

Full Length Research Paper

Safe storage guidelines for black gram under different storage conditions

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Received 1 December, 2014; Accepted 9 February, 2015

India is the leading producer and importer of pulse in the world. Post-harvest loss is very high in India with losses during storage around 5 to 10%. This situation demands the development of storage guidelines for pulses to provide information to farmers on how long storage is possible without deterioration. Black gram is selected for this study as it is an important pulse used in many traditional specialty products in our country. The major storage conditions that affect any grain are temperature and moisture content. Quality parameters of black gram stored at different initial moisture contents (9, 12, 15 and 18% wet basis) at 20, 30 and 40°C were determined. The storage variables (moisture content of the sample, storage temperature and time of storage) had a negative correlation with germination and a positive correlation with fatty acid value (FAV). The maximum storability of 42 weeks with good seed viability and appreciable microbial stability was found in 9% initial moisture content black gram stored at 20°C. The 15 and 18% black gram stored at 30°C was safe up to 10 weeks hence the post harvest treatment like drying to safe moisture content can be recommended. High risk is involved in storing Black gram at higher moisture contents (15 and 18%) at high temperature of 40°C, beyond 2 to 4 weeks because of loss in seed viability and increase in FAV and the early infection with visible and invisible moulds. The safe storage guidelines chart and safe storage time model developed can be used to predict allowable safe storage time of black gram between moisture content and storage temperature ranges of 9 to 18% w.b. and 20 to 40°C, respectively.

Key words: Black gram, moisture content, storage temperature, safe storage guidelines.

INTRODUCTION

Pulses are a gift of nature; they nourish mankind with highly nutritive food and are major sources of protein especially for vegetarians (Savadatti, 2007). India is the largest producer and consumer of pulses in the world contributing around 25 to 28% of the total global production. The production of total pulses in India is presently about 15 million tons covering an area of about 22 to 23 million hectare (Gol, 2013). In India, variety of pulses are grown like chickpea, pigeon pea, lentil, black

gram, green gram, lablab bean, moth bean, horse gram, pea, and cowpea (Nene, 2006). Among these black gram is one of the important pulses used in everyday diet of south Indians. Due to its fermentation capacity it is used in the preparation of various foods like idli and dosa (Campbell-Platt, 1994). The crop is of great importance as about 70% of world's black gram production comes from India (Singh and Singh, 1992) and 10 to 12% national share among the total Indian pulse production

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(Basu, 2011). India is the largest producer of black gram contributing around 70% of the world's total Black gram production.

Pulses can remain in edible condition for a long time, if properly stored in bags or silos. However, pulses are more difficult to store than cereals and suffer much greater damage from insects and microorganisms resulting in deterioration of quality (Mills, 1994). Most important factors of grain deterioration are the interaction of temperature, humidity and moisture, which are the determining factors in accelerating or delaying the complex degradation reactions (Kreyger, 1972). In general, high temperature and high moisture grain allows a very short time for post harvest operations (Hall, 1980). It becomes essential to determine the allowable time before spoilage for wide range of moisture contents and temperatures. This would help farmers by informing them the number of days before which the grain has to undergo post harvest treatments. This ensures the quality of grain to be maintained throughout the storage period (Nithya et al., 2011). Hence, only good quality grain can be sent for further processing to provide safe and nutritive products for the consumers.

The safe storage guidelines will help farmers to determine the number of days in which the grain has to be dried for a particular temperature moisture combination (Karunakaran, 1999). The time period for which the grain can be stored safely without deterioration or without any significant loss in its quality and quantity is known as the safe storage time (Schroth, 1996). The quality assessment factors of stored grain which could be monitored are the seed germination, fat acidity value, appearance of mould growth, percentage rise in batter volume, and protein changes (Pomeranz, 1992; Mills, 1992). Changes in quality of stored grain have been studied for various grains, oilseeds and pulses (Karunakaran et al., 2001; White et al., 1999). Although safe storage guidelines with respect to moisture content and temperature are available for a pulses like chickpea (Cassells and Caddick, 2000) and red gram (Sravanti et al., 2013), nothing had been reported so far for Black gram. As there are no standard storage guidelines for black gram, an attempt has been made to study the rate of deterioration of black gram by monitoring the quality changes when stored at different moisture and temperature conditions and further developing guidelines for safe storage.

MATERIALS AND METHODS

Sample preparation

The pulse chosen for this study is black gram (ADT-5), procured from local market with initial moisture content of $12.5 \pm 0.5\%$ w.b. The black gram was cleaned and sorted using a pulse cleaner cum grader developed at Indian Institute of Crop Processing Technology (IICPT), Thanjavur. Black gram were conditioned to the required moisture contents in the range of 9.0 to 20% w.b. To obtain lower

moisture content, samples were dried in thin layer in the open for natural air drying for about 8 h. The higher moisture content, samples were conditioned by adding a calculated quantity of distilled water and mixed by passing through a screw conveyor. The conditioned grain samples were then stored in sealed polythene bags in a deep freezer at $-5 \pm 2^\circ\text{C}$ (Singh and Goswami, 1996).

