Controlled atmosphere storage of brown cowpea under nitrogen

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Summary

Laboratory studies were conducted to investigate the efficacy of the long-term controlled atmosphere storage of brown cowpeas under nitrogen in Kano, Nigeria, where hot dry weather prevails, typical for a semi-arid zone. The 30-month storage trial consisted of the application of nitrogen in an experimental 0.65 m^3 mini-silo containing half a tonne of the commodity. The storage in nitrogen completely suppressed insect infestation, and grain quality was maintained during the period. There was a significant difference ($P < 0.05$) in the population of insects in the cowpea stored under nitrogen, and the control. The initial insect population of 15 adults of *C. maculatus*, and one adult of *T. castenium* per kg of cowpea died after 12 months of storage under nitrogen. They however, increased in the control to 1288, and 54 adults per kg respectively before their eventual death. Free fatty acid contents increased from 2.60% to 6.51% under nitrogen, but increased rapidly to 58.60% in the control cowpea. This was attributed to the action of insect activities, mould infection and water activities in the control cowpeas. Viability dropped slightly from 89.75% to 72.84% in nitrogen, but dropped rapidly to 0.00% in the control. The palatability of cowpea did not change adversely in nitrogen, whereas it became very poor in the air-stored commodity. The germinability of cowpea seeds stored in the inert atmosphere silos was maintained above 85% after 12 months of storage, which shows that the technology is effective for seeds storage.

Keywords: storage, brown cowpea, nitrogen, insect mortality, nutritional quality

Introduction

Cowpea (*Vigna unguiculata* L. Walp) is one of the major leguminous vegetable crops in Nigeria and West African countries providing more than half the plant protein in the human diet. In Nigeria, cowpea is commonly consumed in the form of bean pudding, bean cake, baked beans, fried beans and bean soup (Ola Salawu et al., 2014). This crop is prone to the heavy post-harvest damage by *Callosobruchus maculates* F., which is one of the most destructive insect pests of cowpea and legume crops in Nigeria. This insect starts its infestation in the field before the harvest, and continues on the dried, stored seeds through several generations per year (Hashem and Risha, 2000). This insect causes severe losses in seeds. The damage is a result of the larvae feeding inside the seed causing weight losses, reduced seed quality and poor seed viability (Ofuya, 1994).
Effective crop storage is an important aspect of the attainment of food security. According to Adejumo and Raji (2007), a major problem regarding the attainment of food security in Nigeria is an inefficient food preservation and storage. Cowpea, which is one the staple food in Nigeria consumed by the entire populace, is not exempted from this problem. The general approach to the control of cowpea insect pests is the use of chemicals, especially phosphine, which is commonly used for fumigation of grains across the country. This method has, however, proven ineffective due to the peculiar nature of the insect pest problems with cowpea in Nigeria. Resistance of several insect pests to this fumigant, especially in grain storage management, has also been reported (Opit et al., 2012). Chemical residues, which are the traces of a chemical or its breakdown products that remain in or on the treated produce after a particular time, are also a major drawback to the use of chemicals as effective insect pest control (Hashem and Sharaf El- Din, 2000). These synthetic products are not only expensive, but may be unavailable at the time of a critical need, and also pose health hazards to man and livestock. Reports of deaths arising from the consumption of beans have also been documented in the country, because people used the banned substances on the stored beans, which are later sold to the innocent consumers.
In Nigeria, the use of chemicals for the control of insect pest is not properly regulated, and as such, it has been prone to the abuse and misuse by farmers, grain handlers and grain merchants. The most worrisome consequence of this was that the European Union has recently placed a ban on the beans export from Nigeria to any of their countries. The rejected beans were found to contain between 0.03 mg/kg to 4.6 mg/kg of

