Vol. 13(2), pp.6-13, July-December 2022 DOI: DOI: 10.5897/JSPPR2022.0325 Article Number: 9E6280A69737 ISSN 2141-6567 Copyright ©2022 Author(s) retain the copyright of this article

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Journal of Stored Products and Postharvest Research

Full Length Research Paper

Interactive effect of moisture content and storage condition on biochemical seed quality of common bean (*Phaseolus vulgaris L.*) in Ghana

E. A. Adjei^{1*}, B. K. Banful², E. A. Asiedu³, P. Marnoh¹, S. Yeboah¹, K. Agyeman¹, M. Arthur¹ and J. Y. Asibuo¹

¹CSIR-Crops Research Institute, Box 3785, Kumasi, Ghana. ²Department of Horticulture, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. ³TAAT Rice Compact, Africa Rice Centre, 01 BP 2551 Bouake 01, Cote d'Ivoire.

Received 3 February, 2022; Accepted 31st May, 2022

Common bean (Phaseolus vulgaris L.) is consumed by over 200 million people in Sub-Saharan Africa (SSA). CSIR-Crops Research Institute in 2016 officially released the first four commercial varieties. This research was undertaken to determine the interactive effect of moisture content and storage condition on the seed quality of common bean seeds stored in the humid region of Ghana. The moisture content (MC) was 8 and 11%, and the storage conditions were ambient and cold. Treatments were arranged in 2 x 2 factorial in completely randomized design with 3 replicates. Seeds were taken after 2, 4, 6 and 8 months storage period for ash content (%), EC (µS cm⁻¹), K and P leachates (ppm), moisture and protein contents (%) analyses. Seed dried to 8% MC and stored under ambient condition showed significantly (p < 0.001) higher ash content of 5.98% compared to 4.72% at 2 and 8 months, respectively. Seeds stored under ambient showed the lowest K leachates of 1244.6, 1706.4 and 2382.7 ppm at 2, 4 and 6 months after storage, respectively. Seeds stored under ambient condition significantly (p < 0.001) lowered P leachate at all storage periods. After 6 months of storage, seeds dried to 11% MC and stored under ambient showed 27.84% protein content significantly higher (p < 0.001) than 26.89% in seeds dried to 11% MC and stored under ambient. Common bean seeds can be dried to 11% MC and stored under ambient condition in the humid region of Ghana. This will be economical to seed growers compared to seeds dried to 8% MC and stored under cold condition.

Key words: Common bean (*Phaseolus vulgaris* L.), moisture content, ambient, cold, seeds, condition.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) is an important grain legume cultivated globally for its multi-faceted benefits. Common bean is consumed for nutritional quality providing dietary protein for over 100 million people in both rural and urban communities in different

products. Consumption of the bean provides health benefits against major non-communicable diseases such as cardiovascular, cancers and others (Kotue et al., 2018). The World Health Organization (WHO) has estimated that 250 million preschool children are vitamin

*Corresponding author. E-mail: adjei_asamoah@yahoo.com. Tel:+233-244679338.

