EFFECT OF WATER ACTIVITY AND STORAGE TIME ON THE PROXIMATE COMPOSITION OF TWO CHECKPEA CULTIVARS

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ABSTRACT: *The experiment was laid out in Complete Randomized design with three repeats. Chickpea varieties (KK3 and KC98), Storage interval (00, 20, 40 and 60 days) and Water activity (control, 0.5, 0.6 and 0.7* a_w*) were considered as factor 1, 2 and 3 respectively. Both cultivars were collected from Agricultural Research Station, Karak. Initial analysis of water activity and construction of moisture sorption isotherms was done for the samples. Henceforth 50 gram of each sample was modified for water activity and storage. The modified sample was then analyzed for proximate composition and fungal count using the respective procedure. Results obtained for various parameters indicated a significant effect of the treatments on chickpea varieties. In proximate composition a net increase in % moisture content and % ash was observed however % fat and % protein showed a significant decrease. % fiber and nitrogen free extract showed a non significant effect for* a_w *and storage time. It was observed that all the factors alone and their interaction significantly affected the total fungal count.*

KEYWORDS: Chickpea **(***Cicer arientium* L.), Water Activity, Total fungal count, proximate composition, Nitrogen free extract, storage interval.

INTRODUCTION

Chickpea (*Cicer arientium* L.) also known as garbanzo bean or channa is an edible legume. It is an important food source in India, Pakistan, Africa and South America. During 2009-10 Pakistan produced about 741 thousand tonnes of chickpea on an area of 1080 hectares and during the same year the production and area under cultivation in Khyber Pakhtunkhwa was 42 thousand hectares and 20 thousand tonnes, respectively (ASP, 2009).Chickpea has an extensive use in human consumption. It is commonly used in many food items such as salads and soups. Chickpea has two types: Kabuli and Desi. Kabuli type is suited to temperate and Desi type to semi arid regions (Muehlbauer and Singh, 1987). Chickpea is used as a whole, grinded into dhal or finely powdered called

Besan for usage in Indo-Pak sub-continent. The chemical constitution and essential amino acids in both types of chickpeas are same (Viveros et al., 2001).The nutritional value of chickpea is very high. Besan of chickpea is very rich in protein. Many dishes are formed from besan in India and Pakistan. It has a higher amount of protein, fat and fiber as compared to white flour but provides fewer calories (Iqbal et al., 2006). It also contains important minerals and vitamin D. Because of the low amount of carbohydrates in chickpea they offer a healthy nutrition to diabetic patients. Amount of fat in chickpea is also low and mostly in unsaturated form. Chickpea seeds contain about 37-58% carbohydrate, 3% fiber, 4.7 to 5.4% fat, 3% ash and 0.3% phosphorous. Protein and carbohydrate digestibility ranges from 76 – 78% and 57 to 60%, respectively (Hulse, 1991). 100 gm of raw chickpea seed have: 357 calories, 4.5 to 15.7% moisture, 14.9 to 24.6% protein, 0.8 to 6.4% fat, 2.3 to 11.7% fiber and 2 to 4.8% ash (Geervani, 1991). Storage conditions play an important role in germination and shelf life of chickpea (Burr et al., 1968; Garcia and Stanely, 1989; Hentages et al., 1991). The changes in the nutritional and textural characteristics of chickpea seeds depend a lot on the amount of available moisture, which is commonly called as water activity (a_w). The quality attributes which mainly include; texture, aroma, shelf life and flavor are greatly influenced by water activity. It is also important to the stability of pharmaceuticals and cosmetics (labuza et al., 1972). While temperature, pH and several other factors can influence if and how fast organisms will grow in a product, water activity might be the most important factor in controlling spoilage. Most bacteria do not grow at water activities below 0.80 a_w and most molds cease to grow at water activities below 0.70 a_w. By measuring water activity, it is possible to predict which microorganisms will and will not be potential sources of spoilage. Water activity is the most prominent factor in controlling the microbial growth in food items along with the activation of several enzymes which are very important in taste, color and aroma development (Vertucci et al., 1994). Labuza et al., (1972) reported the relationship between a_w and spoilage of foods. They found that reducing the water activity below 0.7 prevent microbial spoilage, however, other deteriorative reactions can still occur. To successfully preserve a food product, the water activity (a_w) should be lowered to a range where the rate of deteriorative reactions is minimal. Karel (1978) studied the relationship between a_w and stability of food and observed that there is an optimum water activity for each food at which it gives relatively increased storage life. At different a_w levels the extent of lipid oxidation, browning production, enzymatic activity and microbial growth is different. Such changes are closely related with water activity rather than moisture content. Lang et al., (1981) determined moisture content after equilibrating against a saturated salt solution by using proximity cell (PEC) and conventional dissector. Same work was done by Landrock and proctor in 1951. Sumbul and Heperkan (1990) reported that low relative humidity level during harvesting and subsequent storage was adequate for obtaining low levels of moisture content and water activity in the products for safe storage. High relative humidity in winter did not increase the moisture content of product and as a result no mould growth was observed. Therefore all storage facilities were deemed safe for duration of one year. This project was initiated to study the effect of water activity (a_w) and storage time on proximate composition and fungal growth of two common chickpea varieties, (Desi and Kabuli). The results of this study might be of great importance to researchers, producers and consumers.

