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Effect of Water Activity and Storage Time on the Nutritional Quality and Microbial Stability of Maize

Asfandyar Alam[1], Shafi Ullah Gul[1], Mohammad Salim[2], Muhammad Rasheed Khan[3], Sajid Ali[4], Hafiz Anwar Ullah[5], Humaira Sarfraz[6], Umair Ahmad Termizi[7], Hassan Ali Shah[7]

[1]Department of Zoology, Government Post Graduate College Karak, KP, Pakistan.
[2]Department of Forestry and Wildlife Management, The University of Haripur, KP, Pakistan.
[3]Department of Agriculture Chemistry, The University of Agriculture, Peshawar, KP, Pakistan.
[4]State Key Laboratory of Tree Genetics and Breeding, Northeast Forestry University, Hexing Road, Harbin, China.
[5]Department of Zoology, The University of Lahore, Punjab, Pakistan.
[6]Department of Chemistry, Minhaj University Lahore (MUL), Punjab, Pakistan.
[7]Department of Horticulture, The University of Haripur, KP, Pakistan.

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Correspondence to: Mohammad Salim, Department of Forestry and Wildlife Management, The University of Haripur, KP, Pakistan.
Email: mohammadsalim@uoh.edu.pk

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ABSTRACT

The aim of this study was to investigate the effect of water activity and storage time on the nutritional quality and microbial stability of maize grains. The research work was conducted in the Department of Agricultural Chemistry, The University of Agriculture Peshawar during 2015. Maize grain sub-samples, each of 50 g were weighed into sterile baby food jars with micro porous caps and rehydrated to 0.85, 0.90 and 0.95aw levels by the addition of distilled water. Jars of the same aw levels were then enclosed in sealed plastic containers together with salt-water solution at the same aw to maintain uniform relative humidity inside the boxes. At 15 days interval, three samples of each aw level were randomly selected and analyzed for proximate composition, starch content, mineral contents and total fungal viable counts. Analysis of the data showed that both water activity and storage time significantly affected the proximate composition, starch, mineral content and total fungal count of the grains. The highest moisture content (30.0%) and starch (75.19%) were recorded at 0.95 aw and 0.85 aw on day 1 whereas their lowest amounts were noted after 60 days of storage in control sample and at 0.95aw, respectively. The average ash content was higher in control sample, which progressively decreased with increasing aw levels over storage period. The crude protein content and nitrogen free extract (NFE) were minimum (10.0 and 39.73%) at 0.95 aw on day 1 of the experiment, which gradually increased to 15.03% at 0.85aw and 67.49% in control sample, respectively after 60 days of storage. Crude fats and crude fibers significantly reduced with storage time whereas the effect of aw was not significant on them. All macro and micro elements including Na, K, Fe, Ca, Zn, Mg and Cu showed maximum values i.e. 604.30, 3468.90, 51.87, 423.09, 55.95, 1639.21 and 22.03 mg/kg at 0.95 aw on day 15 whereas the least amount of these elements i.e. 577.50, 3445.6, 33.65, 403.01, 34.69, 1618.26 and 6.09 mg/kg, respectively were found in control sample after 60 days of storage. The total fungal viable count showed an increasing trend with increasing aw levels over storage period. It was concluded that aw and storage time played key role in maintaining the quality of post-harvest stored grains. It was, therefore recommended that aw of the grains should be kept minimum for extending the storability and nutritional quality of cereal grains.

INTRODUCTION

Maize (Zea mays L.) is commonly known as corn which is an annual cereal crop belonging to the grass family Poaceae (Gramineae). Among the four species of the genus Zea, only species mays which is economically important, with a plant height ranging from 1–4 meters. It is a group of monoecious, cross-pollinated crop bearing male and female flowers separately on the same plant. The maize is a major cereal crop Globally due to its economic value, dietary importance, and contribution to a food security. In Pakistan, it is the second most important Kharif crop which is primarily cultivated in Khyber Pakhtunkhwa and Punjab (Khalil & Jan, 2005). During 2014, it was grown on approximately 1.168 million hectares, producing 4.944 million tons (MINFAL, 2014). However, the national average yield still remains about 33% lower than the

world average this is mainly due to climatic variation and differences in production technologies.

Nutritionally the maize is widely utilized as for food purposes, animal feed, and raw material for oil and alcohol production (Asiedu, 1989). The maize contains 7–13% protein, although the protein quality is poor owing to a deficiency of essential amino acids such as lysine and tryptophan (Lahouar et al., 2000). If we see the maize on dry weight basis, maize comprises approximately 70–75% digestible carbohydrates, 4% lipids, 2% crude fiber, and 1.2% ash (Kulp & Joseph, 2000). It is also a good source of macro- and micro-nutrients including phosphorus, potassium, magnesium, zinc, and iron, as well as vitamins such as thiamin, niacin, and folate (Nuss & Tanumihardjo, 2010).