Selection of storage conditions

Temperature and moisture content are the two important factors determining the storage life of grains after harvest. In general, pulses are harvested after the darkening of the pods at in the moisture range of 14 to 18% w.b. Depending on the weather conditions the moisture will be lower in case of a sunny day and higher in case of a rainy day. In general for safe storage pulses are dried to around 11 to 12% moisture before storage. The different levels of moisture contents for the present study, that is, 9 to 18% w.b. were selected on the basis of storage conditions of pulses throughout the world. During the period of harvest and storage, the grains are subjected to a wide range of temperature (20 to 40°C) depending upon the harvesting season. To simulate temperatures that black gram could undergo during harvest and storage in India, temperatures of 20 , 30 and 40°C were selected.

Experimental setup

All experiments were performed in three environmental chambers (Industrial, Chennai) maintained at 20 , 30 and 40°C with $60 \pm 5\%$ relative humidity. The schematic diagram of the experimental setup is shown in Figure 1. For each set of experiments the conditioned black grams were taken in three different mesh bags. Two bags were of 2.5 kg capacity and the third one was of 5 kg capacity. The 5 kg bag was sandwiched between the two 2.5 kg bags which acts as buffer by preventing any loss of moisture from 5 kg sample bag. About 2 L of KOH of known specific gravity was taken in the plastic pails to maintain the required equilibrium relative humidity (Solomon, 1951). The mesh bag were placed in the plastic pail over a support system to hold the grain sample in the mesh bags (Rajaramanna et al., 2010). A lid was loosely placed on the top of each pail. All experiments were performed in environmental chambers maintained at 20 , 30 and 40°C with 60% relative humidity. Three replicates were done for each temperature and moisture combination.

Determination of quality assessment parameters of black gram under storage

Sampling was done every week and grain samples were collected from the middle bag, after mixing thoroughly, for quality analysis.

Germination

The germinating ability of the grain is the index used to assess the viability of the stored product (Pomeranz, 1992). The change in seed germination over time was tested every week according to the method of Wallace and Sinha (1962). About 10 g of sub sample was used for the germination test. In a 9 cm diameter Petri dish, 25 seeds were placed on a Whatman no. 3 filter paper and saturated using 5.5 ml of distilled water. The Petri dishes were vertically stacked in a stand; and to prevent desiccation of the filter paper the stacked dishes were covered with a polythene bag. This set up was incubated at 25°C for 4 days. The polythene bag was then removed and incubation was continued for another 3 days. The number of seeds germinated after 7 days of incubation was counted and the

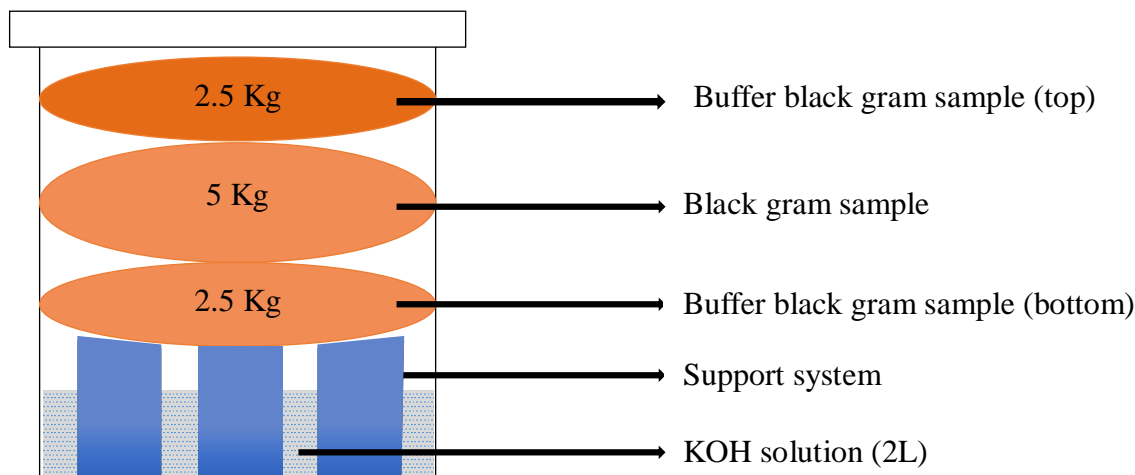


Figure 1. Experimental setup for maintaining equilibrium relative humidity with respect to the moisture content of black gram used for storage studies.

germination (%) was calculated.

Moisture content

The moisture content of the samples was expected to remain constant but needed to be monitored during the study. Therefore, every week, the moisture content was determined by placing approximately 10 g of unground grain in a hot air for 72 h at 130°C (ASAE, 2008). The moisture content of the sample was calculated and expressed in percentage wet basis.

Visible and invisible mould

The deterioration of the grain samples was checked by visual inspection of the samples every week. The presence of invisible mould and the microfloral identification was done every two weeks of storage (Mills et al., 1978). For identification of the invisible microfloral species, 25 seeds were placed in a 9 cm Petri dish with Whattman no. 3 filter paper saturated with 5.5 ml of 7.5% aqueous sodium chloride (NaCl) solution. The Petri dishes were stacked vertically in stands; covered with plastic bags; and incubated at 25°C for 4 days. On the fourth day the polythene bag was removed and incubation was continued for another 3 days. On the seventh day the microfloral species on the grain were identified using a dissection microscope (Leico Microsystems, India). The percentage of infected seed was calculated and microfloral species were identified based on the appearance of microorganisms.