MATERIAL AND METHODS

Samples collection

Composite samples (0.5 kg) of two chickpea cultivars KC-98 and KK-3 were obtained from Agricultural Research Station, Karak. The experiment was conducted in the post-graduate laboratory of Department of Agricultural Chemistry, University Of Agriculture, Peshawar.

Modification of samples' water activity and storage

Chick pea sub-samples (150 g) were weighed into sterile baby food jars with micro porous caps and rehydrated to the required a_w (0.5, 0.60 and 0.70a_w) by the addition of distilled water using the moisture absorption curves of the respective cultivar. Control samples were stored without the addition of water. The jars were initially stored at 4ºC for 48 hrs to modify the seeds to the required a_w. They were regularly shaken to obtain a uniform treatment. Jars with the same a_w were then enclosed in sealed plastic containers together with sodium chloride-water solution at the same a_w to maintain the treatment uniform inside the boxes. All the samples jars were incubated at 25ºC. At 15 days intervals, three samples of each a_w level along with control were destructively sampled and analyzed for the following parameters. The total storage time was 60 days.

Proximate composition

The proximate composition i.e. % moisture content, % ash, % crude protein, % fat and % crude fiber were determined by the standard methods of AOAC (2003). Nitrogen free extract was calculated by difference.

Frequency of isolation and total fungal counts in samples

The total fungal populations and dominant genera were isolated from the samples using MEA by dilution plate method (Christensen, 1957). Accurately weighed 1 g of each sample was put in 9 ml of sterilized water containing 0.01% Tween 80 in glass Universal bottles. The contents were mechanically agitated for 2 minutes. A serial dilution series was made using 1 in 9 ml (10^{-2} to 10^{-4} dilution). 100 µl of the suspension from the appropriate dilutions were transferred to Petri plates containing MEA and were spread plated using sterilized bent Pasteur pipettes. All the Petri plates were incubated at 25^0C for 7-10 days. The numbers of colonies of different fungi were counted and the total fungal viable count per gram of samples was calculated.

RESULTS AND DISCUSSION

Moisture content (%)

The data regarding the effect of Water Activity (a_w), storage time and varietals difference on the moisture content of chick pea is shown in Table 1. The statistical analysis of data showed that a_w and storage time significantly affected the moisture content, while varieties had a non significant effect. The maximum moisture content (10.37%) was found at the interval of 40 days where as minimum moisture content (5.12%) was at the start (0 day) of experiment. The minimum moisture content (5.85%) was found at control level where as the maximum moisture content (8.97%) was noted at 0.7 a_w. The overall effect of storage interval, a_w and cultivars showed that all the factors significantly ($P \leq 0.05$) affected the moisture content of chickpea. In case of interactive effect, higher moisture content (15%) was observed for the interaction of KK3, 40 days interval and a_w of 0.7 while interaction of KK3 with 60 days interval and a_w of control had shown minimum moisture content (2.59%). The results of our findings are in close agreement with those of Shehzadi et al. (2007) who reported an increase in moisture content of chickpea and lentil as a result of storage. The moisture content showed an increase from 0 days to 40 days however after 40 days a net decrease in moisture content was observed.

Table 1. Moisture content (%) of chick pea cultivars at different a_w levels and stored for 60 days.