The importance of maize grains it is highly vulnerable to post-harvest deterioration. The maize grains in tropical and subtropical regions are often stored under hot and humid conditions with limited drying and storage facilities (Weinberg et al., 2008). The hygroscopic nature of maize is to absorb moisture from the environment resulting in deterioration, insect infestation, and fungal contamination (Devereau et al., 2002). The High moisture content (above 11%) particularly encourages the growth of molds and mycotoxigenic fungi, leading to big loss of nutritional quality, reduced grain weight, and economic damage (Barney et al., 1995; Marin et al., 1998). The store fungi are governed by a combination of nutritional, physical, and biotic factors, with water activity (aw) and temperature being the most critical (Miller, 1995).

In developing countries, post-harvest grain losses due to insects and microbial infestation are estimated at $500 million to $1 billion annually (Cuevas et al., 2005). The fungal attacks which is not only reduce the nutritional and market value of maize but also produce harmful secondary metabolites, further threatening food security (Egal et al., 2005). Therefore, maintaining grain quality during storage requires controlling moisture content, temperature, and relative humidity (Nukenine, 2010; Jian & Jayas, 2012).

Given the significance of water activity and storage conditions in determining the biochemical stability and safety of maize, this study was designed to evaluate the effect of aw and storage time on the biochemical composition and storage stability of maize cultivar Azam. The findings will provide valuable insights for a researchers, producers, consumers, and policymakers to adopt effective grain storage strategies for minimizing the big losses and ensuring food security threats.

MATERIALS AND METHOD

1.1 Samples Collection

A composite sample (2 kg) of maize (Zea mays L.) variety Azam was procured from the Agricultural Research Farm, The University of Agriculture, Peshawar. The experimental work was carried out in the laboratory of the Department of Agricultural Chemistry, The University of Agriculture, Peshawar. Prior to storage studies for the samples were analyzed to determine their initial water activity (a_w). Subsequently a moisture sorption of isotherm was constructed for the maize grains to evaluate their moisture adsorption behavior under different environmental conditions

1.2 Water Activity of Samples

The water activity (a_w) of the maize samples was measured using a Novasina Thermoconstanter TH200 (Axaid Ltd., Pfäffikon, Switzerland) by using the following procedure described by (Kashan et al., 1986). The instrument was also switched on and allowed to equilibrate for a minimum of 2 hours prior to measurement. Maize grains were placed in clean plastic sample bowls and positioned within the measuring sensor knob. After securely the closing chamber of the door, the instrument was allowed to reach equilibrium, and a_w values were recorded to three decimal places. All the determinations were performed in a triplicate to ensure accuracy and reproducibility.

1.3 Moisture Sorption Isotherms for the Samples

The moisture sorption isotherm of maize samples was determined following the method of (Alam et al., 2014). The 10 g sub sample of each were prepared in triplicate and placed into glass universal bottles. The distilled water was added 0.5 mL increments (0.5–3.0 mL) to hydrate the samples. The bottles were sealed, shaken thoroughly, and left overnight to ensure complete absorption of water.The water activity (a_w) of the hydrated samples was measured using the Novasina Thermoconstanter TH200, as described earlier. So each sample was then weighed using an analytical balance and transferred to small beakers, with both the weight and corresponding a_w recorded. The beakers were oven dried overnight at 80 °C, after which the samples were reweighed. The moisture content (%) of the grains was calculated using the following equation:

$$\% \text{ MC} = \frac{\text{Weight Day 2- Weight Day 3} \times 100}{\text{Weight Day 2}}$$

So using MS-Excel, graphs were produced relating the percent moisture content to a_w and the amount of added water (ml) vs. a_w of the samples.

1.4 Modification of Samples' Water Activity and Storage

Maize grain sub-samples (50 g each) were weighed into sterile baby food jars fitted with microporous caps and adjusted to the desired water activity levels (0.85, 0.90, and 0.95 a_w) by the addition of distilled water, using the moisture sorption isotherm of the grains (Appendix 1). The sample were prepared without water addition. The jars were stored for 48 h at 4°C to allow the equilibration and achieve the target a_w with the help of periodic shaking to ensure uniform moisture distribution.

The following equilibration, jars corresponding to each a_w treatment were placed in sealed plastic containers which contain saturated sodium chloride water solutions of the same a_w to maintain a equilibrium which relative humidity (ERH). All samples were incubated at 25 °C for the storage study. At 15-day intervals the three jars from each a_w level along with the control they were randomly selected and analyzed for the designated biochemical parameters. The total storage duration is 60 days.