Fatty acid value (FAV)

The fatty acid values were measured at two week intervals. The analysis was carried out according to the American Association of cereal chemist's procedure (AACC, 1962) with some modifications (Schroth et al., 1998; Karunakaran, 1999). The FAV values were determined by using a fat extractor followed by titration with a KOH solution. The whole grains were dried in hot air oven at 130°C for 19 h. About 5 g of grounded dry grain powder was folded in a whattman no. 5 filter paper. This was placed inside a glass thimble/cylinder and was attached to the fat extractor (Pelican, SOCS PLUS Automatic Solvent Extraction System) with 80 ml of

Hexane solvent in beakers. The solvent was heated and allowed to condense, and pass through the same sample for 2 h continuously. The oil was separated from the solvent by heating it again. TAP solution (50% toluene and 50% ethanol with phenolphthalein indicator) of 25 ml was added to the oil. A KOH solution of known normality was used for titration until the appearance of a pale pink color. The calculated FAV was then expressed as mg KOH/ 100 g of dry grain.

Protein content

Protein content of stored beans were tested prior to storage and after storage to study the effect of storage conditions. Control samples and samples at the end of storage period with different initial moisture contents stored at 20, 30 and 40°C were used for protein analysis using AOAC method 990.03 (AOAC, 1999).

Batter volume rise

The most important quality parameter of Black gram is its fermentation ability. So, batter volume rise was calculated to determine the effect of drying temperature and time on fermentation. Black gram (100g) was soaked for 3 h with 200 ml of distilled water. The soaked black gram was ground in a wet grinder for a constant time of 8 min and the batter was collected. For testing the batter volume rise, 100 ml of the batter was kept for fermentation in the measuring cylinder for 24 h at room temperature (33 ± 2) and the rise in volume was noted to calculate the percentage batter volume rise (Tiwari et al., 2008).

Statistical analysis

Statistical analysis was done to check the effect of moisture content, temperature and storage period on germination rate. The analysis of variance (ANOVA) of a three factorial model (4 moisture contents \times 3 temperatures \times 43 weeks) was used to study the effects of temperature, storage period and moisture content on the various dependent variables (germination, FAV, protein and percentage batter volume rise). Least significant difference (LSD) was used for pair wise comparisons between quantitative variables