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dichlorvos pesticide, and the acceptable maximum residue limit is 0.01 mg/kg. This chemical residue constitutes a danger to human and animal health (Premium Times, 2016).
Natural methods for the protection of grains against cowpea beetle, involving the use of plant materials, have also been studied with promising results (Oparaeke and Dike, 2005 and Ilesammi and Gungula, 2016). However, the limited scale of application of this potent material has made it relatively unpopular among grain merchants.
The Controlled atmospheres (CA) storage has been demonstrated as a safe residue–free alternative to chemical (fumigants and protectants) for controlling stored products insect pests (Carli et al., 2010). Controlled atmosphere has been considered a promising method for pest control in grain and other commodities. The following two principal types of atmosphere can be used for the controlled atmosphere storage and disinfestations of grains; low O_2, and high CO_2 atmospheres. This environment kills insects and mite pests and prevents aerobic fungi from growing (Mitcham et al., 2006). However, the studies in Nigeria have shown that an atmosphere of high N_2 is most preferable (Agboola, 2012).
Inert Atmosphere silos (a form of a control atmosphere storage technology) has proven to be an effective storage structure for grains in tropical countries due to its huge technical advantages over the conventional metal silos. The superiority of grains stored in metal silo filled with nitrogen over grains protected by Phostoxin fumigant in silos was demonstrated by Williams et al. (1980), Adesuyi et al. (1980) and Sowunmi et al. (1982). These researchers tested the system of using nitrogen to preserve grains at Ibadan, Nigeria, at 24.8 to 30.3 °C ambient temperature and 72.0 to 80.9% relative humidity. They observed that storage in nitrogen atmosphere protected grain in metal silos from the insect damage, and the quality of the grain was maintained throughout the period of the trial. White and Jayas (2003) have also observed that the controlled atmosphere of nitrogen is used as a periodic treatment to control pests (insects and mites) in stored grain or, less frequently, as a long-term storage environment to prevent pest occurrence. This system of storage eliminated the general problem due to moisture condensation within conventional metal silos. They also demonstrated that the nitrogen atmosphere in the grain silo did not affect the viability of the grains.
This study was designed to investigate the application of the controlled atmosphere storage system of brown cowpea in the semi-arid zone of Nigeria for the effect of different ecological zone (as in northern part of Nigeria) on the biological and organoleptic quality of the stored cowpea. Cowpea was chosen because it is a commonly grown legume in the area where this study was conducted. It is also easily attacked by insect pests in storage with a chemical control proven to be ineffective in conventional silos; hence the efficacy of this system can easily be demonstrated.

Materials and methods

Description of silo and the collection of sample materials

An airtight mini-silo of volumetric capacity 0.65 m^3, used in Kano for this study, was installed under shade, as suggested in earlier research (Adesuyi et al., 1980). The mini-silo is provided with three sampling points located at the bottom, middle and top positions of the metal silo. A dial bi-metallic thermometer probe was also fitted midway of the silo with a sensing probe penetrating to the central axis.
Nitrogen was supplied to the metal silo through a nitrogen distribution system consisting of a pressure cylinder and a gas flow instrument panel in such a way that nitrogen could flow from the silo downwards through the stored grain. A pressure relief valve was fitted at the base of the silo.
The cowpea used in this study was purchased dry and had no insecticide protection. All precautions regarding silo loading with grains, regularity of sampling from the silo and purging with nitrogen were followed. For control, cowpea was stored in air in a metal drum of the capacity of 180 liters kept under shade in the proximity of the mini-silo. Control samples were drawn from the bottom, middle and top of the drum content with a compartmentalized sampler, and were mixed for the analysis.

Seed damage and weight loss

The inert atmosphere silos containing the seed stored under N_2 was observed at 12 months intervals up to 30 months of storage. After completion of each storage period, the seal of the silo was opened and observations on the insect damage (%), weight loss (%), moisture content (%), and germination (%) were recorded. The number of the damaged seeds in each replication was counted after taking a random sample of 100 g seeds and converted to per cent insect infestation. The seed moisture content of the treatments was determined by the oven method. The weight loss due to the insect infestation was calculated by deducting the final weight from the initial weight, and converted to per cent weight loss. Seed germination was measured using the standard paper towel method as per the ISTA rules (1999).

Quality assessment

Oxygen concentration within the silo was checked with the Taylor Servomex oxygen analyzer, type OA 272 fortnightly, and after every purge following grain sampling. The stored grains were sampled from both the silo and the control for the analyses initially during loading, after 12 months and subsequently at 6-month intervals. The samples were subjected to the following tests: insect infestation count, insect damage, proximate composition, organoleptic evaluation and viability of the seed. These tests were carried out with standard methods as described by Williams et al. (1980) and AOAC (2005). Viability tests of stored cowpeas were carried out using fifty seeds per replicate, germinated in the sterilized soil in the laboratory. The organoleptic evaluation was carried out on the traditional beans dish prepared by cooking the stored cowpeas in water and other ingredients were added. This was performed initially, and at the end of the experiment by staff of the Nigerian Stored Products Research Institute, Kano.

