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Full Length Research Paper

Effect of storage on nutrient composition and mycoflora of sundried soyabean (*Glycine max*.)

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Sundried *Glycine max* were stored for 20 weeks. During storage, monthly analyses were carried out to determine the effect of storage on the nutrient composition, minerals and mycoflora present in the sample. The mycoflora isolated from sundried *Glycine max* during storage were *Aspergillus niger, Aspergillus candidus, Aspergillus glaucus, Aspergillus versicolor, Aspergillus flavus* and *Rhizopus* sp. The results of the proximate of stored sun dried *G. max* showed that moisture content increased from 6.80 to 8.34%, ash content from 5.07 to 6.45% and crude protein from 40.94 to 42.33% as the storage time increases while the fat decrease from 19.15 to 18.37%, fibre from 5.72 to 5.35% and carbohydrates from 22.33 to 19.18% with the storage time. The results of mineral analysis indicated that all the mineral elements; sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn) and phosphorus (P) were found to decrease as the storage time increased while potassium (K) and zinc (Zn) increased as the storage time increased but copper (Cu) and cadmium (Cd) were detected in the first two months of storage in the samples. Some of these isolated fungi play important role in the soil, *Aspergillus* spp. are common mould found in soil and the second most commonly recovered fungus in opportunistic mycoses while *Rhizopus* sp. is responsible for the damage of blood vessels, nerves and cause necrosis of the infected tissues. These mineral elements found in the plant serves an important role in the body system, such as teeth formation by calcium, formation of haemoglobin by iron (Fe) and regulation of acid base balance of the body by phosphorus (P). Finally, this work has shown that there is urgent need to develop a better drying method for local use since the drying methods encouraged contamination by air microflora and spores of fungi present in the air.

Key words: Mycoflora, crude protein, chemical composition, soya bean, storage.

INTRODUCTION

Glycine max Merr. is an annual leguminous plant grown in tropical and temperate regions with damp summer weather (Halima, 2000; Sneller, 2003). It belong to the family Fabaceae and genus *Glycine* which was derived from the Greek word 'glykys' meaning 'sweetness' (Crawford, 2006). It is also grown as pasture, forage for food crop used as either hay or silage production, and green manure. The crop is raised from seeds and has an average life span of 120 days (Sneller, 2003). It is also the leading agricultural export in the United States and the bulk of the crop is grown for oil production, with the protein defatted and toasted (Miniello, 2003).

Soya bean has been promoted by natural food companies for its mild nutty flavor, better texture, larger size, higher protein and lower oil content. It is also rich in minerals, vitamins, carbohydrate and fibre (Gottstein, 2003). However, storage conditions have effect on the proximate and chemical composition of the stored soya bean because of the growth of some spoilage fungi that strive in such conditions (Abaka and Norman, 2000). The fungi that invade stored product are generally grouped into two categories namely: field fungi which attack developing and matured seeds in the field and storage fungi which are predominantly species of *Aspergillus* and *Penicillium* which attack the stored products (Fagbohun et al., 2010).

The conditions of the stored product determine the extent of invasion of the stored product. The environmental factors that aid the development of fungi in stored products include content (Amusa et al., 2002), temperature (Abaka and Norman, 2000), aeration

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(Giampietro, 2004), pH (Aderiye, 2004) and relative humidity (Kuku, 1979). However, the effect of this storage fungi on stored products include deterioration and spoilage of stored products (Abaka and Norman, 2000; Ekundayo and Idzi, 2005), reduction of market value (Muller, 1991) and production of chemical substances that are toxic (Richard and Wallace, 2001). The preventive measures that can be employed for the growth of the storage fungi are biological control (Aderiye, 2004), chemical control (Rice, 2002) and physical control (Aderiye, 2004).

The objectives of this investigation were to study the effect of storage on the chemical composition and the mycoflora of *G. max*.

MATERIALS AND METHODS

Collection of samples

The samples of the seeds of *G. max* were collected from Oja-oba, the main market in Ado Ekiti, Ekiti State, Nigeria. The bean were spread in a tray and sun dried for one week. The samples were stored for six months in an insect free container, labeled and kept in the laboratory. The samples were examined for the changes in the mycoflora and nutrients composition after each month of storage.

Isolation of fungi from the stored sun dried soya bean

Direct plating

From the sundried soya bean, 10 beans were examined randomly for external mouldness. They were surface sterilized with ethanol and later washed with sterile distilled water. Using a sterile dissecting forceps, the surface of the stored dried soya bean were scrapped and was plated aseptically on potato dextrose agar (PDA) plate and incubated at 28°C for 5 to 7 days as described by Amusa (2001) and Arotupin and Akinyosoye (2001). The fungi cultures were subcultured until pure colonies were obtained by successive hypha tip transfer (Egbebi et al., 2011). The cultures were examined under the microscope for fruiting bodies, hyphae to determine the common fungi present.