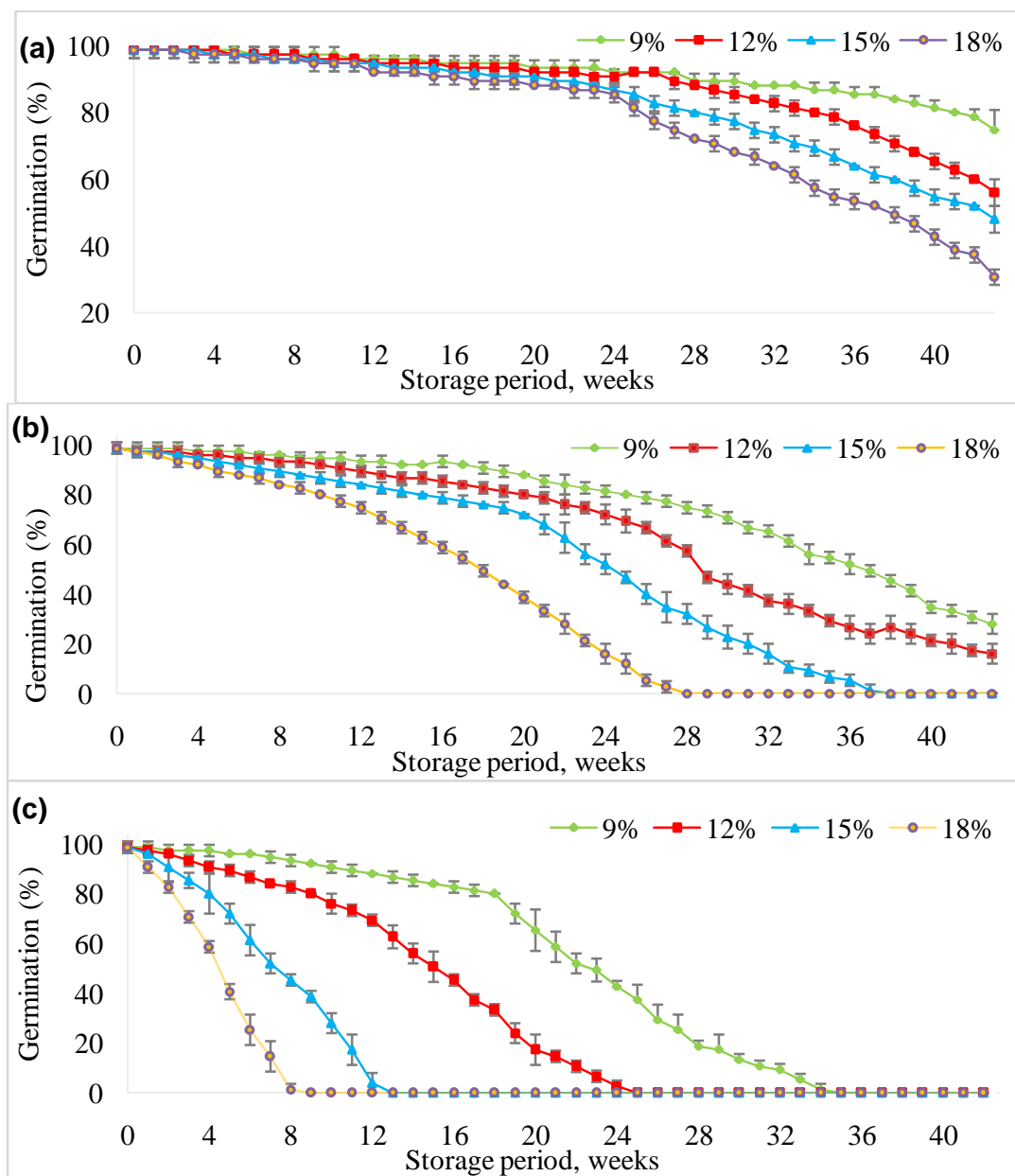


Figure 2. Changes in germination of black gram with different initial moisture contents stored at (a) 20°C, (b) 30°C and (c) 40°C with respect to storage period.

during storage trials. The differences within each level under each variable were tested at a 95% confident interval. General linear models (GLM) procedure in Statistical Analysis System software (SAS version 9.1, Statistical Analysis Systems Institute, Inc., Cary, NC) was used for all the analysis.

RESULTS AND DISCUSSION

Germination

The germination of black gram was used as an indicator

for deterioration during storage as germination is more sensitive to quality changes. Germination is the first factor that gets affected due to improper storage conditions. Figure 2 shows the changes in germination of black gram stored at three different temperatures of 20, 30 and 40°C, with 9, 12, 15 and 18% w.b. initial moisture contents combinations.

The initial germination was around 98.6 % for all the initial moisture contents of black gram stored at different temperature. The grain sample with initial moisture content of 9, 12, 15 and 18% w.b. stored at 20°C did not

lose their viability till 25 weeks of storage; however at 40°C, germination reached zero in the 3rd week of storage. The changes in germination at 30°C were in between these two conditions.

At 40°C, the 12, 15 and 18% initial moisture content samples had a significant decrease in germination after a week of storage. Germination trends decreased and reached 0% germination after 35 weeks of storage for the 9, 12, 15 and 18% initial moisture content samples. The highest moisture content (18%) black gram reached 82.6% germination after 2 weeks of storage. Germination of 80% was reached in the initial moisture content black gram of 9, 12 and 15% after 18, 9 and 4 weeks of storage, respectively. The high moisture content samples 15 and 18% reached 0% germination after 13 and 9 weeks of storage whereas, lowest moisture content samples of 9 and 12% samples reached 0% germination after 35 and 25 weeks of storage (Figure 2a).

In black gram samples stored at 30°C, all the initial moisture content samples were viable up to 10 weeks of storage. The germination of low moisture content samples of 9 and 12% was 78.6% after 26 and 21 weeks, respectively. The 15 and 18% initial moisture content samples germination was 78.6 and 80.0% after 16 and 10 weeks of storage, respectively. The 15% initial moisture content black gram lost all germination after 38 weeks of storage. The 18% moisture content black gram followed similar trends and reached 0% germination after the end of the 28 week of storage (Figure 2b).

At 20°C, the entire moisture content black gram was viable up to 25 weeks of storage. The germination rate of 15 and 18% initial moisture content sample was 81.3 and 78.6% after 29 and 25 weeks of storage, respectively. For 12% initial moisture content samples, germination rate was 78.6% after 35 weeks. The 9% initial moisture content samples germination was 78.6% after 42 weeks of storage (Figure 2c).

According to Pomeranz (1992), germination is the most important factor to assess the quality of grain during storage. The effect of storage parameters like moisture content, temperature and storage time had significant effects ($\alpha = 0.05$) on the germination. The germination decreased with increasing time, temperature and moisture content. This correlates with the results of Christensen and Kauffmann (1969) who reported that increased storage temperatures cause injury or death to most type of grain. The grain samples with low moisture content were susceptible to spoilage at higher temperature of 40°C. Wallace and Sinha (1962) reported that there exists a negative correlation between germination and storage temperature.

Moisture content

The minimum variation in the moisture content of the samples over time was observed in all the initial moisture

content samples. The changes in the initial moisture content of the black gram samples stored at 20, 30 and 40°C with respect to time are shown in Figure 3.

At 20°C, moisture contents of all the black gram samples remained almost constant. The moisture contents of 9, 12, 15 and 18% initial moisture content black gram after 43 weeks of storage increased to 9.9, 12.8, 16.3 and 19.0%, respectively (Figure 3a). At 30°C, the high initial moisture content (15 and 18%) black gram gained moisture over time and increased to 16.2 and 18.1%, respectively after 24 weeks of storage. The buffer samples were replaced to maintain the initial moisture content of the test sample. In 12% initial moisture content samples, there was a gain in moisture content to 13.1% by the end of 43 weeks. The lowest moisture samples (9%) remained almost constant with time and increased to 10.3% moisture content at the end of 43 weeks storage period (Figure 3b).

