



Original Research Article

Biodeterioration of Soybean (*Glycine max* L.) seeds during storage by Fungi

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ABSTRACT

Keywords

Soybean,
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Physical
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Soybean (*Glycine max* L.) is native of southeastern Asia and is an important legume crop in the Far East. Seeds of soybean are used in extracting oil, which is edible with very high levels of proteins and soluble carbohydrates. Two varieties of freshly harvested soybean seeds viz. PK-262 and PK-472 were collected from Adilabad district of Telangana and stored for six months in cloth bags. Seeds were analyzed at the interval of thirty days for seed mycoflora by employing blotter, agar plate and dilution plate methods, Physical parameters like germination percentage and moisture content and biochemical parameters such as protein content by Lowry's method, total oil content by Meara's method, free fatty acids by Zenely and Coleman, iodine number and saponification value by A.O.A.C. methods were explored. Thirty one fungal species belonging to thirteen different genera were isolated from PK-262 and thirty nine fungal species belonging to eighteen genera were isolated from PK- 472. There was gradual decrease in germination percentage, moisture content, protein, total oil, iodine number and increase in free fatty acids and saponification value in both the varieties. All the test analysis performed concluded that PK-472 variety showed maximum deterioration in seed constituents during storage.

Introduction

Soybean (*Glycine max* L.) is an important legume used in extracting oil, which is edible with high levels of proteins and soluble carbohydrates. The seeds are rich in calcium, phosphorous and vitamin B complex. India is the fifth largest producer of Soybean in the world. Madhya Pradesh is the largest producer of soybean in the country Singhal, (1996). Soybean is ranked as second oil seed crop in India and constitutes 33% of the world's edible oil

consumption. In Telangana the total oil seed production is 2542 thousand tones cultivated in 3118 thousand hectares of land.

Soybean cultivation in tropical countries like India is characterized by high temperature and high relative humidity resulting in genetic integrity leading to rapid deterioration of seeds. According to Shelar et al., 2008 deterioration is one of the basic reasons for the low productivity leading to

the loss of 25% annually. Seed deterioration can be defined as “deteriorative alterations occurring with time that increase the seed exposure to external challenges and decrease the ability of the seed to survive” (Malik and Jyoti, 2013). Manoharachary and Kunwar, (2006); Kapoor et al., (2011) described deterioration as catabolic process which involves cytological, physiological, biochemical and physical changes in seeds. According to Christensen, (1957) deterioration occurs more rapidly in stored grains due to the invasion of microorganisms and losses caused by them are referred to as biodeterioration. The world’s stored grain is damaged mainly by the activity of fungi than other microorganisms was stated by Neergard, (1977). Fungi have assumed great economic significance, as they not only cause spoilage of grains, during pre and post-harvest stages of production, but also produce various toxins (mycotoxins).

The present study was aimed to study the seed mycoflora and assess the changes in germination percentage, moisture content, protein content, total oil content, free fatty acids, iodine number and Saponification value in soybean seeds during the storage.

Materials and Methods

Two varieties of soybean PK 262 and PK 472 were collected from fields of Adilabad district of Telangana. The collected seeds were stored in cloth bags for six months at room temperature for the study of storage fungi and for biochemical analysis. Isolation of seed mycoflora was done by blotter method De Tempe, (1953). Agar plate method by Muskett, (1948) and Dilution plate method by Peterson, (1959). Germination percentage and moisture content were determined as per the standard methods (Neergard, 1977; AOAC,

1947). Estimation of protein content (Lowry et al., 1951), Total oil content (Meara, 1955), Free fatty acids (Zenely and Coleman, 1938), Iodine number and Saponification value by A.O.A.C (1960). These investigations were carried out in freshly harvested seeds samples as well as during their subsequent storage at an interval of 30 days for six months.

Results and Discussion

Seed mycoflora

Thirty one fungal species belonging to thirteen different genera were isolated from PK-262 and thirty nine fungal species belonging to eighteen genera were isolated from PK- 472 (table-1). *Alternaria alternata*, *Alternaria humicola*, *Aspergillus flavus*, *Aspergillus fumigatus*, *Aspergillus candidus*, *Aspergillus niger*, *Cladosporium* sp, *Curvularia lunata*, *Drechslera holodes*, *Mucor varians*, *Penicillium chrysogenum*, *Rhizopus nigricans*, *Trichoderma album* were commonly isolated from all the three methods in both the varieties. The dominance of a particular species of the fungi seems to depend upon the moisture requirements of that species. It was observed that there was a gradual decrease in field fungi and increase in the storage fungi as the storage period increased.