Statistical Analysis

The analysis of variance was obtained using SPSS statistical software. The Duncan's Multiple Range Test (DMRT) was used for testing the significance of means in all conducted experiments.

Results and discussion

Temperature, relative humidity and moisture content of cowpea inside the silo

Temperatures recorded in the silo were in the range of 21 °C (in the night) to 35 °C (in the day) with a mean of 28.5 °C. The ambient temperature ranged from 14 °C to 43 °C (day) with a mean of 33.2 °C. The ambient relative humidity ranged from 19 to 100%, with a mean of 44.3%. The interstitial atmosphere within the silo was maintained below 0.1% oxygen by purges after grain or insect samplings. This was in agreement with temperature data reported by Ajayi et al., 2016 which showed that the temperature variations in the inert atmosphere silos (using nitrogen) were minimal.

Moisture contents of brown cowpeas stored under nitrogen, and in air for 30 months are shown in Table 1. After the storage period in nitrogen, the initial moisture content 9.66% of cowpeas reduced slightly to 8.65%. On the other hand, the control increased steadily to 19.25%. This showed that the moisture content of grains in the inert atmosphere was maintained below the safe level throughout the storage period, whereas those stored in the control (air storage) have steadily exceeded their safe moisture level. The technology was able to maintain the moisture content of the grains, thus preventing spoilage of the grains through other means other than the activities of insects, such as mould growth and seed rot.

Grain damage and insect infestation

The initial damage of cowpeas averaged 3.3% (Table 2), and the insect population was low (Table 3). The level of the grain damage increased rapidly in the control. The cowpea showed initial infestation with *Callosobruchus maculatus* (F), 15 living adults per kg of grain weight and *Tribolium castaneum* (Herbst), 1 living adult per kg of grain. There was a significant difference in the insect population during the storage period. All insects were recorded dead after 12 months of storage in nitrogen, whereas a living insect was found in the control. This is due to the lack of oxygen, which is a major reason for insect mortality, because feeding activities of the insects generally reduce as oxygen level reduces; it increases the level of acidity in the form of lactic acid and causes poisoning, which is in agreement with the reports of Can et al. (2012). Although similar observation was made in the control with all *C. maculatus* found dead, there was an enormous increase of insect population to 1288 per kg before their eventual death. An increase in the population of *T. castaneum* to 54 adults per kg was also observed in the control, and they eventually died after 12 months. The moisture increase in the control might have made the environment lethal to insects. These results were in agreement with those by Mbata and Reichmuth (1996).

Table 1. Variation in the moisture content of cowpea during storage

Storage medium		Moisture content of stored cowpea (%) in months				
		0	12	18	24	30
Inert Atmosphere Silo(Nitrogen)	Top	9.66	8.67	7.76	8.00	8.12
	Middle	9.66	8.63	7.71	8.89	8.78
	Bottom	9.66	8.66	7.83	8.07	8.02
	Mean	9.66	8.65	7.77	8.65	8.31
Ambient (Air)		9.66	14.92	12.55	19.25	20.04

Table 2. Insect damage of brown cowpea stored under nitrogen and in air for 30 months

Storage medium	Insect damage (%) in months				
	0	12	18	24	30
Inert Atmosphere Silo(Nitrogen)	3.3	3.1	3.4	3.2	3.3
Ambient (Air)	3. 1	88.0	100.0	100.0	100.0

Insect count on 1kg samples

Table 3. Insect infestation of brown cowpea stored under nitrogen and in air for 30 months

Storage period	Storage medium	Population of insect species per 1kg of grain weight			
		T. castaneum	*C. maculates*	Parasite	Total
0	*Nitrogen*	0	15A, 25a	0	15A, 25a
	Air	1A	0	0	1A
12	*Nitrogen*	0	1a	1a	2a
	Air	54A	1288a	0	1342a
24	*Nitrogen*	0	3a	0	3a
	Air	16a	916a	0	932a
30	*Nitrogen*	0	1a	0	1a
	Air	4a	408a	0	408a