Dilution plate method

This method was used to determine the type of fungi present in the stored sun dried soya bean. About one gram of the sample was sterilized with ethanol and grinded with 10 ml of sterile distilled water. This was shaken thoroughly and 1 ml of suspension was pipetted into a sterile test tube containing 9 ml of distilled water. This was thoroughly mixed together. The sample was serially diluted and 1 ml each of aliquots of 10^{-5} and 10^{-6} were added to molten PDA plates. The plates were swirled gently to obtain thorough mixing and were allowed to solidify and incubated at room temperature for 5 to 7 days. The fungal colonies were counted every 24 h. Successive hyphae tip were transferred until pure cultures of each of fungus was obtained.

Washing method

This was carried out by weighing one gram of the soya bean into 10 ml of sterile distilled water in a beaker. This was shaken thoroughly and drops of suspension of contaminated water were introduced into Petri dishes containing potato dextrose agar. This was evenly spread on the agar plate with aid of a sterile glass spreader. The plates were incubated at 28°C for 5 to 7 days and were observe for visible fungi growth.

Identification of mycoflora

The associated fungi were identified by their cultural and morphological features (Alexopoulous et al., 1996). The isolates were examined under bright daylight for the colour of the culture and further examination was carried out.

Needle mount preparation method

The method was carried out according to Tuite (1961), Crowley et al. (1969) and Egbebi et al. (2011) whereby fragments of the sporing surface of the initial culture was taken midway or between the centre and the edge of the colony. This was teased out in drop of alcohol on a sterilized glass slide using a botany needle. The fragments were stained by adding a drop of lactophenol blue. A cover slip was applied and the preparation was examined under X10 and X40 objective lens of the microscope.

Slide culture technique

From a plate approximately 2 mm deep, 1 cm^2 PDA was cut and placed on a sterile glass slide. Fungus was inoculated into the four vertical sides using a sterile needle. A sterile cover slip was placed on it so that it over lapped the medium on all sides. The preparation was placed on a suitable support in a Petri dish containing blotting paper soaked in 20% glycerol in water. The preparation was kept moist at 28°C until adequate growth was observed. After removing the medium with scalpel, the fungus adhering to both cover slip and slide was examined (Crowley et al., 1969). A drop of alcohol was added followed by a drop of lactophenol blue and the preparation was covered and examined under the low power objective of microscope.

Proximate analysis

The proximate analysis of the samples for moisture, ash, fibre and fat were done by the method of AOAC (2005). The nitrogen was determined by micro-Kjeldahl method as described by Pearson (2002) and the percentage nitrogen was converted to crude protein by multiplying 6.25. All determinations were performed in triplicates.

Mineral analysis

The mineral was analyzed by dry ashing the samples at 550°C to constant weight and dissolving the ash in volumetric flask using distilled water, deionized water with a few drop of concentrated HCl. Sodium and potassium were determined by using a flame photometer (Model 405 Corning, UK) with NaCl and KCl standards. Phosphorus was determined colometrically using Spectronic 20 (Gallenkap, UK) as described by Pearson (2002) with KH_2PO_4 as standard. All other metals were determined by atomic absorption spectrophotometer (Pekin-Elmar Model 403, Norwalk CT, USA). All determinations were done in triplicates. All chemicals used were analytical grade (BDH, London). Earlier, the detection limit of the metals has been determined according to Tuite (1961). The optimum analytical range was 0.1 to 0.5 absorbance unit with a coefficient of variation of 0.87 to 2.20%. All the proximate values were reported as percentage while the minerals were reported as mg/100 g.

Table 1. Fungi isolated from stored – dried *Glycine max* using different methods.

Fungal species	Period of storage (Weeks)											
	0			4			8			12			16			20		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Aspergillus candidus	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus flavus	+	+	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+
Aspergillus glaucus	+	+	-	-	-	-	+	+	+	+	+	+	+	+	+	+	+	+
Aspergillus niger	-	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+
Aspergillus versicolor	+	-	-	+	-	-	+	-	-	+	-	-	+	-	+	+	-	+
Rhizopus sp.	-	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+

+ = Present (isolated); - = Absent (not isolated); *A = Direct plating method; B = Dilution plate method; C = Washing method.

Table 2. The variations in the mineral contents (mg/100 g) of sun dried *Glycine max* during 20 weeks of storage.

Mineral	Period of storage (Weeks)					
	0	4	8	12	16	20
Sodium	27.68	27.76	28.27	28.31	40.22	27.42
Potassium	46.21	48.45	51.54	60.68	81.20	61.20
Calcium	218.50	218.91	219.04	220.11	221.50	205.36
Magnesium	158.94	159.30	160.21	160.23	162.21	158.54
Zinc	6.38	8.25	8.58	8.60	8.32	8.37
Iron	7.21	7.12	7.20	7.24	7.31	6.50
Copper	0.14	0.12	ND	ND	ND	ND
Manganese	1.36	1.33	1.35	1.38	1.40	1.25
Phosphorus	586.12	588.22	591.20	591.51	591.75	560.03
Cadmium	0.2	0.11	ND	ND	ND	ND

ND = Not detected.