Varieties (V)	Water Activity (a_w)	Interval (I) (days)				Mean
		00	20	40	60	V x a_w
KK3	Control	5.2	8.7	4.7	2.6	5.27
	0.5	5.5	9.7	8.0	5.5	7.15
	0.6	5.5	9.0	11.0	6.2	7.93
	0.7	5.5	10.0	15.0	7.9	9.60
KC-98	Control	4.8	7.7	8.0	5.2	6.42
	0.5	4.8	8.7	9.7	7.1	7.55
	0.6	4.9	9.3	12.7	8.4	8.81
	0.7	4.9	8.0	14.0	6.4	8.33
		V x I				
KK3		5.4	9.3	9.7	5.55583	7.49
KC-98		4.8	8.4	11.1	6.7675	7.78
		a_w x I				
	Control	4.97	8.17	6.33	3.92	5.85 c
	0.5	5.12	9.17	8.83	6.28	7.35 b
	0.6	5.18	9.17	11.83	7.29	8.37 a
	0.7	5.2	9	14.5	7.16	8.97 a
Mean		5.12 d	8.87 b	10.37 a	6.12 c	

LSD value for interval at $p \leq 0.05$ = 0.63
LSD value for Water Activity (a_w) at $p \leq 0.0$ = 0.63
LSD value for varieties x interval at $p \leq 0.05$ = 0.88
LSD value for varieties x Water Activity (a_w) at $p \leq 0.05$ = 0.88
LSD value for interval x Water Activity (a_w) at $p \leq 0.05$ = 1.25
LSD value for varieties x interval x Water Activity (a_w) at $p \leq 0.05$ = 1.77

Ash content (%)

The data about the effect of Water Activity and storage time on ash content of two chick pea cultivars is shown in Table 2. The mean values for chick pea cultivars showed that it significantly ($P \leq 0.05$) affected the ash content. However higher ash content (4.1%) was present in KK3 cultivar as compared to KC98 which had 3.5 % ash. The results for a_w suggested that it significantly ($P \leq 0.05$) affected the ash content. It was examined that the ash content gradually increased with storage time. The combined effect of varietals difference, a_w and storage time showed that all these factors significantly ($P \leq 0.05$) affected the ash content. The results obtained for ash content showed similarity with those stated by Shehzadi et al. (2007) who reported a significant effect of storage on the ash content of chickpea. In terms of interaction, KK3 combined with 60 days interval and a_w of 0.5 showed higher ash content (6.66%) while KC98 with 0 day interval and a_w of 0.7 revealed lower ash content (1.5%).

Storage conditions play a very important role in determining the nutritional profile of a particular food. Significant changes in ash and moisture content of chickpea occur as a result of storage (Shehzadi et al., 2007). Storage of chickpeas and lentils for 6-18 months resulted in decrease of ash (4.3-19.4%) (Naghmana, 1997). The content of ash in chickpea seed also depend on the physiological condition of seed. A net decrease in ash and moisture content was reported in chickpea seeds as they near maturity; however other parameters did not exhibit a notable change (Shakra et al., 2006).

Table 2. Ash content (%) of chick pea cultivars at different a_w levels and stored for 60 days.

Varieties (V)	Water Activity (a_w)	Interval (I) (days)				Mean
		00	20	40	60	V x a_w
KK3	Control	4.1	5.0	3.8	2.8	3.9
	0.5	3.7	4.0	5.3	6.7	4.9
	0.6	3.7	2.5	5.5	3.6	3.8
	0.7	3.4	1.5	5.2	5.2	3.8
KC-98	Control	4.0	3.2	4.0	5.1	4.1
	0.5	3.8	2.5	3.0	4.7	3.5
	0.6	3.7	2.0	4.1	2.8	3.2
	0.7	3.6	1.8	3.7	3.5	3.2
		V x I				
KK3		3.7	3.3	4.9	4.6	4.1 a
KC-98		3.8	2.4	3.7	4.0	3.5 b
		a_w x I				
	Control	4.1	4.1	3.9	3.9	4.0 ab
	0.5	3.7	3.3	4.1	5.7	4.2 a
	0.6	3.7	2.3	4.8	3.2	3.5 b
	0.7	3.5	1.7	4.4	4.3	3.5 b
	Mean	3.8 a	2.8 b	4.3 a	4.3 a	

LSD value for variety at $p \leq 0.05$ = 0.41
LSD value for interval at $p \leq 0.05$ = 0.58
LSD value for Water Activity at $p \leq 0.05$ = 0.58
LSD value for interval x Water Activity (a_w) at $p \leq 0.05$ = 1.15
LSD value for varieties x interval x Water Activity (a_w) at $p \leq 0.05$ = 1.63

Fat content (%)