The grain samples with initial moisture contents of 9, 12, 15 and 18% stored at 40°C, lost moisture over storage time due to drying. The lower initial moisture content of 9 and 12% samples moisture content decreased to 8.3 and 11.4% at the end of 34 and 23 weeks of storage, respectively. The moisture content of higher initial moisture samples (15 and 18%) also decreased with storage time and reached 14.8 and 17.7%, respectively at the end of 12 and 8 weeks (Figure 3c) respectively.

According to Solomon (1951), controlling the relative humidity in biological experiments using chemical solutions like potassium hydroxide has been in practice for a long time. Errors in the humidity control arise if the graded humidity solutions lose too much water through absorption of water vapor by materials enclosed with them or if they absorb water vapor from damp materials. Generally, solutions tend to give too low humidity at elevated temperatures. Furthermore, the equilibrium humidity will deviate from the expected value if the solution is at different temperature than the ambient air temperature above it. This might be the reason for the change in relative humidity inside the storage containers and hence there is a change in moisture contents of the black gram samples over time (Sathya et al., 2008). However by replacement of buffer bags the required initial moisture content of black gram samples was maintained like in the previous studies by Sravanthi et al. (2013) and Rani et al. (2013).

Fatty acid value (FAV)

The fatty acid value (FAV) increases with increase in moisture content of stored black gram. Fatty acid is an intermediate products of hydrolytic reaction caused by the enzymatic secretions of micro-organisms in stored grain. These fatty acids produced in grains have characteristic off-odors. Hence, the FAV has been used

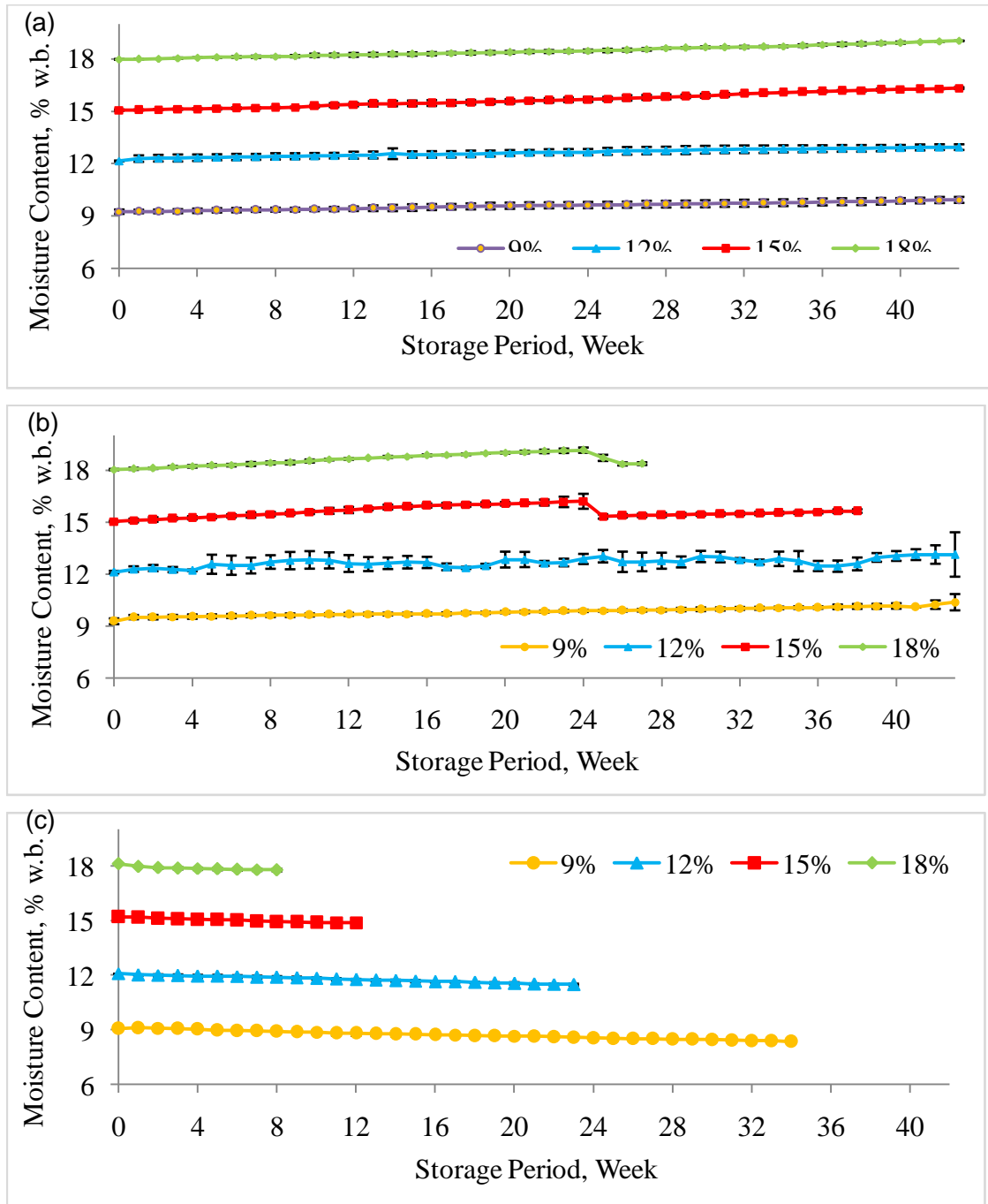


Figure 3. Changes in moisture content of black gram with different initial moisture content stored at (a) 20°C (b) 30°C (c) 40°C with respect to storage period.