RESULTS AND DISCUSSION

A total of six fungi were isolated from stored sundried soya bean based on their cultural and morphological characteristics. The fungi include *Aspergillus niger*, *A. candidus, A. flavus, A. glaucus, A. versicolor* and *Rhizopus* sp. The summary of the fungi isolated from stored sundried soya bean using various methods are shown on Table 1. In addition, results of the proximate and mineral analysis are shown on Tables 2 and 3 respectively. The fungi isolated in this study could be from the air, soil, storage house and or improper handling of the products. The *Rhizopus* is as common and as cosmopolitan as the aspergilla. They are so called general contaminant which so frequently find on *Glycine* and other fruits, on jellies and preserves and on other foodstuffs that have become contaminated with their spores (Rice, 2002).

The results showed that *Aspergillus niger*, *Aspergillus candidus, Aspergillus flavus, Aspergillus glaucus, Aspergillus versicolor* and *Rhizopus* sp. were found to be associated with the stored sundried soya bean seeds, most of which are known to be surface contaminants of agricultural products. They cause decay of agricultural produce thereby reducing their market and nutritional value (Amusa et al., 2002). The fungi isolated using washing method are those capable of growing in the bean. The fungi isolated by any of the three methods used could therefore be field or storage fungi (Ogundana, 1990).

In this study, there was an increase in the numbers of fungi isolated as the study progressed. Four fungi *A. candidus, A. flavus, A. glaucus* and *A. versicolor* were isolated at the first week of the study using direct plating method while others joined the number as the study progressed. This result is in agreement with the findings of Broadbent et al. (1969) who reported the isolation of a total number of eighty eight fungi from stored palm kernel. Similarly, this result also agreed with the findings of Fagbohun et al. (2010) also reported the isolation of *A. flavus, A. niger, Rhizopus* spp., *Penicillium* spp. from sun dried plantain chips that was stored for sixteen weeks.

The results of this study also showed that all the six fungi were isolated throughout the study using the three methods of isolation except for *A. versicolor* that was not isolated during the first twelve weeks but tend to surface at the sixteenth week of the study. Likewise, *A. glaucus* was not isolated during the first four weeks of the study

Table 3. The biochemical composition (%) of sun dried *Glycine max* during 20 weeks of storage.

Weeks of storage	Ash	Moisture content	Crude protein	Fat	Fibre	Carbohydrate
Freshly collected (0)	5.07	6.80	40.94	19.15	5.72	22.33
4 weeks	5.13	6.83	41.15	18.89	5.69	22.31
8 weeks	5.25	6.89	41.19	18.78	5.64	22.25
12 weeks	6.14	7.16	42.36	18.55	5.61	20.18
16 weeks	6.36	7.49	42.48	18.42	5.59	19.66
20 weeks	6.44	8.34	42.33	18.37	5.35	19.18

but appears at the eighth week of the study. This may be as a result of the availability of the metabolized nutrient by other fungi present all through the study and the environmental conditions that determine the nature and density of the colonizers (Okigbo, 2003).

Some of the fungi associated with stored products are capable of producing toxin metabolites or chemicals that are detrimental to the health of consumers. However, consumption of excessive amount of these chemicals in the stored products can cause illness or death (Mirocha et al., 2003). Based on the concern of the hazard to livestock and man, concerted effort is now being directed at finding every cheap and reliable methods of minimizing aflatoxin formation in stored products (Bankole and Adebanjo, 2003).

The result of the biochemical composition of stored soya bean revealed that the fresh soya bean had ash content of 5.07%, moisture content 6.80%, crude protein 40.94%, fat content 19.15%, fibre content 5.72% and carbohydrate 22.33% (Table 3). However, after six months of storage the percent ash, moisture content and crude protein increased to 6.44, 8.34 and 42.33 respectively. On the other hand the fat, fibre and carbohydrate contents were decreased to 18.37, 5.35 and 19.18% respectively during storage. Similar observations were reported earlier by Fagbohun et al. (2010) in stored sundried plantain chips. The increase in moisture content, crude protein and ash content along with variation in other nutrients may be attributed by the degrading activity of different mycoflora during storage (Abaka and Norman, 2000; Egbebi et al., 2011).

The mineral analysis of the soya bean during storage also showed variation with respect to storage period (Table 2). Elements like Na, Ca, Mg, Fe, Mn and Cd of stored soya bean decreased during storage, whereas K, Mg and P contents were increased during storage of soya bean. Ekundayo and Idzi (2005) reported that Na, Ca, Mg, Fe, Mn and Cd content in soya bean decreased after two weeks of storage. This variation is explained by the group of fungi which degrades the stored soya bean and either liberates the minerals or utilizes during the process (Smith et al., 1996).

Conclusion

Soya bean are of great economic importance and in order to maintain the quality, they should be stored under proper controlled conditions to prevent them from fungal deterioration. The present study enlighten on the effect of storage of soya bean on the chemical composition and mycoflora. The isolated fungi can degrade the soya bean as substrate thereby posing threat to immunecompromised individual. There is an urgent need to design a good means of reducing this contamination so as to meet the international standards of good manufacturing practice.

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