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A deficient (VAD) and it is likely that in VAD areas a substantial proportion of pregnant women are VAD.Also an estimated 250 000 to 500 000 VAD children become blind every year, half of them dying within 12 months of their sights (http://www.who.int/nutrition/topics/vad/en/ accessed on 26/11/19). The leaves and dry pods of the bio-fortified common bean varieties contain enough vitamin A or beta carotene needed by the most vulnerable including pregnant women, children and lactating mothers. The cultivation of the crop has moved from subsistence level and attained commercial importance due to its potential for job creation in the production for export, industrial and other uses (Tumsa et al., 2014). It is also considered as climate smart crop due to its short duration and tolerance to wide temperature (Yeboah et al., 2021). Until recently, Ghana depended on importation of the crop to meet its needs. The first four commercial common bean varieties have been released in Ghana by the Council for Scientific and Industrial Research-Crops Research Institute (CSIR-CRI) in 2016 and are being promoted for adoption and cultivation locally. The adoption and productivity of the released varieties in terms of achieving the potential yields is still low (Yeboah et al., 2021). In order to achieve the potential benefits of the crop the quality of seeds produced and supplied to farmers will be of much importance for improved productivity. In the humid tropics, characterized with high temperature and relative humidity. Seed Growers face challenges in maintaining initial seed quality in storage. The high temperature and relative humidity conditions lead to seed aging which can be regulated by the moisture content and storage condition (Silva et al., 2018). Stored common bean seeds undergo changes in the quality caused by increase in the seeds' respiration rate and enzymatic activity which could be increased by 2-3 times when the moisture content and the temperature increase (Ferreira et al., 2017). Determining the best seed moisture content for seed storage will reduce seed deterioration in storage. Knowledge of seed moisture is useful in seed testing to prevent the imbibition injury during germination and also standardize results of conductivity and accelerated aging tests for vigour (Bakhtavar et al., 2019). Increases in seed moisture content, storage duration or temperature of storage condition rapidly increase the rate of deterioration (Claudio et al., 2019; Rugut et al., 2010).

This study was therefore undertaken to determine the interactive effect of moisture content and storage condition on the biochemical seed quality of common bean seeds stored in the humid region of Ghana.

MATERIALS AND METHODS

Study area and seed source

The study was conducted at the research field of the Council for Scientific and Industrial Research-Crops Research Institute (CSIR-CRI) located at Fumesua (01°36'W; 06°43'N.), in the Ejisu

Municipality in the Ashanti Region. The area falls in the forest agroecological zone. Seeds of common bean variety *Nsroma* (local parlance which means star), (red seeded)), was sourced from CSIR-Crops Research Institute Legumes Division. Seed multiplication field was established in May, 2019. Planting was done at a spacing of 60 cm × 20 cm three seeds per hill and thinned to 2 seedlings two weeks after planting. All the good agronomic practices were done and harvesting was done at maturity when pods have turned brown. Manual threshing was done by beating harvested pods in sacks, seeds dried to 11% moisture content (MC) and sorted for the commencement of the storage studies.

Experimental procedure on seed storage

The experimental design for the seed storage studies was 2 x 2 factorial (two moisture contents and two storage conditions) in a completely randomized design, replicated three times. The moisture contents were 8 and 11%, and the storage conditions being ambient and cold storage. Dehumidification of seed from 11 to 8% MC was done using a Munter's model MX 1500E dehumidifier. Samples were taken periodically to check the MC with an agraTronix MT-16 Grain Moisture Tester. Seeds dried to 11 and 8% MC were packaged in moisture-proof polythene bags, 10 x 10 cm and 0.2 mm thick, heat-sealed. Sets of packaged seeds of each lot were stored in a warehouse constructed for ambient seed storage at 27-32°C and another portion stored in cold room at 15 ± 2°C for 8 months. Both facilities were located about 50 m apart at Kwadaso (245 m asl N 06° 40. 696 W001 40, 321°) in the Forest Agro-Ecological Zone. The seeds were arranged on wooden shelves and a hygro-thermometer fixed for the collection of data on temperature and relative humidity (Figure 1) during the entire 8 months period. Samples were taken at 2, 4, 6, and 8 months for the following analyses:

Ash content (%)

Ash content was determined using AOAC (2000, method 923.03) after carbonizing the common bean flour sample weighing 2 g and by igniting in a muffle furnace (Gallenkamp, Model FSL 340-0100, U.K) at 550°C until ashing was completed. Then, the ash content was estimated by the formula:

Total ash (%) =
$$\frac{(W2 - W1)}{(W1 - W)} \times 100$$

Where, W = mass of empty dish in grams; W1 = Mass of the dish plus sample in grams (dry matter basis (db)); W2 = Mass of the dish plus ash in grams.