The data exhibiting the effect of Water Activity (a_w) and storage time on the fat content of two chickpea cultivars (KK3, KC98) is shown in Table 3. It was examined that the fat content gradually decreased with the storage time. The mean values for cultivars showed that fat contents did not significantly (P≤0.05) vary between the cultivars. The Water Activity significantly (P≤0.05) affected the fat content however; at 0.5 and 0.6 a_w the fat contents were found the same. Similarly the fats content at 0.7 a_w was same as that found in control samples. The combined effect of varietals difference, a_w and storage time showed that all these factors had non-significantly (P≤0.05) affected the fat content of chickpea. Our results exhibited a fair similarity with those of Esmat et al., (2001) who stated that the fat content decrease significantly as a result of storage. Similar results were reported by Shehzadi et al., (2007).

Table 3. Fat content (%) of chick pea cultivars at different a_w levels and stored for 60 days.

Varieties (V)	Water Activity (a_w)	Interval (I) (days)				Mean
		00	20	40	60	V x a_w
KK3	Control	4.8	4.4	4.1	3.6	4.22
	0.5	4.9	4.5	4.1	3.6	4.26
	0.6	4.9	4.5	4.0	3.7	4.27
	0.7	4.9	4.5	4.0	3.6	4.26
KC-98	Control	4.8	4.3	4.1	3.6	4.20
	0.5	4.9	4.5	4.1	3.6	4.30
	0.6	4.9	4.5	4.0	3.6	4.26
	0.7	4.9	4.4	4.0	3.6	4.24
		V x I				
KK3		4.9	4.5	4.0	3.6	4.3
KC-98		4.9	4.4	4.1	3.6	4.3
		a_w x I				
	Control	4.8	4.4	4.1	3.6	4.2 b
	0.5	4.9	4.5	4.1	3.6	4.3 a
	0.6	4.9	4.5	4.0	3.6	4.3 a
	0.7	4.9	4.5	4.0	3.6	4.2 b
Mean		4.9 a	4.4 b	4.0 c	3.6 d	

LSD value for interval at p≤ 0.05 = 0.042
LSD value for Water Activity at p≤ 0.05 = 0.04

Protein content (%)

The data reported for the effect of Water Activity and storage time on the protein content(%) of two chick pea cultivars i.e. (KK3, KC98) is shown in Table 4. It was examined that the protein content gradually decreased with storage time; however, the decrease was non-significant (P≤0.05). Similar observations were found for Water Activity where maximum protein content (17.0 %) was noted at 0.5 a_w and minimum (14.5 %) in control samples. The overall mean values for cultivars showed that KC98 had more protein content (16.5 %) as compared to KK3 which had 15.1 % protein. The combined effect of varietals difference, a_w and storage time showed that all these factors non-significantly (P≤0.05) affected the protein content. These results are opposite to those of Shehzadi et al. (2007) and Esmat et al. (2010) who reported significant changes in protein content of chickpea as a result of storage.

Table 4. Protein content (%) of chick pea cultivars at different a_w levels and stored for 60 days.

Varieties (V)	Water Activity (a_w)	Interval (I) (days)				Mean
		00	20	40	60	V x a_w
KK3	Control	20.9	16.5	12.8	11.4	15.4
	0.5	20.4	16.5	12.4	14.4	16.0
	0.6	21.2	16.2	13.1	12.3	15.7
	0.7	20.1	10.5	12.6	10.8	13.5
KC-98	Control	18.2	13.3	12.2	10.5	13.5
	0.5	19.6	24.5	12.5	15.7	18.1
	0.6	19.2	24.1	12.4	14.5	17.5
	0.7	18.6	24.7	13.3	11.5	17.0
		V x I				
KK3		20.7	14.9	12.7	12.2	15.1
KC-98		18.9	21.6	12.6	13.0	16.5
		a_w x I				
	Control	19.6	14.9	12.5	10.9	14.5
	0.5	20.0	20.5	12.5	15.1	17.0
	0.6	20.2	20.2	12.7	13.4	16.6
	0.7	19.4	17.6	12.9	11.2	15.3
Mean		19.8	18.3	12.7	12.6	

Fiber content (%)

Table 5 represents the data regarding the effect of a_w and storage time on the fiber content of chickpea cultivars. It was examined that fiber content significantly increased (P≤0.05) with storage time. The highest value of fiber (7.93%) was noted at 40 days interval whereas the lowest (7.37%) at 0 day. The same pattern was observed for Water Activity a_w in which the highest fiber content (7.82%) was examined at 0.7 a_w and lowest fiber (7.50%) was found at control level. However, the effect of a_w was not significant. The means value for cultivars showed that high fiber content (9.03%) was found in KC98 as compared to KK3 which had 6.38 % fiber. The overall effect of these three factors showed that they had non-significantly ($P \leq 0.05$) affected fiber content in chick pea. Our results are in fair agreement with those of Shehzadi et al. (2007) who stated that the fiber content of chickpea is non significantly affected by storage and processing.