1.5 Moisture Content

The moisture content of maize samples was determined

using the oven-drying method.Empty Petri dishes were first weighed, after which the samples were added and their initial weights recorded using an electronic balance. The partially covered Petri dishes were then placed in a hot-air oven at 105 °C for 4–6 h. The following drying and the dishes were cooled in a desiccator and reweighed. The percentage moisture content was calculated using the following formula:

$$\% \text{ Moisture} = \frac{V1 - V2}{\text{Weight of sample}} \times 100$$

Where,

V_1 = Initial weight of Petri dish + sample weight

V_2 = Final weight of the Petri dish + sample weight

1.6 Ash Content

The ash content of maize samples was determined using the direct ignition method. The clean, dried and crucibles were pre-weighed, after which 1 g of sample was placed into each crucible. The samples were initially charred by using a Bunsen burner flame with the help of aid of a blowpipe to ensure complete combustion. The crucibles also contain the charred samples were then transferred to a muffle furnace at 600 °C until complete ignition was achieved. The fallowing ashes has been and crucible were cooled in a desiccator and reweighed. The percentage ash content was calculated using the following formula:

$$\% \text{ Ash} = \frac{\text{Wt. of ash}}{\text{Weight of sample}} \times 100$$

1.7 Crude Fat

The crude fat content was also determined by using a Soxhlet apparatus. Approximately there is 1 g of sample was accurately weighed, wrapped in filter paper, and placed in a thimble. The thimble was inserted into the extraction tube of the apparatus. A pre-weighed beaker containing petroleum ether was attached and the extraction carried out through repeated siphoning. After completion, the beaker was removed and placed in an oven to evaporate the solvent. The beaker was then cooled and reweighed. The percentage of crude fat was calculated as:

$$\% \text{ Crude fat} = \frac{\text{Weight of beaker +oil} - \text{Weight of empty beaker}}{\text{Weight of sample}} \times 100$$

1.8 Crude Protein

Crude protein was determined using the Kjeldahl method. Approximately 1 g of sample has been digested with 12 mL concentrated H₂SO₄ and a digestion mixture of K₂SO₄:CuSO₄ (7:1, 8 g) in the presence of pumice stones which prevent the bumping. The digest was diluted to 100 mL with distilled water. So for distillation, 10 mL NaOH was added to 10 mL of the digest, and the released ammonia was collected in 20 mL of 4% boric acid containing a few drops of modified methyl red indicator, which turned yellow upon absorption of ammonia. The solution was titrated against 0.1 N HCl, and nitrogen content was calculated using the following formula:

$$\% \text{ N} = \frac{(S-B) \times N \times 0.014 \times D}{\text{Wt. of sample} \times V} \times 100$$

$$\text{Crude Protein (\%)} = \%N \times 5.6$$

Where:

S= Sample titration reading

B= Blank titration reading

N= Normality of HCl

D= Dilution of sample after digestion

V= Volume taken for titration

0.014= Mille equivalent wt. of Nitrogen

1.9 Fiber Crude

The Crude fiber was also determined following the standard acid–alkali digestion method. Accurately the weighed of 2 g of ground sample was placed in a beaker containing 200 mL of 1.5% HCl and heated in a water bath at 100 °C for 2 h. The contents were filtered through a muslin cloth and the residue was transferred to another beaker containing 200 mL of 1.5% NaOH and again heated at 100 °C for 2 h. After the completion all the contents were filtered and the residue washed sequentially with hot water and acetone, followed by oven-drying. The dried residue is also then ignited in a muffle furnace at 600 °C until complete ashing. The percentage crude fiber was calculated based on the weight loss of carbonaceous material using the following formula:

$$\%\text{Crude Fiber} = \frac{(\text{Wt. of oven dried residue - Wt. after ignition})}{\text{Wt. of sample}} \times 100$$

2.0 Nitrogen Free Extract

The total digestible carbohydrate that was represented by nitrogen free extract (NFE) was calculated by using following formula.

NFE = 100 - % (Proteins + Fats + Ash + Crude Fiber)

2.1 Mineral Analysis

The dried powdered of a maize samples were digested using a mixture of nitric acid and perchloric acid. The digest was analyzed for micro-minerals (Cu, Zn, Mg, Fe, and Mn) using an Atomic Absorption Spectrophotometer (AAS), while Na and K were determined using a flame photometer.

2.2 Preparation of Acid Digest

The Wet digestion of samples was carried out following the method. Accurately weighed 1 g of powdered sample was placed in a digestion tube with 10 mL concentrated HNO_3 and kept overnight in the dark. The next day 5 mL concentrated $HClO_4$ was added, and the tubes were heated gradually up to 200 °C until the dense white fumes disappeared, indicating completion of digestion. The digests were cooled to room temperature and filtered through Whatman No. 42 filter paper, so the diluted with distilled water to a final volume of 100 mL in a volumetric flask. The prepared digests were stored in a refrigeration until analysis.

2.3 Sodium and Potassium Content

Flame photometer was used for the determination of sodium and potassium by the method of Khalil and Ullah (2004).

A. Preparation of standard curves:

The standard solutions of a sodium and potassium were prepared from NaCl and KCl salts in 100 mL volumetric flasks. The emission intensities of the standards were measured by using a flame photometer with a potassium filter (786 nm) and a sodium filter (589 nm). The Standard curves for a sodium and potassium were then constructed

by plotting emission intensity against concentration using MS Excel.