Electrical conductivity (µS cm⁻¹)

Electrical conductivity test as a measure of seed membrane integrity and vigour was measured at the end of each storage period. It was measured in 100 ml deionized water soaked with 3.5 g of each seed lot for 24 h using the conductivity meter-dip cell. This instrument measures the electrical current passing through the water as a result of the leakage of electrolytes from weak or damaged embryonic cells into the surrounding water. The measurement was expressed in micro-seconds per centimeter per gram of seed ($\mu S \text{ cm}^{-1} \text{ g}^{-1}$).

Potassium leachate (ppm)

The amount of potassium (K) was determined by flame photometry

by comparing the intensities of radiation emitted by K atoms in the seed leachate into a series of standard solutions. Seed samples were weighed and 3.5 g, put into 250 ml glass bottles and 100 ml of deionized water added. The bottles and their contents was incubated for 24 h and then filtered through a fine filter paper. Extracts was taken to the flame analyzer and the emission readings taken. A calibration curve of K-emission against concentration was drawn. Potassium was estimated as the product of sample in ppm of the extracting ratio. Potassium extract content was expressed in milligram of potassium per kilogram of seed (mg K/kg).

Phosphorus leachate (ppm)

Contents of Phosphorus (P) in the seed leachate were estimated as additional measure of vigour of the common bean at the end of each storage period. For phosphorus estimation, 5 ml of the seed leachate from each seed sample, together with blanks and standard series pipetted into test tubes, after which 10 ml of color reagent was added slowly and mixed. A pinch of L-ascorbic acid was added and thoroughly mixed. Absorbance was measured with 10 mm Ø cuvettes at 880 nm after 30 min, but within 24 h with a spectrophotometer. A graph relating the absorbance to the amount of phosphate present was plotted, and P estimated as a product of sample extract in ppm and extracting ratio. The estimation was expressed in milligram phosphorus per kilogram of seed.

Moisture content (%)

The moisture content was determined by the oven method. A metal container and its cover were weighed and 4.5 g of the sample evenly distributed over the surface of the container. The weight of the container and its content before drying were measured. The oven was set to a temperature of 130°C and container with the samples heated for 1 h. The content was then removed from the oven and placed in a desiccator for 30 min to cool at ambient temperature. The percentage moisture content was determined using the following formula:

Moisture content (%) =
$$\frac{\text{M2 - M3}}{\text{M2 - M1}} \times 100$$

Where, M_1 = weight of container and its cover (g); M_2 = weight of container, its cover and seed sample before drying (g); M_3 = weight of container, its cover and seed sample after drying (g)

Protein content (%)

For the extraction of proteins, the testa of seeds was removed by scratching it with a small file. Seeds devoid of testa were crushed using a pair of pliers and then ground to fine powder with a pestle and mortar. Ten grams of seed powder was put into separate Eppendorf tubes to which 400 µl Sodium dodecyl sulphate (10%) detergent was added. The mixtures were vortexed for 15 s and left for 1 h. They were then placed in a warm water bath for 1 h at 100°C. The mixture was centrifuged at 5000 rpm for 15 min, and the supernatant stored at -4°C. A sample of 1 µl from each of the 12 supernatants was used to determine the protein content by the Biuret methods (Mitchell et al., 2009). Data analysis was performed using Statistix 9.1 statistical software package. Tukey's Honestly Significant Difference (HSD) test was used to separate treatment means.

RESULTS

Temperature and relative humidity of the storage environments

Figure 1 (a and b) gives the temperature and relative humidity readings taken during the storage period. The highest temperature under the ambient condition was observed in November, 2019 (33.40°C) and the lowest in July, 2020 (25.43°C). The highest RH was 85.80% recorded in July, 2020, whilst the lowest was 38.53% in November, 2019. Under the cold storage condition, the highest temperature was observed in May, 2020 (15.58°C) and the lowest in January, 2020 (16.81°C). The highest RH under the cold condition was 70.30% in October, 2019 whilst the lowest was 68.54% in January, 2020.

