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Research Article

SEED QUALITY DETERIORATION OF SOYBEAN (*GLYCINE MAX*: LINN): ROLE OF FUNGAL LIPASES

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ABSTRACT
Cultivars of Soybean viz. JS 335, Prasad and Puja yielded *Alternaria tenuis, Aspergillus flavus, A. fumigatus, A. niger, A. terreus, Chaetomium sp., Cladosporium herbarum, Curvularia lunata, Fusarium moniliforme, Mucor mucedo, Penicillium sp., Rhizoctonia sp.Rhizopus stolonifer, Verticillium albo-atrum*. The study revealed that the dominant fungi from seeds of soybean were *F. moniliforme, R. stolonifer* and *A. niger*. It also revealed that these fungi synthesized lipases responsible for deterioration in storage. There was drastic reduction in total oil content in all the three cultivars due to seed borne fungal incidence. With increase in time of storage the quantity of total oil content decreased. The qualitative estimation for free fatty acids and glycerides indicates the decrease in glycerides with increase in time and accumulation of free fatty acids with storage period.

Keywords: *Soybean, Oil, Lipases, Free Fatty Acids*

INTRODUCTION
Soyabean is a globally important crop plant providing oil and protein. India ranks fifth largest soyabean producer in the world. In India, Maharashtra and Madhya Pradesh are the major soyabean producing states. Seed deterioration is loss of seed quality and seed viability (Kapoor *et al.*, 2010). Seed-borne fungi cause losses in terms of seed quality and quantity in all oilseed crops. Seeds of many oil seed crop are known to harbour large amount of mycoflora. These fungi also reduce the germination and storability of the seed. They are responsible for seed rot, seedling blight, root/stem rot, foliar infection as well as pod blight diseases and affect adversely the seed germination, vigour and quality and quantity of oil (Ward and Diener, 1961; Lambat and Ram, 1969; Kadian and Suryanarayana, 1972; Agrawal and Joshi, 1972; Agrawal and Singh, 1974). This alteration in oil quality and quantity during storage and the incidence of fungi on seeds had been shown by Sugiura, (1970) where he related the effect of the product formed during lipolysis by *Mucor* lipases. Seed deterioration is one of the major reasons for the low crop productivity (Shelar, 2008).
A little work has been done on fungi associated with oil seeds and deterioration of oil quantitatively and qualitatively. Therefore, work was undertaken on quantitative and qualitative deterioration of oilseeds in soybean as a model oilseed.

MATERIALS AND METHODS
Cultivars: Three cultivars of soybean viz. cv. JS 335, cv. Prasad, cv. Puja were obtained from the Oilseed Research Station (Marathwada Agricultural University, Latur, Maharashtra). The seeds were stored at 22°C in cloth bags and used whenever needed.
Seed Mycoflora: Seed mycolfora was isolated following standard blotter test method and agar plate method. The isolated fungi were identified following early recorded one.
***Lipase Production*:** The isolated fungi were grown on specially designed medium for production of lipases. Czapek medium-broth was added with soybean oil instead of sucrose as carbon sources. The pH was adjusted to 6.0 by adding dilute NaOH solution.
Lipid Content: Fat estimation was made with ether extraction method. Ten gm of seeds were wrapped in Whatman's No. 1 placed in a thimble in the butt of Soxhlet extractor. The sample was extracted with petroleum ether for 2 hours without interruption by gently heating. Then, solvent ether along with the seeds fat was taken in preweighed flask, it was then kept in hot water bath. Ether was evaporated under

hood. The residue was dried over night in an oven at 60°C. Then, the flask was immediately transferred in a desiccator and on cooling, it was weighed. The amount of fat extracted was reported as % crude fat.

Qualitative Analysis of Lipids: The separation of lipid was done by TLC using silica gel – Silver nitrate method developed by Barrett *et al.*, (1963). Precoated silica gel G (20X20cm, 0.4 mm thickness, Darmstadt, Germany) plates were purchased from local market. The plates were loaded with oil samples and were run with Petroleum ether: Diethyl ether (1:1 v/v) as the solvent system.

Preparation of Enzymes: Fifty ml of medium was poured in 250 ml flasks and was inoculated with 0.5 ml of spore suspension of the fungi. The flasks were incubated for 27±2°C for 10 days. Flasks were drawn after regular time interval and the contents were centrifuged at 10000 rpm for 10 minutes to remove spore and suspended matter. The filtrate was dialysed against running tap water for 24 hours and was used as crude enzyme preparation and stored at 2-4°C for further use.

Measurement of Lipase Activity: Triacylglycerol acyihydrolase (EC 3.1.1.3) was determined by method given by Jayaraman (1981). One unit of enzyme is defined as the quantity of fatty acid released in unit time, measured by the quantity NaOH required to maintain pH constant. The milliequivalent of alkali consumed was taken as a measure of the activity of the enzyme.

RESULTS AND DISCUSSION

It is now well established that storage fungi bring about deleterious effect in a number of ways like reduction in seed germination, discolouration and biochemical changes in the seeds. Many worker established the fact that store seeds develop various types of molds like species of *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria*, *Macrophomina* etc. which ultimately affected germination and germination and variability of seeds, discolouration of seeds, reduction of oil content and loss of quality, development of mycotoxin problem, loss in grain weight and unfit for sowing and human consumption (Singh and Sinha, 1975; Nandi *et al.*, 1982; Mahajabin *et al.*, 2015).

Table 1: Change in Oil Content in Storage of Soybean

Time	% Oil Content		
	JS 335	Puja	Prasad
Initial	23.6	21.9	25.0
30	22.6	20.5	24.4
60	21.4	19.2	23.1
90	20.0	18.3	22.4
120	19.2	16.9	21.3
150	17.8	15.8	20.1
180	15.9	13.3	19.7
210	14.2	12.4	17.8
240	13.1	11.4	15.6
270	11.2	10.7	14.2

Experiments conducted to asses the quantity of the oil in the soybean cultivars exhibited interesting results.