B. Sample assay:

An aliquot the 10 mL of the sample digest was analyzed using a flame photometer, and the emission intensities of sodium and potassium were recorded as described for the standards. The concentrations of Sodium and Potassium were calculated from the respective standard of curves and expressed as mg/kg of sample.

2.4 Determination of micro minerals

The sample digests were analyzed using an Atomic Absorption Spectrophotometer (Hitachi model 170-10). Specific hollow cathode lamps were used for each element. The standard solutions of the respective minerals were run before and during analysis for calibration and instrument check. A dilution factor of 100 was applied for all minerals except Mg. For calcium determination, 1.0 mL lithium oxide solution was added to the digest to prevent interference from Mg. Mineral concentrations were calculated using the following equation:

$$\text{Concentration } (\mu g/g) = \frac{Absorbance reading \times dilution factor \times 100}{Weight of Sample}$$

2.5 Determination of Total Fungal Counts

The total fungal population was estimated using Malt Extract Agar (MEA) medium (Christensen, 1957). One gram of sample was added to 9 mL of sterilized water containing 0.01% Tween 80 in universal bottles and mixed thoroughly by mechanical agitation for 2 minutes. A serial dilution (10^{-3}–10^{-4}) was prepared, and 100 µL from the appropriate dilution was spread on MEA plates using sterilized bent Pasteur pipettes. The plates were incubated at 25°C for 7–10 days so after incubation, the fungal colonies were counted, and the viable fungal population per gram of sample was calculated.

2.6 Starch Determination

Starch content was determined following the method of (Potzi et., al 2006). A 5 g of sample was washed sequentially with 10% and 30% alcohol on a filter paper and the residue was transferred to a beaker which containing 50 mL of water. The suspension was heated for 15 minutes with constant stirring to gelatinize the starch. After cooling to 50°C the 0.03 g of diastase enzyme (dissolved in 5 mL of water) was added, and the mixture was incubated at 60°C for 1.5 hours. The suspension was then heated to 100°C and filtered. The residue was hydrolyzed with 0.1 N HCl for 2 hours then cooled and neutralized with 0.1 N Na_2CO_3, and the volume was made up to 500 mL with distilled water.

The reducing sugars were estimated using the Lane and Eynon (1923) method. For this, 5 mL of Fehling's solution A and 5 mL of Fehling's solution B were boiled with the test solution until the blue color disappeared. A few drops of methylene blue were then added, and continued titration until a brick red end point was observed which indicating complete reduction.

$$\text{Dextrose} = \frac{500 \text{ ml} \times 0.1200 \times 100}{\text{Sample titration in ml} \times \text{Sample wt. in gram}}$$

2.7 Statistical Analysis

The data were analyzed for Analysis of Variance (ANOVA) through Statiscal Package Statistix 8.1 using Completely Randomized Design (CRD) with two-factors. Means were separated through Least Significant Difference (LSD) test. All the means were calculated from triplicate values.

RESULT AND DISCUSSION

The Maize samples were adjusted to a different water activity (aw) levels (0.85, 0.90, and 0.95) and stored in sealed jars for 60 days. The Samples were also taken every 15 days, along with a control, and analyzed for the biochemical parameters and fungal counts. Results are presented in tables and figures.

3.1 Moisture Content

The water activity and storage time significantly affected the moisture content of maize grains ($p < 0.05$). Moisture was the highest at 0.95 aw (20.73%) and lowest in the control (10.36%). Storage time also reduced moisture from 21.72% on day 1 to 12.8% after 60 days. The combined effect showed the highest value (30.0%) at day 1 and 0.95 aw, while the lowest (10.7%) was in the control at 15 days. These findings agree with previous reports (Dorsey-Redding et al., 1990; Ullah et al., 2010; Adeyeye et al., 1992). The high moisture will also reduces the storability and promotes mold growth which highlighting the need for proper storage of maize.

Table 3.1.

Moisture content (%) of maize grains at different a_w level during storage for two months.

Water activity (a_w)	Storage time (Days)					Mean
	1	15	30	45	60	
Control	11.20	10.70	10.40	9.80	9.70	10.36 d
0.85	21.50	19.20	18.83	14.83	11.50	17.17 c
0.90	24.17	18.67	18.13	18.00	13.50	18.49 b
0.95	30.00	22.83	17.33	17.00	16.50	20.73 a
Mean	21.72 a	17.85 b	16.18 c	14.91 c	12.80 d	

Means followed by same letters in each row and column are not significantly different at $p \leq 0.05$

LSD value for Storage time = 1.2923

LSD value for a_w = 1.1559

LSD value for Storage time × a_w = 2.584

3.2 Ash Content

Ash content of maize was significantly affected by storage time ($p < 0.05$) but not by Water activity. It decreased from 1.95% on day 1 to 1.23% after 60 days, with the lowest value (0.95%) at 0.95 aw after 60 days. Overall, ash content declined with increasing aw and storage duration, likely due to microbial activity. These results agree with earlier findings (Sawhney et al., 1995; Ullah et al., 2010).