as a measure of deterioration of the stored grain (White et al., 1999). The changes in FAV of black gram at three different temperatures of 20, 30 and 40°C, for different initial moisture contents are shown in Figure 4.

The initial free Fatty Acid Value (FAV) of black gram was found to be 2.69 mg KOH/100 g. A positive correlation exists between moisture content, storage

temperature, and storage period with the FAV of stored beans as found in previous studies (Karunakaran et al., 2001; Rajaramanna et al., 2010; Nithya et al., 2011; Rani et al., 2013; Sravanthi et al., 2013). The storage period and moisture content of the grain were found to have a significant effect ($\alpha=0.05$) on the FAV values and there was an increase in the FAV values with increase in

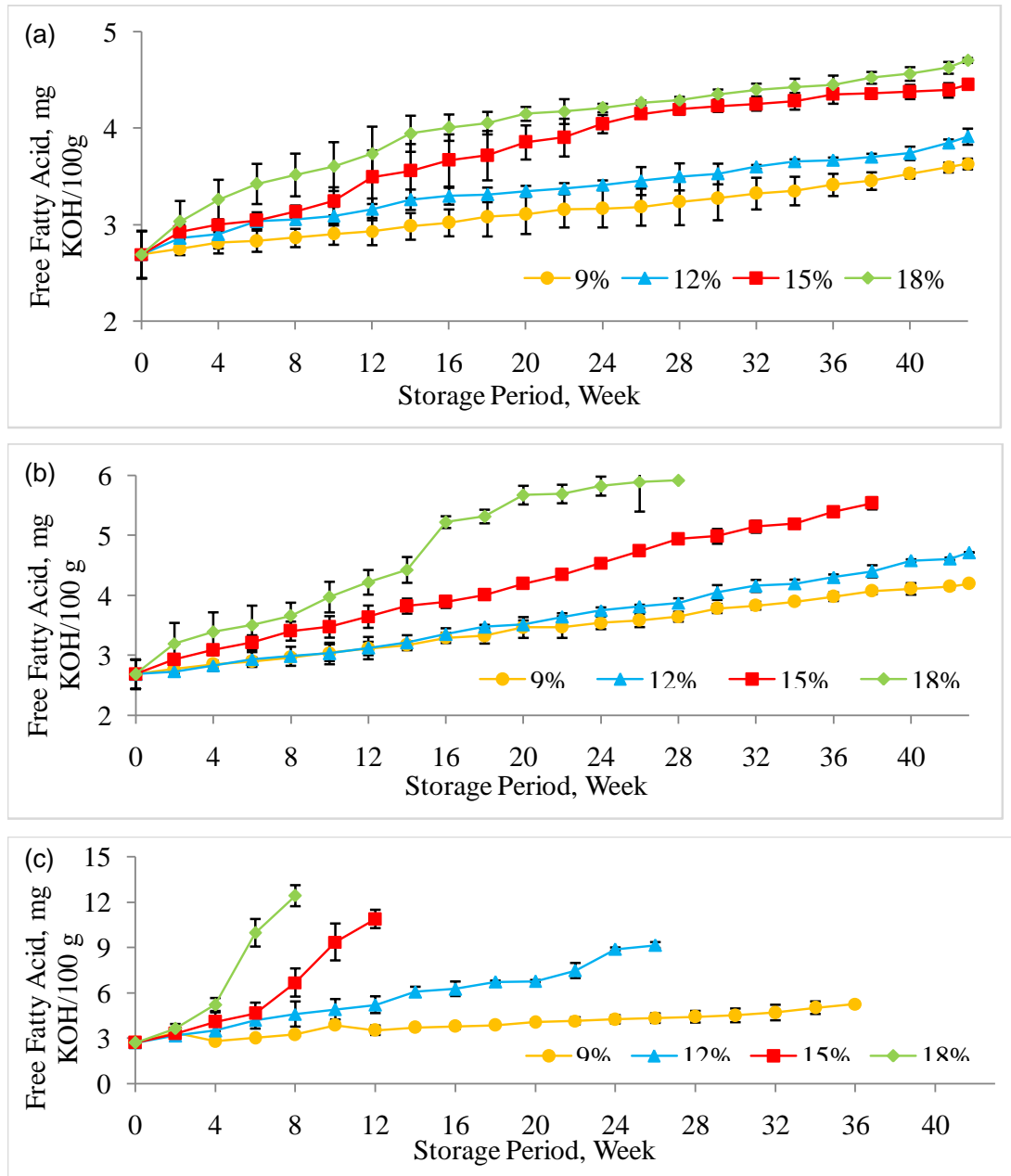


Figure 4. Changes in FAV of different initial moisture content black gram stored at (a) 20°C (b) 30°C (c) 40°C with respect to storage period.

moisture content and storage time. The samples of 15 and 18% initial moisture content stored at 40°C, the FAV values were 11.1 and 12.7 mg KOH/100 g respectively. At 20°C, FAV varied from 3.6 to 4.2 mg KOH/100g for 9 to 18% initial moisture content samples respectively. For the samples stored at 30°C, the FAV of 9 to 18% initial moisture content samples ranged from 4.5 to 5.9 mg KOH/100 g, respectively.