Table 5. Fiber content (%) of chick pea cultivars at different a_w levels and stored for 60 days.

Varieties (V)	Water Activity (a_w)	Interval (I) (days)				Mean
		00	20	40	60	V x a_w
KK3	Control	6.19	6.04	6.50	6.27	6.25
	0.5	6.21	6.50	6.49	6.28	6.37
	0.6	6.21	6.52	6.44	6.33	6.37
	0.7	6.98	6.46	6.43	6.29	6.54
KC-98	Control	8.43	7.81	9.44	9.34	8.76
	0.5	8.43	9.51	9.40	9.28	9.15
	0.6	8.30	9.53	9.34	9.26	9.11
	0.7	8.20	9.55	9.39	9.24	9.10
		V x I				
KK3		6.40	6.38	6.46	6.29	6.38 b
KC-98		8.34	9.10	9.39	9.28	9.03 a
		a_w x I				
	Control	7.31	6.93	7.97	7.81	7.50
	0.5	7.32	8.01	7.95	7.78	7.76
	0.6	7.26	8.02	7.89	7.80	7.74
	0.7	7.59	8.01	7.91	7.77	7.82
	Mean	7.37 b	7.74 a	7.93 a	7.79 a	

LSD value for varieties at $p \leq 0.05$ = 0.21
LSD value for interval at $p \leq 0.05$ = 0.24
LSD value for varieties x interval at $p \leq 0.05$ = 0.42

Nitrogen free extract (%)

Table 6 represents the data on the effect of a_w and storage time on the nitrogen free extract (NFE) content of two chickpea cultivars i.e. KK3 and KC98. It was examined that all the factors significantly ($P \leq 0.05$) affected the NFE contents of chickpea cultivars. However, the combined effect of these factors was found non-significant. Chemical analyses showed that KK3 had more nitrogen free extract content (62.62 %) as compared to KC98 (58.94 %). The same outline was shown for storage time in which gradual increase of nitrogen free extract content was observed. The highest amount of nitrogen free extract (65.50%) was noted on 60 days interval where as the minimum nitrogen free extract (57.84%) was observed on 20 days interval. The mean values for the effect of a_w showed that it significantly ($P \leq 0.05$) affected the nitrogen free extract value which gradually decreased from 63.97% in control level to 59.39% at 0.5 a_w. However, nitrogen free extract did not significantly vary at 0.5, 0.6 and 0.7 a_w. The results are supported by the studies of Shehzadi et al. (2007) who reported variation in nitrogen free extract from 80.67% to 82.35% as a result of storage.

Table 6. Nitrogen free extract (%) in chick pea cultivars at different a_w levels and stored for 60 days.

Varieties (V)	Water Activity (a_w)	Interval (I) (days)				Mean
		00	20	40	60	V x a_w
KK3	Control	58.80	59.34	68.23	73.3	64.9
	0.5	59.27	58.85	63.75	63.5	61.4
	0.6	58.49	61.30	59.97	67.9	61.9
	0.7	59.11	67.03	56.73	66.2	62.3
KC-98	Control	59.75	63.83	62.31	66.2	63.0
	0.5	58.50	50.29	61.31	59.6	57.4
	0.6	58.99	50.57	57.51	61.5	57.1
	0.7	59.80	51.52	55.68	65.7	58.2
		V x I				
KK3		58.92	61.63	62.17	67.75	62.62 a
KC-98		59.27	54.05	59.20	63.25	58.94 b
		a_w x I				
	Control	59.28	61.58	65.27	69.76	63.97 a
	0.5	58.89	54.57	62.53	61.58	59.39 b
	0.6	58.74	55.93	58.74	64.70	59.53 b
	0.7	59.46	59.28	56.21	65.96	60.22 b
Mean		59.09c	57.84 d	60.69 b	65.50 a	