Cost of storage under ambient condition and cold condition

During the study period, the cost of storing 1mt common bean seeds under ambient storage condition was \$5/month, whereas that of cold storage was \$10/month. This means that, for the eight month storage period the cost was \$40 and \$80 for ambient and cold storage, respectively. Unlike ambient storage, breakdown of cold system due to frequent power outages poses high risk in maintaining the initial seed quality.

Ash content (%)

There were highly significant (p < 0.001) differences in the moisture content x storage condition treatment interactions for the percentage ash content at two, six and eight months after storage (Table 1). After two months, seeds dried to 8% MC and stored under ambient condition showed significantly (p < 0.001) higher ash content (5.98%) than the other treatment interactions, with the least observed in seeds dried to 11% MC and stored under cold. After six months of storage, seeds dried to 8% MC and stored under cold storage condition showed significantly (p < 0.001) higher ash content, 1.24 times higher than the least observed in seeds dried to 11% MC and stored under cold storage condition. Eight months after storage seeds dried to 8% MC and stored under ambient condition had 4.72% higher ash content, 1.06 times higher than the least observed in seeds dried to 11% MC and stored under ambient condition.

Electrical conductivity (EC (µS cm⁻¹))

There were highly significant (p < 0.001) differences in the moisture content x storage condition treatment

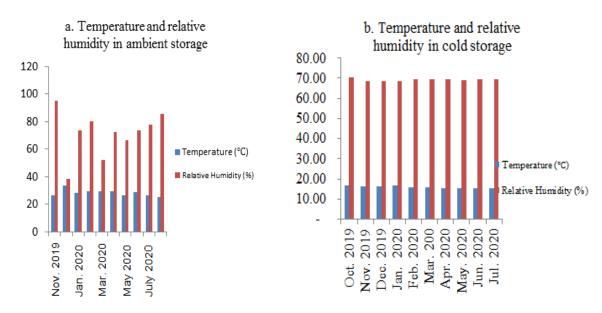


Figure 1. Temperature and relative humidity under the ambient and cold storage conditions. Source: Recorded in the storage facilities using HT 160 Thermohygrometer Data Logger

Table 1. Interactive effects of moisture content and storage condition on ash content (%) of common bean seeds during storage.

	Ash content (%) Storage condition							
Moisture content								
		Ambient		Cold				
	Two months	Six months	Eight months	Two months	Six months	Eight months		
8%	5.98 ^a	4.13 ^b	4.72 ^a	5.22 ^b	4.26 ^a	4.47 ^c		
11%	5.03 ^b	3.94 ^c	4.45 ^c	4.61 ^c	3.79 ^d	4.62 ^b		
Means	5.51 ^a	4.04 ^a	4.58 ^a	5.51 ^a	4.02 ^a	4.55 ^b		
HSD (0.05)	0.306	0.093	0.036	0.306	0.093	0.036		

Source: Data taken from seeds stored in the storage facilities. Mean with the same letter (s) in column do not differ at 5% significance level

interactions for the electrical conductivity at two, four and six months after storage (Table 2). After two months, seeds dried to 11% MC and stored under cold condition showed significantly higher electrical conductivity than the other treatment interactions. Seeds dried to 11% MC and stored under cold condition had 250.93 µS cm⁻¹ which was 1.46 times higher than the least 171.32 µS cm⁻¹ observed in seeds dried to 11% MC and stored under ambient condition. Four months after storage, seeds dried to 8% MC and stored under cold condition had EC of 246.45 µS cm⁻¹ which was 1.79 times higher than the least 138.06 µS cm⁻¹ observed in seeds dried to 11% MC and stored under cold condition. After six months storage period, seeds dried to 11% MC and stored under ambient condition showed significantly (p < 0.001) higher EC of 225.44 µS cm⁻¹ which was 1.24 times higher than the least 182.38 µS cm⁻¹ observed in seeds dried to 8% MC

and stored under ambient condition.