The percent oil content was determined from fresh harvested seed and subsequently at regular time interval upto 270 days. A record percentage of incidence of fungi was also made. In all the three varieties of soybean there was a decrease in percent oil content with increase in time of storage. The initial oil content ranged between 21.9-25% whereas the decrease after 270 days recorded was 10.7-14.2% (Table 1).

Attempts made to determine the change in various fatty acids and lipids by storage fungi showed intriguing observations. The extracted oil was subjected to separation for various fatty acid and lipids present in the oil after 90, 180 and 270 days of storage. The presence or absence of specific fatty acids and lipids was recorded.

In all the three varieties of soybean, there was slight variation in the types of fatty acids and lipids while marked difference was recorded with the increase in storage period. There was increase in higher fatty acids with respect time whereas lower fatty acids decreased. Similarly, there was also a decrease in diglycerides and triglycerides with respect storage period (Table 2).

Table 2: Change in Types of Lipids/Fatty Acid during Storage in Soybean

Lipids/Fatty Acid	JS 335			Puja			Prasad		
	90	180	270	90	180	270	90	180	270
Myristic acid	+	+	+	+	+	+	+	+	-
Lauric acid	+	-	-	+	+	-	+	+	+
Palmatic acid	+	+	-	+	-	-	+	+	-
Stearic acid	+	+	++	+	+	+	+	+	+
Arachidic acid	+	+	++	+	+	+	+	+	+
Oleic acid	+	+	++	+	+	+	+	+	+
Linoleic acid	+	+	-	+	-	-	+	+	-
Monoglycerides	++	+	-	++	+	-	++	+	-
Diglycerides	++	+	-	++	+	+	++	+	-
Triglycerides	++	+	+	++	+	-	++	+	-

- = Absent + = Present

Table 3: Production of Lipase by *Aspergillus Niger, Rhizopus Stolonifer* and *Fusarium Moniliforme* on Soybean Oil Containing Medium

Age of Culture Filtrate (Days)	Lipase Activity (U/ml)		
	Aspergillus Niger	***Rhizopus Stolonifer***	***Fusarium Moniliforme***
1	0.00	0.00	0.00
2	0.00	0.02	0.00
3	0.00	0.06	0.00
4	0.02	0.06	0.05
5	0.04	0.08	0.06
6	0.07	0.12	0.10
7	0.10	0.18	0.12
8	0.13	0.24	0.13
9	0.15	0.23	0.12
10	0.10	0.23	0.12

Fungi are known for their capacities to synthesize a variety of enzyme depending upon availability of substrate. A series of experiments were undertaken to assess the ability of fungi to degrade lipids present in the soybean seed by secretion of lipase. *Aspergillus niger*, *Rhizopus stolonifer* and *Fusarium moniliforme* were most dominant fungi and therefore, selected for further investigations. They were grown on soybean oil containing medium and 8 days old culture filtrate was used as crude enzyme source. *Aspergillus niger*, *Rhizopus stolonifer* and *Fusarium moniliforme* synthesized lipase in both the media. The synthesis increased with increase in time of incubation in the media; however, the amount of enzymes varied. Enzymes were secreted in soybean oil containing medium (Table 3). The quantity of enzyme secreted in medium increased during the study period, the maximum amount (4-0.18 U/ml, 5-0.25 U/ml and 6-0.12U/ml) was detected in 9 days.

Stored oilseeds may undergo physical, physiological and chemical changes even under ideal storage conditions. Some of the changes may or may not have a negative effect on the final use of oilseeds depending on the degree of change. One common indicator of chemical change in stored oilseeds is the

level of free fatty acid (FFA) present. An increase of FFA above 1% may translate into lower quality of its oil content. Oilseeds are rich in oil therefore, the process of deteriorarion by fungi may be attributed to lipase production by fungi (Chavan and Kakde, 2010).

The assessment of oilseeds in storage for the quantities of oil present in the seeds revealed that there was drastic reduction in oil content in all the three varieties. With increase in time of storage the quantity of oil decreased. However, the maximum loss of oil was recorded at the end of nine months. The loss of oil content in most of the case was more or less 50%. The dominant fungi associated in all the cases were internal seed borne fungi. This indicates there role in deteriorating oil quality by secretion of lipases.

The qualitative estimation for free fatty acids and glycerides indicates the decrease in glycerides with increase in time and accumulation of free fatty acids with storage period.

This indicates the lipolytic abilities of the dominant fungi associated with the respective seeds. The study of the dominant fungi for synthesis of lipases revealed that they were ardent producers of lipases. So, the deterioration of oil can be attributed to the lipolytic ability of fungi. Similarly, the decrease in glycerides and free fatty acids indicates their utilization as substrate by these fungi.

Thus, the lipolytic enzymes produced by the seed borne fungi plays and decisive role in deterioration of oilseed in storage. The seed borne fungi in groundnut may reduce the oil content and cause a change in its colour, induce an unpleasant odour and lead to hydrolytic rancidity. The increase in fatty acids in groundnut extracted from the seed-borne fungi infected seeds was observed by Lalithakumari *et al.*, (1971).

According to Zeeleny and Coloman (1938) acids were produced by action of lipids. The increase in fatty acids was not quantitative. The products of hydrolysis of lipids serve as substrates for growth of fungi and for increased metabolic processes in seed. The largest and earliest increases occur in the free fatty acids. Changes in lipids during oilseed storage has been shown that there are changes in the lipid components of seeds that are associated with seed deterioration (Vertucci, 1992).

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