Table 3.2.

Ash content (%) of maize grains at different a_w level during storage for two months

Water activity (a_w)	Storage time (Days)					Mean
	1	15	30	45	60	
Control	1.98	1.70	1.60	1.57	1.57	1.68
0.85	1.95	1.87	1.55	1.43	1.33	1.63
0.90	1.92	1.73	1.67	1.30	1.07	1.54
0.95	1.93	1.70	1.57	1.47	0.95	1.52
Mean	1.95 a	1.75 b	1.60bc	1.44 c	1.23 d	

Means followed by same letters in each row and column are not significantly different at p ≤ 0.05
LSD value for Storage time =0.1798
LSD value for a_w= 0.1608LSD value for Storage time × a_w =0.3596

3.3 Crude Protein Content

The Crude protein content of a maize was significantly affected by both water activity and storage time. It was highest at 0.85 aw (15.02% after 60 days) and lowest at 0.95 aw (10.0% on day 1). The Proteinous content increased with storage up to 45 days (13.40%), then slightly decreased (12.83% at 60 days). So the overall the lower aw maintained higher protein levels which have longer storage initially increased and later reduced protein content. These results are consistent with earlier reports (Mepba et al., 2007; Sawhney et al., 1995; Zarkadas et al., 2000).

Table 3.3

Crude Protein (%) of maize grains at different a_w level during storage for two months.

Water activity (a_w)	Storage time (Days)					Mean
	1	15	30	45	60	
Control	10.13	10.95	12.64	14.04	11.21	11.79 b
0.85	10.03	10.69	13.06	14.07	15.03	12.58 a
0.90	10.10	11.40	12.04	13.08	13.36	12.00 b
0.95	10.00	11.69	12.73	12.40	11.72	11.71 b
Mean	10.07 d	11.18 c	12.62 b	13.40 a	12.83a b	

Means followed by same letters in each row and column are not significantly different at p ≤ 0.05
LSD value for Storage time = 0.6056
LSD value for a_w = 0.5417
LSD value for Storage time × a_w = 1.2112

3.4 Crude Fat content

The study assessed how the Azam variety of maize's crude fat content was affected by storage time and water activity (an). The percentage of Crude fat was significantly impacted by storage time but not by water activity (p > 0.05). Crude fat content rose from 15.33% on day 1 to 15.77% on day 15. After that, it gradually decreased and reached its lowest point at 12.23% on day 60. With the highest crude fat (16.20%) recorded at 0.95a_n on day 15 and the lowest (11.46%) at 0.85a_n on day 60, the relationship between a_n and storage time was also significant. The Long term storage also reduced crude fat overall, particularly at lower a_n levels.These results are consistent with earlier observations showing variations in fat content in cereal grains and imply that enzymatic lipolytic activity (lipase and lipoxidase) may be the cause of decreases during storage. The slightly increases under the greater a_n could be related to the mold growth and contributing small amounts of lipid.

Table 3.4.

Crude fats (%) of maize grains at different a_w level during storage for two months

Water activity (a_w)	Storage time (Days)					Mean
	1	15	30	45	60	
Control	15.33	15.80	13.93	13.20	12.74	14.20
0.85	15.33	15.23	13.97	13.03	11.47	13.81
0.90	15.33	15.87	14.13	13.33	12.33	14.20
0.95	15.33	16.20	14.17	13.65	12.37	14.34
Mean	15.33 a	15.77 a	14.05 b	13.31 c	12.23 d	

Means followed by same letters in each row and column are not significantly different at p ≤ 0.05
LSD value for Storage time = 0.6301
LSD value for a_w = 0.5636
LSD value for Storage time × a_w =1.2602

3.5 Crude Fiber Content

Crude fiber of maize was not significantly affected by aw but decreased significantly with storage time. It was 3.00% at day 1 and declined to 1.38% after 60 days. The highest value (3.33%) was recorded at day 15 (0.90–0.95 aw), while the lowest (1.00%) was at 60 days in control and 0.95 aw. The reduction which occur in fiber may be due to microbial activity and fermentation, consistent with previous reports.

Table 3.5.