Potassium leachates (ppm)

There were highly significant (p < 0.001) differences in the moisture content x storage condition treatment interactions for the potassium leachates at all the sampling periods (2, 4, 6 and 8 months in storage). After two months in storage the potassium leachates from seeds dried to 11% MC and stored under cold condition were 1401.5 ppm, 1.15 times higher than the least leachates observed in seeds dried to 8% MC and stored under cold condition (Table 3). After four months in storage, the K leachate from seeds dried to 11% MC and stored under cold storage were 1884.6 ppm which was 1.14 times higher than the least observed in seeds dried

Table 2. Interactive effect of moisture content and storage condition on electrical conductivity (µS cm⁻¹) of common bean seeds during storage.

	Electrical conductivity (μS cm ⁻¹) Storage condition								
Moisture content									
		Ambient		Cold					
	Two months	Four months	Six months	Two months	Four months	Six months			
8%	232.33 ^b	223.5 ^b	182.38 ^d	171.32 ^d	246.45 ^a	213.56 ^b			
11%	203.1 ^c	166.12 ^c	225.44 ^a	250.93 ^a	138.06 ^d	207.29 ^c			
Means	217.72 ^a	194.81 ^a	203.90 ^b	211.12 ^b	192.26 ^b	210.42 ^a			
HSD (0.05)	4.292	4.534	5.044	4.292	4.534	5.044			

Source: Data taken from seeds stored in the storage facilities. Mean with the same letter (s) in column do not differ at 5% significance level

Table 3. Interactive effects of moisture content and storage condition on the potassium leachates (ppm) during storage.

			F	Potassium lead	chates (ppm)					
Moisture content	Storage condition									
	Ambient				Cold					
	Two months	Four months	Six months	Eight months	Two months	Four months	Six months	Eight months		
8%	1262.7 ^b	1716.8 ^b	2145.9 ^c	2496.4 ^b	1223.8 ^b	1651.7d	2732.3 ^a	2685.2 ^a		
11%	1226.5 ^b	1696.0 ^c	2619.6 ^b	2684.3 ^a	1401.5 ^a	1884.6 ^a	2125.0 ^c	2115.4 ^c		
Means	1244.6 ^b	1706.4 ^b	2382.7 ^b	2590.3 ^a	1312.6 ^a	1768.2 ^a	2428.6 ^a	2400.3 ^b		
HSD (0.05)	61.513	16.993	72.533	183.94	61.513	16.993	72.533	183.94		

Source: Data taken from seeds stored in the storage facilities. Mean with the same letter (s) in column do not differ at 5% significance level

to 8% MC and stored under cold condition. Six months after storage, K leachates of seeds dried to 8% MC and stored under cold condition were significantly (p < 0.001) higher (2732.3 ppm) compared to the least observed in seeds dried to 11% MC and stored under cold condition (2125 ppm). Seeds dried to 8% MC and stored under cold condition were 1.29 times higher than seeds dried to 11% MC and stored under cold condition. After eight months storage period, the K leachates of seeds dried to 8% MC and stored under cold condition were significantly (p < 0.001) higher than the other treatment combinations (Table 3). Potassium leachates of seeds dried to 8% MC and stored under cold condition were 1.27 times higher than the amount observed in seeds dried to 11% MC and stored under cold condition.

Phosphorus leachates (ppm)

Very highly significant (p < 0.001) differences in the moisture content x storage condition treatment interactions were observed for the phosphorus leachates at two, six and eight months after storage (Table 4). Two months after storage, the phosphorus (P) leachates of

seeds dried to 8% MC and stored under ambient condition were 172.16 ppm, which were 1.13 times higher than the least 152.26 ppm observed in seeds dried to 11% MC and stored under ambient condition. After six months storage period, the P leachates of seeds dried to 11% MC and stored under ambient condition were higher than the other treatment combinations. After eight months storage period, P leachates of seeds dried to 11% MC and stored under ambient condition was 262.74 ppm, which was 1.08 times higher than the amount observed in seeds dried to 8% MC and stored under ambient condition.