Roy et al., 2001 had isolated 63 genera and about 108 fungal species from soybean seeds. *Aspergillus flavus* and *Fusarium moniliforme* were two dominant fungi screened from sunflower and safflower seed (Neeti Saxena, 2006). *Aspergillus* was the dominant genus represented by twelve species in both the varieties of soybean. According to Pitt, (1980) climatic conditions of tropical and sub-tropical countries favor the growth of *Aspergilli*.

Germination percentage

There was gradual decrease in germination percentage from freshly harvested seed samples to storage samples PK- 262 (98.0-85.0%); PK- 472 (96.0-79.5%). Loss of germination percentage of soybean seeds was observed by Agarwal and Gupta, (1989). There was gradual decline in germination in stored pea seeds were observed by Saxena and Karan, (1985).

Moisture content

Moisture content of seed during storage is the most persuasive factor affecting the longevity of the seed. In the present study freshly-harvest seed samples of both varieties stored for six months showed the maximum moisture content in initial months and the moisture content was decreased with the increase of storage period PK- 262 (13.50-10.3%); PK- 472 (14.9-10.40%).

The loss of moisture was high in PK- 472 when compared to PK- 262 varieties. Vijayakumari, (1983) also observed a decrease in moisture content during storage in coriander and ajowan.

Protein estimation

Protein content of the PK- 262 and PK- 472 was presented in the table 2. There was a decrease in protein in from freshly harvested seed samples to storage seed samples of sixth month of storage PK- 262 (43.30-40.7%); PK- 472 (43.20-40.00%).

Syed et al., (2011) observed decrease in protein content with an increase in storage period in pigeon pea, chick pea and green gram. Karan and Saxena, (1991) also reported in decrease in protein content in stored sesame and sunflower see[ds.

Total oil content

The total oil content (Table 2) was observed to be decreasing in stored samples compared to freshly harvested seed samples of both the varieties PK- 262 (20.20-17.4%); PK- 472 (20.0-17.3%). Similar observations was made by Saxena and Karan, (1988) in stored white and black sesamum seeds. Singh and Prasad, (1977) had studied the biochemical changes in sunflower seeds due to seed borne fungi.

Free fatty acids

Fungal attack on stored seeds results in an increase in free fatty acids (FFA) and it is considered as a measure of deterioration. Free fatty acid content of the seed samples of two varieties is presented in the table 2. Seed samples showed an increase in free fatty acid content during storage. There was a gradual increase in free fatty acid content with an increase in the storage period recording maximum at six months storage period. Free fatty acid values were least in the freshly harvested seed samples and PK- 472 variety (1.83-4.75mg/g) showed a maximum increase in free fatty acid content. Raja Kumari and Reddy, (1993) also reported increase in free fatty acids in fennel stored for six months in cloth bag (0.64-0.98%), pot (0.64-0.86%) and tin (0.68-1.18%). Increase in free fatty acids in caraway seeds during storage was also reported by Mary Regina and Tulasi Raman, (1992).

Iodine number

Iodine number of freshly harvested samples of PK- 262 was found to be 123.8 which reduced to 118.2 after six months of storage and in samples of PK- 462 it was found to be 124.6 which reduced to 117.8 after six months of storage. (Table 2).

Oil extracted from stored sesamum seeds showed a decrease in Iodine number and increase in saponification value was observed by Lalithakumari, (1971) and Sharma, (1981).

Saponification value

Saponification value of freshly harvested samples of PK-262 was found to be 190.2 which later increased to 193.6 at the end of six months and in samples of PK-462 was found to be 189.3 which later increased to 194.0 at the end of six months. (Table 2). The oil extracted from infested seeds of *Withania somnifera*, *Phoenix sylvestris* and *Indigofera enuaphylla* showed lower Iodine values and increased saponification values by Bhakare, (1993).

Seed mycoflora

Storage conditions in most parts of India are very conducive for mould invasion, proliferation and elaboration of mycotoxins (Girish and Goyal, 1986). The oil seeds crops are associated with a large number of storage mycoflora and it is one of the major factors for the low productivity of oil seeds in India and soybean seeds are highly susceptible to micro organisms and quickly lose their viability (Chandra et al., 1981). Delouch et al., (1973) stated that fungi were the major deteriorating agents which are known to affect the capacity of seeds to germinate, besides discoloring the parts, loss in luster, heating, mustiness and changes in oil content, free acids and protein content etc. Seed borne Mycoflora plays an important role in determining the quality and longevity of seeds. Microbial invasions can lead to the rotting and loss of seed viability, vigour, germination and oil quality Nagaraja and Krishnappa, (2009).