Crude fiber (%) of maize grains at different a_w level during storage for two months

Water activity (a_w)	Storage time (Days)					Mean
	1	15	30	45	60	
Control	3.00	2.50	2.33	2.17	1.00	2.20
0.85	3.00	2.83	2.33	2.17	2.00	2.47
0.90	3.00	3.33	2.17	1.67	1.50	2.33
0.95	3.00	3.33	2.67	1.50	1.00	2.30
Mean	3.00 a	3.00 a	2.38 b	1.88 c	1.38 d	

Means followed by same letters in each row and column are not significantly different at p ≤ 0.05
LSD value for Storage time = 0.4910
LSD value for a_w = 0.4392
LSD value for Storage time × a_w = 0.9821

3.6 Nitrogen Free Extract (NFE)

The NFE concentration of maize was reported in 3.6 table as considerably (p<0.05) impacted by both storage period and water activity (aw). NFE was highest in the control group (58.32%) and lowest at 0.95 aw (50.76%). NFE rose from 48.45% on day one to 60.47% after 60 days in storage. The highest NFE (67.49%) in the control group after 60 days and the lowest (39.73%) at 0.95 aw on day 1, the interaction effect was particularly noteworthy. In general, NFE rose with storage time but fell with increasing aw. These findings are consistent with a previous research showing NFE values of 79.56–83.75% in corn hybrids and 63.0–70.1% (Aerts et al., 1976). Increased α-amylase activity may be connected to the decrease in NFE with greater aw (Lasekan, 1996).

Table 3.6.

NFE of maize grains at different a_w level during storage for two months.

Water activity (a_w)	Storage time (Days)					Mean
	1	15	30	45	60	
Control	60.39	49.35	56.33	58.03	67.49	58.32 a
0.85	48.18	58.21	50.25	54.46	58.67	53.96 b
0.90	45.48	49.00	60.16	52.62	58.24	53.10 b
0.95	39.73	44.24	51.54	60.82	57.46	50.76 b
Mean	48.45 c	50.20 c	54.57 b	56.48 b	60.47 a	

Means followed by same letters in each row and column are not significantly different at p ≤ 0.05

LSD value for Storage time = 3.8240
LSD value for a_w = 3.4203
LSD value for Storage time × a_w = 7.6481

3.7 Starch content

The effect of water activity and storage time on starch content of maize was shown in Table 3.7. Both water activity and storage time significantly ($p<0.05$) affected the starch content of the maize. The lowest value of the starch content (74.81 %) was recorded at 0.95aw while the control sample showed maximum value of starch content (74.93 %). So regarding storage, the highest value of a starch (75.12 %) was observed at 1st day, which progressively decreased with storage time. The lowest starch content is also recorded as (74.57 %) after 60 days of a storage. The interactive effect of aw and storage time was also found significant ($p<0.05$). Control sample showed the highest value of starch content (75.19 %) at 0.85 aw while lowest value of starch content (74.33 %) was observed at the end of the experiment (60 days) at 0.95aw. The result showed that average starch content of the maize was decreased by increasing both water activity and storage time. The present results are supported by previous findings. (Kent, 1982) reported that maize contains 80.2% starch."Similarly, (Idikut et al., 2009) reported that maize contained 69.29-73.71%starch which fairly supports our results. In another study, (Adeyeye et al., 1992) analyzed major cereal grains such as maize, rice, sorghum, millet and examined that the starch content ranged from 70.7 to 82.4 %. The starch content in maize sample was significantly affected by both water activity and storage time in the current study. Higher amount of water provided favorable condition for microbes and due to more feeding of microbes the decrease occurred in starch content. During storage time the decrease was also observed in starch content, which might be due to endogenous amylolytic activity (Rehman and Shah, 1999).

Effect of aw and storage time on the minerals profile of maize

The NFE content of maize was significantly affect both aw and storage time. It was lowest (50.76%) at 0.95 aw and highest (58.32%) in control. The over storage, NFE increased from 48.45% (day 1) to 60.47% (day 60). The maximum value (67.49%) was observed at day 60 in control, while the minimum (39.73%) was at day 1 under 0.95 aw. So overall the NFE will decreased with increasing aw but increased with storage time, in line with earlier reports.

Table 3.7.
Starch content of maize grains at different a_w level during storage for two months

Water activity (a_w)	Storage time (Days)					Mean
	1	15	30	45	60	
Control	75.08	74.99	74.93	74.80	74.86	74.93 a
0.85	75.19	75.05	74.72	74.69	74.61	74.85 ab
0.90	75.10	75.02	74.91	74.58	74.47	74.82 b
0.95	75.11	74.91	74.86	74.83	74.33	74.81 b
Mean	75.12 a	74.99 b	74.85 c	74.73 d	74.57 e	

Means followed by same letters in each row and column are not significantly different at $p \leq 0.05$
LSD value for Storage time =0.1090
LSD value for a_w = 0.0975
LSD value for Storage time × a_w =0.2179

3.8 Sodium content

Na content of maize was significantly affected by both aw and storage time. It is also increased with higher aw but decreased with storage. The maximum value (604.30 mg/kg) was recorded at 0.95 aw after 15 days, while the minimum (577.50 mg/kg) was observed at 0.85 aw after 60 days. These results agree with earlier findings (Ullah et al., 2010).