Seed moisture content (%)

Very highly significant (p < 0.001) differences in the moisture content x storage condition treatment interactions were observed for the seed moisture content (SMC) at two months after storage (Table 5). Seeds dried to 11% MC and stored under cold condition had the highest SMC 11.77% whereas the least of 9.46% was observed in seeds dried to 8% MC and stored under ambient condition. Seeds dried to 11% MC and stored

259.38^b

258.82^a

2.085

Moisture content		Phosphorus Leachates (ppm)								
		Storage Condition								
		Ambient			Cold					
	Two months	Six months	Eight months	Two months	Six months	Eight months				
8%	172.16 ^a	237.76 ^b	243.05 ^c	169.66 ^a	226.38d	258.27 ^b				

262.74^a

252.89^b

161.13^b

165.40^a

4.455

237.05^c

242.26

0.550

258.98^a

237.82^b

0.550

152.26^c 162.21^b

4.455

Table 4. Interactive effects of moisture content and storage condition on the phosphorus leachates (ppm) during storage.

2.085 Source: Data taken from seeds stored in the storage facilities. Mean with the same letter (s) in column do not differ at 5% significance level

Table 5. Interactive effects of moisture content and storage condition on the moisture and protein contents at two and six months after storage.

	Storage condition						
Majatura aantant	Moisture co	ntent (%)	Protein content (%)				
Moisture content	Ambient	Cold	Ambient	Cold			
	Two mo	onths	Six months				
8%	9.46 ^c	9.48 ^c	27.84 ^a	27.44 ^{ab}			
11%	11.35 ^b	11.77 ^a	26.89 ^c	27.21 ^{bc}			
Means	10.41 ^b	10.62 ^a	27.37 ^a	27.33 ^a			
HSD (0.05)	0.084	0.084	0.406	0.406			

Source: Data taken from seeds stored in the storage facilities. Mean with the same letter (s) in column do not differ at 5% significance level

under cold condition had 0.77% increase whereas seeds dried to 8% MC and stored under ambient condition had 0.46% increase after the two months storage period.

Protein content (%)

11%

Means

HSD (0.05)

Very highly significant (p<0.001) differences in the moisture content x storage condition treatment interactions were observed for the protein content after six months storage period (Table 5). Seeds dried to 8% MC and stored under ambient condition had the highest protein content of 27.84% compared to the least 26.89% observed in seeds dried to 11% MC and stored under ambient condition.

DISCUSSION

Effects of moisture content and storage condition on ash content (%)

Seeds dried to 8% MC and stored under ambient condition showed significantly higher ash content at two and eight weeks after storage. Seeds dried to 8% MC

consistently showed higher ash content in all the interactive effects and also among the MC whereas seeds dried to 11% MC showed lower ash content levels. Ash content is an indication of inorganic minerals in the seed and could be affected by deterioration of seeds in storage. The higher levels observed in seeds dried to 8% MC and kept under ambient condition could be attributed to the reduction of respiration and enzymatic activities in seeds with low moisture content as observed by Ferreira et al. (2017). Higher levels of ash is a good seed quality indication (Wołosiak et al., 2018), hence the common bean seeds can be stored under the ambient condition for eight months without loss of quality. Also irrespective of the treatment factor the seeds aged with increase in duration as similarly reported by Wolosiak et al. (2018).

Effects of moisture content and storage condition on electrical conductivity (EC (µS cm⁻¹)

After two months, seeds dried to 11% MC and stored under cold condition had higher EC than the least observed in seeds dried to 11% MC and stored under ambient condition. However, after six months storage period, seeds dried to 11% MC and stored under ambient

condition showed significantly higher EC than the least observed in seeds dried to 8% MC and stored under ambient condition. Higher conductivity value is an indication of lower seed vigour due to loss of cell membrane integrity (De Medeiros et al., 2019). The lower EC recorded in interactions involving ambient storage condition will be economically beneficial to seed growers who will spend 50% less the cost of storing under cold condition for the 8 month period and still maintain good seed quality.