In the present study, variation was observed in seed mycoflora from freshly stage to at the end of the storage period. The change in the predominance of various molds changes with seed moisture and on inter-specific competition as described by Reddy and Reddy, (1982). PK-472 recorded the high percentage of fungi when compared to PK-262. The difference is may be due to the age of the seed, seed type and with the individual seed in the seed lot. Storage fungi were present in low percentage in freshly harvest seed samples and became dominant as the storage period increased. Storage fungi require high osmotic pressure and no water (Manoharachary and Kunwar, 2006). The results obtained from the present study suggest that employing more than one method is useful to note the complete fungal spectrum of seeds.

Germination percentage

In the present study, germination percentage was decreased in during the storage. According to Gidrol et al., (1998) reduction in germination is due to degradation of mitochondrial membrane, leading to reduction in energy supply necessary for germination.

Moisture content

Moisture content of seed plays an important role in influencing the fungal colonization during pre-harvest, post-harvest and in storage. Variations in the chemical composition of the seeds are known to be influence by the seed moisture content. There was a decrease in moisture content in seed samples during storage was noted in the current study. According to Shelar et al., 2008 they absorb or lose moisture till the vapour pressure of seed moisture and atmospheric moisture reach equilibrium.

Table.1 Fungi isolated from the varieties PK-262 and PK-462 of Soybean

Mycoflora	PK -262						PK-462					
	B.M		AGP.M		DP.M		B.M		AGP.M		DP.M	
	U	S	U	S	U	S	U	S	U	S	U	S
<i>Alternaria alternata</i>	+	+	+	+	+	+	+	+	+	+		+
<i>Alternaria humicola</i>	+	+	+	+	+	+	+	+	+	-	-	-
<i>Alternaria tenuis</i>	+	-	-	-	+	+	+	+	+	+	+	-
<i>Aspergillus flavus</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Aspergillus flavipes</i>	+	-	+	-	+	-	+	+	+	+	+	+
<i>Aspergillus fumigatus</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Aspergillus candidus</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Aspergillus niger</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Aspergillus nidulans</i>	+	-	-	-	+	+	+	-	-	+	-	+
<i>Aspergillus ochraceus</i>	+	+	+	+	+	-	+	-	+	-	-	-
<i>Aspergillus sulphureus</i>	-	-	+	+	-	-	-	-	+	+	+	-
<i>Aspergillus sydowii</i>	-	-	-	-	-	-	-	+	+	+	+	+
<i>Aspergillus tamaritii</i>	+	-	+	-	-	+	+	-	+	-	+	-
<i>Aspergillus terreus</i>	-	-	-	-	-	+	+	-	-	+	+	+
<i>Aspergillus versicolor</i>	-	-	-	-	-	-	-	+	+	+	+	+
<i>Cephalosporium sp</i>	+	-	+	-	+	+	+	+	+	-	+	-
<i>Chaetomium cochloides</i>	+	-	+	-	+	-	+	+	+	+	+	+
<i>Chaetomium globosum</i>	+	+	-	-	+	+	+	+	+	-	+	+
<i>Chaetomium herbarum</i>	+	+	+	+	-	-	+	+	+	+	-	-
<i>Chaetomium murorum</i>	+	+	+	+	-	-	+	-	-	-	-	-
<i>Cladosporium sp</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Cochliobolus spicifer</i>	+	-	+	+	+	+	+	+	+	-	+	-
<i>Curvularia lunata</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Drechslera hawaiiensis</i>	+	-	-	-	+	+	+	-	+	+	+	-
<i>Drechslera holodes</i>	+	+	+	+	+	+	+	-	+	-	+	-
<i>Fusarium moniliforme</i>	+	-	+	-	+	-	+	-	+	-	+	-
<i>Fusarium Oxysporum</i>	+	+	-	-	+	+	+	-	+	+	-	-
<i>Fusarium roseum</i>	-	-	+	-	+	-	-	-	+	+	-	+
<i>Mucor varians</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Myrothecium roridum</i>	-	-	-	-	-	-	+	+	+	+	+	+
<i>Nigrospora gossypii</i>	-	-	-	-	-	-	+	+	+	+	+	+
<i>Penicillium chrysogenum</i>	+	+	+	+	+	+	-	-	-	-	+	+
<i>Penicillium citrinum</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Penicillium notatum</i>	+	-	+	-	+	+	+	+	+	-	+	-
<i>Rhizopus nigricans</i>	+	+	+	+	+	+	+	+	+	+	+	+
<i>Stachybotrys atra</i>	-	-	-	-	-	-	+	-	+	-	+	-
<i>Syncephalastrum sp</i>	-	-	-	-	-	-	+	-	+	-	+	-
<i>Trichothecium roseum</i>	-	-	-	-	-	-	+	+	+	-	+	-
<i>Trichoderma album</i>	+	+	+	+	+	+	+	+	+	+	+	+