Table 3.8.
Sodium content (mg/kg) of maize grains at different a_w level during storage for two months

Water activity (a_w)	Storage time (Days)					Mean
	1	15	30	45	60	
Control	591.17	587.80	585.57	586.00	585.53	587.21 c
0.85	591.20	595.70	586.50	582.60	577.50	586.70 c
0.90	590.77	599.80	591.30	587.40	582.80	590.41 b
0.95	590.73	604.30	597.40	592.30	587.50	594.45 a
Mean	590.97 b	596.90a	590.19b	587.08c	583.33d	

Means followed by same letters in each row and column are not significantly different at $p \leq 0.05$
LSD value for Storage time = 0.9000
LSD value for a_w = 0.8050
LSD value for Storage time × a_w =1.8000

3.9 Potassium Content

The potassium content of a maize was significantly influenced on both aw and storage time. It increased with higher aw, with the maximum value (3460.60 mg/kg) at 0.95 aw, while the lowest (3452.26 mg/kg) was in the control. So during storage, K content peaked at 15 days and then gradually decreased, reaching 3448.48 mg/kg after 60 days. These results are consistent with (Ullah et al., 2010).

Table 3.9.
Potassium content (mg/kg) of maize grains at different a_w level during storage for two months

Water activity (a_w)	Storage time (Days)					Mean
	1	15	30	45	60	
Control	3458.70	3456.60	3452.30	3448.60	3445.10	3452.26 d
0.85	3458.40	3462.50	3455.60	3449.40	3445.60	3454.30 c
0.90	3458.50	3466.60	3459.50	3454.30	3449.70	3457.72 b
0.95	3458.90	3468.90	3463.50	3458.20	3453.50	3460.60 a
Mean	3458.63b	3463.65a	3457.73c	3452.63d	3448.48e	

Means followed by same letters in each row and column are not significantly different at $p \leq 0.05$
LSD value for Storage time = 0.8251
LSD value for a_w = 0.7380
LSD value for Storage time × a_w = 1.6502

4.0 Iron content

The Fe content is also increased with higher aw, with the

maximum (45.15 mg/kg) at 0.95 aw and the lowest (37.73 mg/kg) in the control. Storage time also had a significant effect; Fe peaked at 15 days (46.42 mg/kg) and then declined up to 60 days. These results are in agreement with (Ullah et al., 2010).

Table 4.0.

Iron content (mg/kg) of maize grains at different a_w level during storage for two months

Water activity (a_w)	Storage time (Days)					Mean
	1	15	30	45	60	
Control	42.00	39.67	37.56	35.79	33.65	37.73 d
0.85	42.00	45.65	41.79	38.56	35.82	40.76 c
0.90	42.00	48.50	44.66	41.59	38.62	43.07 b
0.95	42.00	51.87	47.56	43.65	40.66	45.15 a
Mean	42.00 c	46.42 a	42.89 b	39.90 d	37.19 e	

Means followed by same letters in each row and column are not significantly different at p ≤ 0.05
LSD value for Storage time = 0.8251
LSD value for a_w = 0.7380
LSD value for Storage time × a_w =1.6502

4.1 Calcium content

The Calcium content is also increased with higher aw, reaching 415.64 mg/kg at 0.95 aw, while the control had the lowest (403.01 mg/kg). Storage time showed a rise up to 15 days (max 423.09 mg/kg) followed by a decline till 60 days. These results agree with who reported 410 mg/kg in Azam maize.

Table 4.1.

Calcium content (mg/kg) of maize grains at different a_w level during storage for two months

Water activity (a_w)	Storage time (Days)					Mean
	1	15	30	45	60	
Control	408.00	412.05	409.06	406.05	403.01	407.63 d
0.85	408.00	415.06	412.09	409.07	406.06	410.06 c
0.90	408.00	418.09	414.09	411.08	408.09	411.87 b
0.95	408.00	423.09	419.03	416.03	412.06	415.64 a
Mean	408.00 d	417.07 a	413.57 b	410.56 c	407.31 d	

Means followed by same letters in each row and column are not significantly different at p ≤ 0.05
LSD value for Storage time = 0.8251
LSD value for a_w = 0.7380
LSD value for Storage time × a_w = 1.6502

4.2 Zinc Content

The zinc content is also increased with higher aw, reaching 48.64 mg/kg at 0.95 aw, while the control had the lowest (39.81 mg/kg). So during storage, Zn rose up to 15 days (max 55.95 mg/kg at 0.95 aw) and then declined, reaching 36.60 mg/kg after 60 days. These values agree with Ullah et al. (2010), who reported 45.2 mg/kg in Azam maize.

Table 4.2.