Effects of moisture content and storage condition on potassium leachates (ppm)

The least potassium leachates were observed in seeds dried to 8% MC and stored under cold condition at 2, 4 and 6 months after storage. However, 8 months after storage potassium leachates of seeds dried to 8% MC and stored under cold condition were 1.27 times higher than the amount observed in seeds dried to 11% MC and stored under cold condition. Higher leachates of K⁺ are an indication of loss of membrane integrity due to imbibition damage or seed age and loss of nutrient reserves (Wu et al., 2015). The lower K leachates observed in the early storage period (2-6 months) is due to the lower seed MC, and temperature in the cold condition which slows down seed aging, reduce respiration thereby conserving nutrients and loss of oxygen. Seeds stored at high moisture content results in increased respiration, generating heat and fungal infection leading to deterioration (Jyoti and Malik, 2013; Rani et al., 2013).

Effects of moisture content and storage condition on phosphorus leachates (ppm)

The highest P leachates were observed in seeds dried to 11% MC and stored under ambient condition after 6 and 8 months storage period. The high seed moisture content and temperature in the ambient condition lead to loss of membrane integrity with seed age resulting in leachate of solutes upon imbibition. Rapid imbibition into the outer cells of the cotyledons, leads to disruption of cell membranes resulting in high solute leakage from the embryos (Matthews and Powell, 2006).

Effects of moisture content and storage condition on seed moisture content (%)

Seeds dried to 11% MC and stored under cold condition had the highest SMC 11.77% whereas the least of 9.46% was observed in seeds dried to 8% MC and stored under ambient condition. This means that seeds dried to 8% MC and stored under ambient condition had the highest marginal increase of 1.46% compared to 0.77% in seeds

dried to 11% MC and stored under cold condition. Seeds are hygroscopic and will attract or loose moisture until an equilibrium is established (Ashok et al., 2019; Agha et al., 2004). Absorption moisture for seeds with low initial moisture in humid ambient condition to establish equilibrium will be more compared to seeds with high initial moisture content. The rate of absorption or adsorption will depend on the initial moisture content; hence the results indicated that 8% moisture content had higher values than the 11% MC (Silva et al., 2018).

Effects of moisture content and storage condition on protein content (%)

Seeds dried to 8% MC and stored under ambient condition had the highest protein content of 27.84% compared to the least 26.89% observed in seeds dried to 11% MC and stored under ambient condition after six months of storage. This is an indication that lower seed moisture content is able to maintain nutrient reserve better than high moisture content. This is due to reduction in metabolic activities such as respiration, insect and microbial attack which involve the use of nutrient reserves. Similarly, the protein content of stored black gram seeds decreased with increase in storage temperature and moisture content (Scariot et al., 2017).

Conclusion

Common bean production is relatively new in Ghana and supply of quality seeds will enhance the adoption and increase in productivity. Irrespective of the treatment factor seed aged with increase in duration. The study revealed that common bean seeds dried to 8 or 11% MC can be stored for a period of 8 months in the humid region of Ghana. After 6 months of storage, seeds dried to 11% MC and stored under ambient showed 27.84% protein content compared to 26.89% in seeds dried to 11% MC and stored under ambient. Drying the seeds to 11% MC and storing for a period of 8 months under ambient condition is recommended. This will be economical to seed growers compared to seeds dried to 8% MC and stored under cold condition. The findings will be useful to the seed sector industry stakeholders in the production, distribution and marketing of this newly introduced legume in Ghana.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENTS

This research was partly conducted under the project

Improving Bean Productivity Marketing in Africa (IBPMA) of the International Center for Tropical Agriculture (CIAT) Pan Africa Bean Research Alliance (PABRA). Funding of the work was received from the Global Affairs Canada.

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