Legend: A = Living insects; a = Dead insects

Proximate composition of stored cowpea

As shown in Table 4, there was an increase from 24.68 to 35.20%. In the crude protein content of cowpeas stored under nitrogen. The crude protein content of cowpea stored in air increased more rapidly to 48.41%. This might have been due to the effect of N_2 in decreasing the insect infestation in the beans stored under N_2.This observed increase in the control is only apparent, as explained by Shahetal et al. (2009) and Nikolova and Georgieva (2015) who indicated that the analytical results of the crude protein of the infected grain would be inflated by the contribution of the excretory product of insect nitrogen metabolism to total nitrogen (N_2) content. In particular, the previous works on the insect infestation of cowpea reported by Sowunmi (1980) and Sheata et al. (2009) showed that poorer quality protein increases in infested cowpea, thus making the crude protein content of infested cowpeas to be greater than the uninfested. The free fatty acid (FFA) content of cowpea increased from 2.67% (initial value) to 6.51% in 30 months, while the control (air stored) cowpea increased rapidly to 58.60%. The observed increase in the fatty acid of cowpea stored in air (hence infested) can be attributed to the action of insect activities, mould infection, water activity and other biodeteriogens. There was a slight decline in the oil content of cowpea from 1.98% to 1.44% stored under nitrogen, but the control showed a rapid rise to 2.35%.

Table 4. Variations in proximate composition of stored brown cowpea

Storage medium	Proximate composition (%)	Storage period (months)				
		0	12	18	24	30
Nitrogen	*Crude protein*	24.68	31.86	32.12	36.33	35.20
	Free fatty acid	2.67	6.62	6.38	6.40	6.51
	Oil content	1.98	1.31	1.28	1.47	1.44
Air	*Crude protein*	24.68	37.49	44.20	49.08	48.41
	Free fatty acid	2.67	40.48	48.18	55.65	58.60
	Oil content	1.98	2.84	2.44	2.26	2.35

Table 5. Variations in weight loss, viability and palatability of stored brown cowpea

Storage medium	Parameters	Storage period (months)				
		0	12	18	24	30
Nitrogen	*Weight loss(%)*	0	3.13	3.52	3.51	3.51
	Viability (%)	89.75	86.4	71.70	72.84	71.64
	Palatability	Good	n.a.d*	n.a.d	n.a.d	n.a.d
Air	*Weight loss(%)*	0	35.40	51.74	56.81	56.78
	Viability (%)	89.75	11.32	0.00	0.00	0.00
	Palatability	Good	v.p**	v.p	v.p	v.p

Legend: * n.a.d = not adversely affected; ** v.p = very poor

Germinability of stored cowpea

There was a slight loss of germinability of cowpea from 89.75 to 71.64% after 30 months of storage under nitrogen, while a much higher loss of viability to 0.00% was observed in the control set-up (Table 5). The low percentage germination observed in the control in this study could be due to the insect infestation which might have damaged seed embryo, thus rendering the seeds implantable. Similar findings have been reported by Guptal et al. (2014) who found that paddy seed can be stored safely with 11% moisture content at least up to 12 months under modified atmosphere (up to 80% CO_2) without much reduction in the seed viability. Jayas and Jeyamkondan (2002) have also concluded in their study that modified atmosphere does not cause any detrimental effects on the functional characteristics of grains, and helps in maintaining seed germination and viability. This is an indication that the inert atmosphere silos can be used for seed storage provided the conditions of the system can be maintained at an optimum level and with the storage period not exceeding 12 months.

Sensory properties of stored cowpea

The palatability of cowpea stored in nitrogen did not change adversely in the 30 months storage period. However, the palatability of the air stored commodity became very poor (Table 5).

Conclusions

This present work has demonstrated that nitrogen can preserve cowpeas of moisture content of 10% or less, stored in airtight containers for up to thirty (30) months without adverse effect on the nutritional qualities. The technology is effective in controlling insect infestation and maintaining the moisture content of cowpea during storage. The technology can also be used for the storage of seeds without loss of viability for a period of 6 months. Because of the poisonous nature of the degraded materials from Phostoxin tablets and the high cost of chemical control of cowpea insect pests coupled with the associated challenges of mis-use and their abuse, the use of Inert Atmosphere storage technology is recommended to farmers and grain merchants in Nigeria as a safe and economically viable alternative.

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