Fatty acids are formed by the hydrolytic reaction caused by the enzymatic secretions of micro-organisms on stored grain. At higher moisture levels grain

undergoes drastic changes due to the proliferation of moulds resulting in production of more free fatty acids at higher moisture and temperature conditions (Nithya et al., 2011).

Visible and invisible mould

The presence of visible mould in grain during storage is always used as an index of deterioration. Increasing the temperature and moisture content favors the growth of

Table 1. Time of first appearance of visible mould on stored red lentil (week) and respective germination (%) of black gram.

Initial moisture content (% wb)	Weeks germination	Storage temperature (°C)		
		40	30	20
9	Week	23		
	g (%)	49.3 ± 4.6	-	-
12	Week	16		
	g (%)	52 ± 13.8	-	-
15	Week	9	25	
	g (%)	38.6 ± 2.3	46 ± 2.3	-
18	Week	5	18	33
	g (%)	40 ± 3.0	49.3 ± 2.3	61 ± 2.3

fungi and presence of visible mould. The visible mould appeared in all high moisture samples irrespective of the temperature. The mould growth was first noticed after the germination dropped well below 80% in all the conditions. The appearance of visible mould can be observed mainly around the hilum of the beans and on the cracks in seed coat. The infected seeds were observed under microscope to ensure the presence of mould. There was no mould growth detected for 9% initial moisture content samples stored at 20 and 30°C. Visible moulds appeared in 18% initial moisture content samples stored at 30°C after 18 weeks of storage. At 40°C moulds growth were visible in 5, 9 and 16 weeks in 18, 15 and 12% initial moisture content black gram samples respectively. The time of appearance of visible mould and the respective germination of the black gram samples at that time is given in Table 1.

Different groups and species of moulds was found in the stored black gram at different periods of storage and at different storage temperatures. The commonly found species in black gram were *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus*. Other microorganisms presented in stored black gram samples were *Penicillium*, *Chaetomium*, *Fusarium* and Bacteria. Some of these mould groups might have infected the black gram in the field and their growth and proliferation can be controlled by lowering the storage temperature. Moisture content of the sample is very critical for the development of visible mould. Even at high temperatures, the low moisture samples were devoid of visible mould.

Invisible mould growth was observed after or along with the visible moulds. The results are in accordance with Christensen and Kaufmann (1969) who reported that the invasion of grains by storage fungi is a direct cause of germination loss and some kinds of grains can survive a long time at rather high moisture contents and moderate temperatures, if kept free of storage fungi. Field fungi may present in the freshly harvested grain and storage fungi develop on the stored grain if the storage

conditions are poor (Muir and White, 2001).

Protein content

The protein content of the black gram samples stored at different temperatures was analyzed by the AOAC - 990.03 method (AOAC, 1999). The storage ended for 9, 12, 15 and 18% initial moisture content samples stored at 40°C at 35, 25, 13 and 9 weeks when germination became zero. Similarly 38 and 28 weeks for 15 and 18% initial moisture content samples stored at 30°C. The initial level of protein for 9 and 18% moisture content samples were 24.3 and 22.0%, respectively. The protein content decreased with increase in storage temperature for all the initial moisture contents of stored Black gram. There was a significant difference in protein content of black gram with respect to storage period for all storage temperatures. The protein content of black gram also varied with respect to moisture content ($\alpha=0.05$). Similar results were reported for pinto beans (Rani et al., 2013) and red gram (Sravanthi et al., 2013).

Batter volume rise

Batter volume rise is an important quality parameter as black gram is used in making many fermented products. The decrease in percentage batter volume rise was statistically significant with respect to temperature and moisture content ($\alpha=0.05$). The initial batter volume rise varied from 82 to 86% with increase in moisture from 9 to 18% respectively. As temperature (20 to 40°C) increased, the batter volume rise decreased with storage period. The 20°C stored samples showed a comparatively lesser reduction in batter volume rise. The results can be related to the research by Tiwari et al. (2008), reporting a decrease in fermentation on heat treatment of black gram due to the heat labile muco-protein and inactivation of

