, non-septate, erect hyphae, spore-forming
Saccharomyces spp	Yellowish-white, circular colony	Budding, non spore forming colony
Fusarium spp.	Wooly-white to yellow colony	Mycelium is septate, branched hyphae, spore-forming
Penicillium glabrum	Bluish-green colony	Mycelium consists of septate and branched hyphae.
Penicillium spp	Bluish-green colony	Mycelium consists of erect, septate and branched hyphae.

The mean proximate composition of fresh and stored *Sorghum bicolor* grains is shown in Table 5. The fresh grain was rich in nutrients such as protein, carbohydrate, fat and oil. Carbohydrate

was the most abundant proximate component in the freshly procured grain. During 5 months of storage, the ash, moisture, protein, fat and fiber content were progressively depleted with increased time of storage.

The moisture content decreased from 11.72 % to 11.36 % in the stored grain. This range of moisture content with corresponding water activity was high enough to support the growth of storage fungi and deterioration of *Sorghum bicolor.* The minimum water activity is the limit below which a microbe or group of microorganisms cannot reproduce. Pathogenic bacteria cannot grow below a water activity of 0.85–0.86, whereas yeast and molds are more tolerant of a reduced water activity of 0.80, but usually no growth occurs below a water activity of about 0.62 [29]. Besides, the storability of the grains decreased at the observed moisture level. This view is in agreement with [30] that field fungi such as *Alternaria* spp, *Cladosporium* spp. and *Fusarium* spp. colonize grains on the field when the moisture content, water activity (a_w) and relative humidity is high. However, storage fungi such as *Aspergillus* spp. and *Penicillium* spp. invade grains at lower moisture content, water activity and relative humidity.

The crude protein component decreased from 14.98 to 9.67 g/100g. It was the barest organic matter after storage. Fungi produce enzymes

that use protein as a source of nitrogen. The metabolic pathway that utilizes protein may be less complicated than those required for the utilization of other substrates in *Sorghum bicolor*. Also, the product(s) from protein digestion may be a precursor to the metabolic pathways which utilize other nutrients. The ash content decreased from 2.65 to 1.54 g/100g. The decrease in ash content indicates the loss of minerals present in the grain as storage period increased [10].

The fat content also decreased from 3.21 to 2.33 g/100g and the fiber content decreased from 2.92 to 2.54 g/100g. Dietary fiber is a combination of lignin and polysaccharide components of plants that are indigestible by enzymes in the human gastrointestinal tract [31]. Insoluble fiber occurs in cereals. It absorbs fluid thereby increasing stool weight and promoting the growth and activity of the gut bacteria coupled with gut health [32].

The starch content increased after 20 weeks of storage. Starch in the grain was resistant to hydrolytic enzymes secreted by the colonizing fungi. An alternative metabolic pathway could have been employed by the fungi to provide their carbon source. This could be responsible for the observed increase in the carbohydrate component. This result was as previously reported in stored wheat by the findings of [33] and it was attributed to an increase in insoluble amylose content.

Filamentous fungi produce enzymes that break down starch which is the primary storage component in plants and other complex polysaccharides such as cellulose, hemicellulose and pectin in the plant cell wall [34]. Besides, affirmed that starch is a polymer comprising repeating units of amylase, a linear glucan with α-1,4 glycosidic bonds and amylopectin, a highly branched glucan with α-1,4 and α-1,6 linkages bonds. Non–waxy sorghum starch is highly resistant to enzymatic digestion than the waxy grain because the amylase component is comparatively higher. Thus the higher the amylase content of the starch the higher the resistance to enzymatic digestion and the lower the digestion [35].

In addition, considering the anatomy of *Sorghum bicolor*, the interaction between starch and protein could be partly responsible for low raw starch digestibility of the grain. Starch granules are embedded in a protein matrix which constitutes a physical barrier preventing access of amylases to the starch [36]. The consumption of resistant starch in place of digestible starch can also reduce postprandial glycemia and insulinemia [37]. Resistant starch has been used in food products to deliver all health benefits of dietary fiber, mainly to prevent obesity and diabetes [38]. Resistant starch functions as a prebiotic. Colonic bacteria can ferment it in the large intestine to short-chain fatty acids such as acetate, butyrate and propionate [39].

Also, [34] affirmed that filamentous fungi such as *Aspergillus* species produce starch degrading enzymes such as α-amylases, glucoamylases and α-glucosidases. They hydrolyze starch synergistically to produce a monomeric glucose unit. α-amylases hydrolyze α-1, 4-glycosidic chains endolytically to produce maltose while α-glucosidases and glucoamylases hydrolyze maltose and α -1,4- bonds exolytically from non-reducing ends to form glucose. Gluco-amylase

also hydrolyzes α-1, 6 linkages at branch connections.