LSD value for varieties at $p \leq 0.05$ = 0.81
LSD value for interval at $p \leq 0.05$ = 1.14
LSD value for Water Activity (a_w) at $p \leq 0.05$ = 1.14
LSD value for varieties x interval at $p \leq 0.05$ = 1.61
LSD value for interval x Water Activity (a_w) at $p \leq 0.05$ = 2.28
LSD value for varieties x interval x Water Activity (a_w) at $p \leq 0.05$ = 3.22

Total fungal count

Figure depicts the total fungal count in selected chickpea cultivars as affected by a_w and storage time. It was observed that all the factors alone and interactively significantly affected the total fungal count. Generally KC98 was more prone to fungal attack than that of KK3. A gradual increase in total fungal count was observed in both the cultivars with storage time. At the start of experiment (0 day) the total fungal count in KK3 and KC98 were examined to be 11.15 x 10^3 and 13.13 x 10^3 CFUs/g, respectively whereas highest level of total fungal count (i.e. 210 x 10^3 and 224.50 x 10^3 CFUs/g, respectively) were observed after 60 days of storage. Similar pattern of results were observed for a_w. In control samples of KK3 the total fungal viable count was observed to be 102.14 x 10 ^{3} CFUs/g whereas at 0.7a_w the total fugal count was examined to be 120.22 x 10 ^{3} CFUs/g. Similarly, in KC98 the lowest value (109.77 x 10 ^{3} CFUs/g) was counted in control samples whereas the highest value (125.20 x 10 ^{3} CFUs/g) was examined at 0.7a_w. The results of our findings are fairly supported by the work done by Monach et al., (1988), Klich, (2007), Rupela et al., (2008) and Alam and Shah (2009).

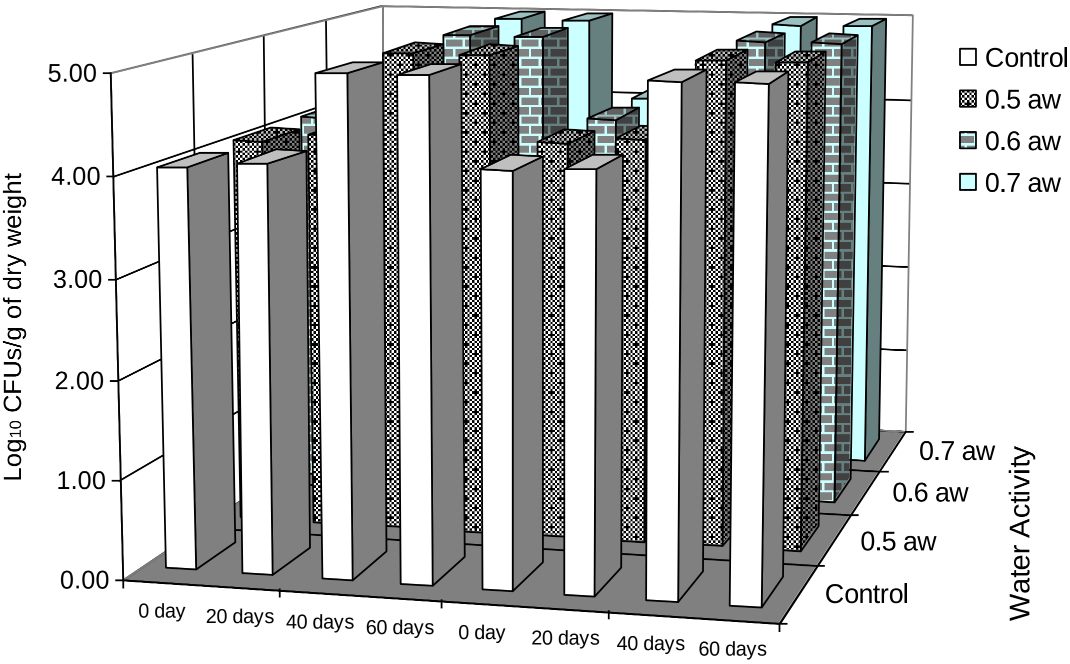

Figure: Total fungal count of two chickpea cultivars as affected by a_w and storage time.

CONCLUSIONS

Finally we were able to infer as;

- Protein and fat contents showed a significant decrease with increasing a_w and storage time.
- Ash, moisture and crude fiber exhibited a significant increase with increasing a_w and storage time.
- Total Fungal Count was significantly increased in both the cultivars during the 60 days storage interval.
- KK3 (Desi chickpea) was more resistant in term of nutrients stability as compared to KC98 (Kabuli).

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