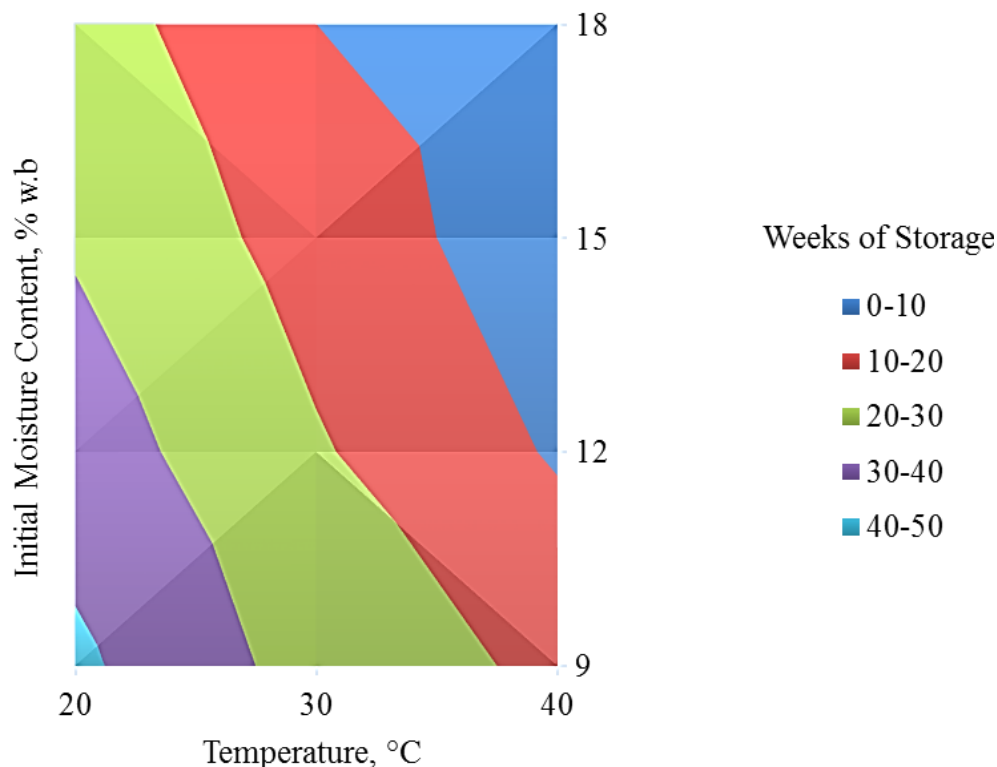


Figure 5. Safe storage guidelines chart for different initial moisture content of black gram stored at different temperature.

enzymes, responsible for fermentation.

Estimated safe storage life of black gram

The estimated safe storage guidelines for black gram were developed based on the decrease in germination rate and appearance of visible mould. When germination was more than 80% of the initial germination (98.8%) and visible mould was absent the black gram is in the safe zone. The number of weeks the samples remained above 80% initial germination without any visible mould was plotted against the initial moisture content and storage temperature to get the estimated safe storage-life guideline (Figure 5). The rate of deterioration increased with increasing initial moisture content and temperature. If the moisture content of the stored grain can be maintained at a sufficiently low level, then that grain can be stored for many years without any significant loss in quality (Tipples, 1995). The time (number of weeks) available for the post-harvest drying treatments before the start of deterioration of stored grain, at a particular moisture and temperature for black gram can be easily determined with the safe storage guidelines chart. The available safe storage time was shown to decrease with increase in moisture content and temperature of the grain during storage.

Conclusion

The storage variables (moisture content of the sample, storage temperature and time of storage) have a negative correlation with germination and a positive correlation with FAV. Germination of the Black gram at 9 and 12% moisture content were maintained above 78.66% at storage temperatures of 20°C for 42 and 34 weeks respectively. But there was significant decrease in germination for higher moisture content (15 and 18%) samples stored at 20, 30 and 40°C with an increase in storage period along with an increase in FAV. The black gram at higher moisture contents (15 and 18%) stored at higher temperature (40°C) lost their viability completely by the end of the 4th and 2nd week of storage with a significant increase in FAV.

The invasion of visible mould around the hilum of the seeds was common in the higher moisture content samples of 15 and 18% irrespective of storage temperature. The lower moisture content samples (9, 12 and 15%) stored at 20°C were free of mould growth for the entire period of storage. *Aspergillus* and *Rhizopus* spp. was the predominant microflora found in the grain. *Penicillium* and *Fusarium* spp. was also common along at lower storage temperature of 20°C. Moisture content, temperature and the storage periods significantly ($\alpha=0.05$) affected the protein content and batter volume

rise of black gram.

Samples with high moisture stored at high temperatures showed visible mould from the beginning of storage. The predominant microfloral species (% of seeds infected) found were *Aspergillus*, *Rhizopus* and *Penicillium*. The percentage of infested grains increased with storage time. The maximum storability of 42 weeks with good seed viability and appreciable microbial stability was found in 9% initial moisture content black gram stored at 20°C. The 15 and 18% black gram stored at 30°C, were safe up to 10 weeks of storage period. The higher moisture contents 15 and 18% at high temperature of 40°C, were safe up to 2 to 4 weeks, respectively. The safe storage guidelines chart and safe storage time model developed can be used to predict allowable safe storage time of black gram between moisture content and storage temperature ranges of 9 to 18% w.b. and 20 to 40°C, respectively.

Conflict of Interest

The authors have not declared any conflict of interest.

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