Zinc content (mg/kg) of maize grains at different a_w level during storage for two months

Water activity (a_w)	Storage time (Days)					Mean
	1	15	30	45	60	
Control	45.23	42.21	39.35	37.56	34.69	39.81 d
0.85	45.23	48.69	43.39	39.65	36.60	42.71 c
0.90	45.23	51.56	47.74	43.77	38.69	45.40 b
0.95	45.23	55.95	51.87	46.79	43.36	48.64 a
Mean	45.23 b	49.60 a	45.59 b	41.94 c	38.33 d	

Means followed by same letters in each row and column are not significantly different at p ≤ 0.05
LSD value for Storage time =0.8522
LSD value for a_w = 0.7623
LSD value for Storage time × a_w= 1.7045

4.3 Magnesium content

The Mg content is also increased with higher aw (max 1632.25 mg/kg at 0.95 aw) and decreased with storage time, with the highest value (1639.2 mg/kg) at 15 days and the lowest (1618.3 mg/kg) after 60 days. These findings are consistent with (Ullah et al., 2010), who reported 1625 mg/kg Mg in Azam maize.

Table 4.3.

Magnesium content (mg/kg) of maize grains at different a_w level during storage for two months.

Water activity (a_w)	Storage time (Days)					Mean
	1	15	30	45	60	
Control	1624.67	1627.56	1625.61	1622.23	1618.26	1623.67 d
0.85	1624.67	1632.56	1628.62	1624.32	1621.23	1626.28 c
0.90	1624.67	1637.56	1632.20	1629.21	1626.11	1629.95 b
0.95	1624.67	1639.21	1635.55	1632.30	1629.52	1632.25 a
Mean	1624.67 d	1634.22a	1630.50b	1627.01c	1623.78e	

Means followed by same letters in each row and column are not significantly different at p ≤ 0.05
LSD value for Storage time = 0.8231
LSD value for a_w = 0.7362
LSD value for Storage time × a_w = 1.6

4.4. Copper Content

The Cu content increased with higher aw (max 17.66 mg/kg at 0.95 aw) and decreased with storage time so by peaking at 22.03 mg/kg after 15 days and dropping to 9.09 mg/kg at 60 days. These results agree with (Ullah et al.,2010) who reported 14.03 mg/kg Cu in Azam maize.

Table 4.4.

Copper content (mg/kg) of maize grains at different a_w level during storage for two months

Water activity (a_w)	Storage time (Days)					Mean
	1	15	30	45	60	
Control	14.02	15.09	13.02	11.08	9.09	12.46 d
0.85	14.02	17.06	15.02	13.09	11.05	14.05 c
0.90	14.02	19.09	17.06	15.07	13.06	15.66 b
0.95	14.02	22.03	20.09	17.09	15.09	17.66 a
Mean	14.02 c	18.32 a	16.30 b	14.08 c	12.07 d	

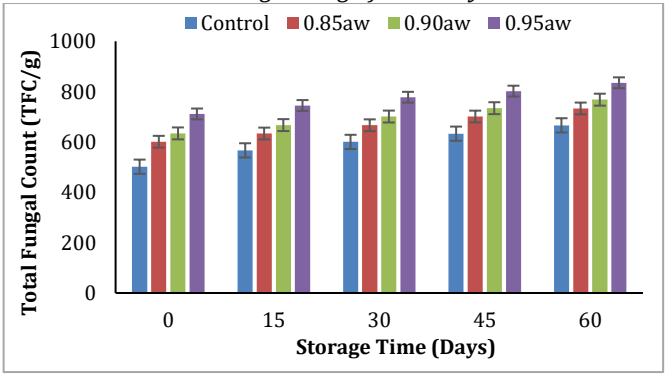
Means followed by same letters in each row and column are not significantly different at p ≤ 0.05
LSD value for Storage time = 0.8042
LSD value for a_w = 0.7193
LSD value for Storage time × a_w = 1.6084

4.5 Total fungal count

The TFC increased significantly with higher aw and longer storage. The lowest count (5.01 ×10^2 CFUs/g) was at day 1 in control, while the highest (8.35 ×10^2 CFUs/g) was at 0.95 aw after 60 days. These findings agree with earlier studies reporting fungal growth and contamination in stored maize.

Figure 4.5

Total Fungal Count (CFUs/g) of maize grains at 0.85, 0.90 and 0.95 aw level during storage for 60 days.



CONCLUSION

It was concluded from the research work that Water activity and storage time significantly affected the nutritional quality and total fungal viable count of maize grains. Ash content, crude fiber and starch content had inverse relation with both water activity (0.85 – 0.95 a_w) and storage time (60 days). Crude protein and NFE had negative correlation with water activity (0.85-0.95a_w) while positive correlation with storage time (60 days). Moisture content was increased with increasing water activity while decreased with storage time. Crude fat content and all macro and micro element including Na, K, Ca, Mg, Fe, Cu and Zn showed increasing trend with increasing water activity (0.85-095a_w). However, with respect to storage time, these constituents showed increasing trend up to 15 days from the beginning of the experiment and then decreased with increasing storage time till the end of the experiment (i.e.60 days). Total fungal count was significantly increased with increasing both water activity and storage time.

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