U- Unsterilized, S- Surface sterilized, B.M- Blotter, AGP.M- Agar plate and DP.M- Dilution Plate Method

Table.2 Physical and Biochemical changes in the soybean seed samples in freshly harvested and during storage

Seed	Variety	Parameters	Storage period in months						
			Freshly 6 Harvested seeds	1	2	3	4	5	
Soybean	PK-262	Germination (%)	98.0	97.0	96.2	94.3	92.4	89.3	85.0
		Moisture content (%)	13.50	12.9	12.3	11.5	11.1	10.9	10.3
		Protein (%)	43.30	43.0	42.4	42.0	41.9	41.2	40.7
		Total oil content (%)	20.20	19.8	19.2	18.9	18.6	18.0	17.4
		Free fatty acids(mg/g)	1.75	1.88	2.23	2.84	3.20	3.90	4.35
		Iodine number	123.60	123.60	122.50	121.20	120.10	119.00	118.20
	PK-472	Saponification value	190.20	190.60	190.30	191.90	192.30	193.00	193.60
		Germination (%)	96.0	92.5	90.3	89.2	87.3	82.3	79.5
		Moisture content (%)	14.9	14.1	13.4	12.7	11.9	11.0	10.4
		Protein (%)	43.20	42.9	42.2	41.7	41.3	40.7	40.0
		Total oil content (%)	20.0	19.4	19.1	18.7	18.2	17.7	17.3
		Free fatty acids(mg/g)	1.83	2.12	2.74	3.24	3.85	4.32	4.75
		Iodine number	124.6	124.00	123.20	122.30	121.50	120.60	117.80
		Saponification value	189.3	189.70	190.40	191.30	192.00	192.80	194.00

Protein estimation

Less amount of protein content was estimated from storage samples when compared to seeds drawn from freshly harvested conditions. Similar observations were made by Sethumadhava et al., (2014) in stored vegetable seeds and Ram Babu, (1994) in stored red and black gram seeds. Sinha and Prasad, (1977) stated that the proteins break down into amino acids due to the action of fungi. Bhadraiah and Rama Rao, (1988) stated that the microorganism are responsible for these changes in the seeds.

Total oil content

Reduction in oil content is one of the important parameter which is influenced by ageing of the seeds. There was a decrease in oil content from freshly seed samples to storage samples was observed Sharma and Chauhan, (1976) reported that the reduction in oil content, rancid smell and change in colour was due to *Aspergillus flavus* and *Cladosporium herberum* and stated that

fungal enzyme lipase is responsible for the reduction in oil content. Whereas Lalitha Kumari et al., (1970) expressed as lypolytic activity of the seed borne fungi is different and their capacity to accumulate the reducing sugars could be the reason for the reduction in oil content.

Free fatty acids

In the present the amount of free fatty acids were observed to increase in the seed samples during the storage when compared to freshly harvested seeds. Free fatty acids are produced by the actions of lipases on fats which break down into free fatty acids and glycerol during the storage. The accumulation of free fatty acids indicates a decline in the cellular p^H and harmful to seed health (Malik and Jyoti, 2013). The deteriorative changes in oilseeds may be either oxidative resulting in typical rancid flavor or hydrolytic resulting in the production of free fatty acids. The results conclusively show that there are certain fungi which are responsible for the reduction of total yield of oilseeds; whereas others

spoil the quality of oil by increasing the free fatty acids. The chemical changes indicate increase in saturated fatty acids, which is undesirable as it causes obesity & cardiovascular disorders (Robinson, 1978). Hence, such seeds are unfit for extracting oil for human consumption and the oil cake as animal feed.

Iodine number

The relation between Iodine number and saponification values is also an important criterion for the assessment of quality of oils. Iodine number indicates the quantity of unsaturated acids present in the oil. On contrary Ward and Diener, (1961) showed an increase in Iodine number in peanut due to fungal invasion. Lalitha kumari, (1971) observed a low Iodine number in oil extracted from *Aspergillus flavus* and *C. herbarum* infested groundnut seeds.

Saponification value

In the present investigation, increase in saponification value was observed in both the seed samples during the storage. According to Shankaram, (1966) saponification value changes due to the formation of number of short chain fatty acid glycerides during the lypolysis of oil by the enzyme lipase.

The study was conducted in two varieties of soybean i.e. PK-262 and PK- 472 in order to investigate physiological and biochemical changes during seed storage. The cumulative study based on results of all the parameters concluded that PK- 472 variety seeds are highly sensitive. According to Indian standard deteriorated oilseeds cannot be satisfactorily used for edible and industrial purposes. A special interest to be taken on nation's oil needs from the angle of nutrition and information regarding the

biodeterioration of soybean oilseeds as it is used for various purposes and to meet their specifications for purity.

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