From a genetic perspective, [40] and [41] affirmed that in *Aspergillus* spp., expression of genes encoding amylolytic enzymes required the transcriptional activator AmyR, a binuclear zinc cluster. Its disruption in *A. oryzae* and *A. nidulans* led to significantly decreased amylolytic enzyme activities and restricted growth on starch medium.

A negative Pearson correlation between crude protein and carbohydrate content was observed (r = - .990) as previously reported in stored wheat by the findings of [33].

The mean mineral composition of *Sorghum bicolor* grains during 5 months storage is shown in Table 6. The rate of deterioration of mineral nutrients in stored *Sorghum bicolor* varied and some minerals were more stable than others. Potassium was the most abundant mineral present in the grain with an initial concentration of 595.02 mg/100g while copper was the least abundant mineral with an initial concentration of 1.45 mg /100g. There was a noticeable decrease in the concentration of all the mineral elements during storage.

The concentration of potassium in stored *Sorghum bicolor* decreased from 595.02 to 465.75 mg/100g. Potassium is an ionic component of intracellular fluid. It is responsible for the regulation of osmotic pressure, the transmission of nerve impulse and co-ordination, acid-base balance, and contraction of muscle. It helps in the transfer of phosphate from ATP to pyruvate. Deficiency of potassium leads to hypokalaemia and it is found in a patient suffering from diarrhea and neuromuscular paralysis [42].

Sodium content of the grain decreased from 19.99 to 16.89 mg/100g. Dietary sodium plays a vital role in the maintenance of osmotic pressure of the body fluids, activation of nerve and muscle function. It is also essential in maintenance of membrane potentials and transmission of nerve impulses [42]. Sodium deficiency in the serum causes hyponatremia and it occurs in severe Addison's disease, vomiting, diarrhea and intestinal obstruction [43].

The calcium content decreased from 10.32 to 5.35 mg/100g. Calcium is a constituent of bones and teeth. It is concerned with the regulation of nerve and muscle function. It plays a vital role in enzyme activation. It is required for membrane permeability, muscle contraction and transmission of nerve impulses. Calcium deficiency causes rickets in children, osteomalacia and osteoporosis in adults [42].
There was a decrease in phosphorus concentration from 69.94 to 60.13 mg/100g. Phosphorus is an essential mineral essential for metabolic and cellular functions. It is a component of bones, teeth, adenosine triphosphate (ATP) and nucleic acids. Deficiency of the mineral causes rickets in children and osteomalacia in adults [42].

Manganese content of the stored grain decreased from 5.09 to 1.94mg/100g. Manganese is a co-factor of some enzymes and it is essential for lipid and carbohydrate metabolism, calcium absorption, blood sugar regulation, cognitive and nervous functions[44].

Iron content decreased from 13.75 to 5.32 mg/100g. Iron can be obtained from both plant and animal sources. Iron in an animal is mainly found in hemoglobin (protein component of the red blood cell) that transfers oxygen in form of oxy-haemoglobin from the lungs to other body parts. It is vital for growth, development and normal cellular functioning. Iron deficiency leads to anemia or a shortage of blood in the body [45].

Copper content decreased from 1.45 to 0.33 mg/100g. It was the barest mineral with 77.24 % reduction. Copper is a constituent of many metallo-enzyme complex and it is important for proper growth, production of iron and consequently red blood cells. Copper deficiency can lead to nervous diseases and anemia. Adequate dietary copper is essential for proper metabolism of iron. Dietary copper and iron play a major role in the prevention of anemia [46].

Magnesium content of the stored grain decreased from 230.62 to 200.74 mg/100g. Magnesium was the most stable mineral in *Sorghum bicolor* on storage with the lowest percentage reduction of 12.96%. It is essential for nervous coordination and enzyme activation which enhances RNA synthesis and DNA replication. It also plays a role in the active transport of calcium and potassium ions across cell membranes during nerve impulse conduction, muscle contraction, and normal heart rhythm. Its deficiency can result in muscular weakness and neuromuscular disorder in man [47].

The zinc content of the stored grain decreased from 7.16 to 3.67 mg/100g. Zinc functions as a co-factor for specific enzymes involved in metabolism and cell growth. This trace element is found in about 300 specific enzymes [48]. Being a component of many enzymes, it takes part in the metabolism of proteins, lipids carbohydrates, and energy. Zinc plays an essential role in many biochemical pathways. It is involved in cell growth and reproduction, protein and DNA synthesis, insulin activity, and liver function [49]. Zn deficiency causes stunted growth of infants and growing children, impaired cognitive and immune functions [50].

4. CONCLUSION

The results of this research work show that some proximate and mineral components in *Sorghum bicolor* were relatively stable while others experienced noticeable depletion during prolonged storage. Mycoflora population and grain deterioration also increased with storage time. Prolonged storage of *Sorghum bicolor* grains is responsible for increased mycoflora population and degradation of vital nutrients in the stored grain. Consumption of nutritionally bare food without fortification with mineral supplements may lead to malnutrition and compromised state of animal and human health.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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