Effect of Storage on Redgram (*Cajanus cajan* /L./ Millsp) and Greengram (*Vigna radiata* /L./ Wilczek) with Particular Reference to Lipid Composition

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Abstract

Gopinath M.R., Sambiah K., Niranjana S.R. (2011): E**ffect of storage on redgram** (*Cajanus cajan* /L./ Millsp) **and greengram** (*Vigna radiata* /L./ Wilczek) **with particular reference to lipid composition**. Plant Protect. Sci., **47**: 157–165.

The effects of storage on the lipid composition of three redgram (TTB-7, BRG-1, and ICP-8863) and three greengram (PUSA BISAKI, KDM-1, and CHINA MOONG) varieties were studied. Environment variables such as temperature and relative humidity were recorded during storage period. Moisture content was found to increase along with the percent incidence of storage insects. The incidence of storage moulds and insects was recorded in all the samples; results showed that storage moulds belonged to *Aspergillus* species and the insect *Callosobruchus chinensis* infested the stored samples. The total fat, triglycerides, phospholipids, free fatty acids and peroxide value were evaluated in the control and in samples stored for three and six months. Results showed that storage depleted total fat (1.94–1.75 g), triglycerides (1.46–1.07 g), whereas phospholipids (0.06–0.21g), free fatty acids (0.002–0.01 g) and peroxide values (2.14–4.46 meq) increased. The fatty acid content of palmitic (26.03–23.56%), stearic (7.4–5.46%), linoleic (56.2–45.2%) and linolenic acids (6.9–4.7%) decreased, but oleic acid content increased (8.3–21.6%) in all the varieties during storage.

Keywords: redgram; greengram; triglycerides; phospholipids; peroxide value; insects; moulds

Pulses, being an important source of proteins, are the major constituent of diet in India, where the consumption of animal protein except milk is still considered a religious and social taboo. However, there is a growing recognition that production alone does not solve the food problem, food grains must be preserved in edible, nutritionally adequate condition until they can be distributed and consumed by those who need it. Pulse grains such as redgram and greengram are important sources of proteins and lipids and also natural isoflavinoid antioxidants such as daidzein, genistein and their glucosides daidzin and genistin, which can protect them against oxidation (Lapcik *et al*. 1999) for human consumption as well as animal feed. During storage these pulses suffer a great deal of damage due to storage moulds and insects, and the most serious results of such damage appear to be both quantitative and qualitative losses. Lipid oxidation is one of the major causes of quality deterioration in natural foods, oxidative deterioration is a large economic concern in the food industry because it affects many quality characteristics such as flavour, colour, texture, and the nutritive value of foods (Chaiyasit *et al*. 2007). Lipid related changes during storage revealed a decline in phospholipids and polyunsaturated fatty acids (Priestly & leoplod 1983a,b). The role of fungi in changes occurring during storage is great and the most frequently reported among these changes were an

increase in free fatty acids, peroxide value and a decrease in total fat, seed germination (Goodman 1950; Doworth & Christensen 1968; Fletcher 1968; Mbata & Osuji 1983; Mbata 1986). Others (Millner & Geddes 1946a,b; Goodman 1950; Modgil & Mehta 1996; Ojimelukwe *et al*. 1999) reported the nutritive changes caused by infestation by storage insects. Very few reports were available with respect to redgram and greengram varieties, which are extensively used in India for various food preparations; hence the present study was carried out to understand the effect of storage moulds and insects on lipid composition during storage of redgram and greengram varieties.

MATERIAL AND METHODS

Collection of samples. Infestation-free samples comprising the redgram variety TTB-7 (Turk Tugari Bangalore), BRG-1 (Bangalore Redgram), ICP-8863 (International Crops Pulses) and greengram varieties Pusa Bisaki, KDM-1 (Karnataka Dharwad Moong) and China Moong (5 kg each sample) were procured from National Seed Project of Bangalore and Dharwad Agricultural Universities. The samples were stored in cotton bags for six months (June–December 2004), allowed for natural infestation under temperature and relative humidity of laboratory conditions. All the chemicals used in this study were of analytical grade and the solvents were distilled before their use.

Monitoring of environment variables during storage. During the storage period the actual weather data on mean maximum and minimum temperature (°C) and relative humidity (%) of the storage room were recorded on all the days. Relative humidity (%) was recorded with wet and dry bulb hygrometer (psychrometer) following the method of Buck (1981).

Determination of moisture content by high constant temperature oven method. Seeds were pulverized and the known amount (5 g) of seeds was taken and the working sample was evenly distributed over the surface of the container. The weight of the container and its cover was recorded before and after filing the sample. The oven was maintained at a temperature of 130–133°C, the sample was dried for 1 hour. At the end of the prescribed period the container was covered and placed in a desiccator to cool for 30–45 minutes. After 1 hour the sample was cooled in a desiccator and the weight was noted down. The percentage loss in weight was calculated according to ISTA (Anonymous 2003).

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

where:

M1 – weight of the empty dish (g)

M2 – weight of dish + powdered seed material (before drying) (g)

M3 – weight of dish + powdered seed material (after drying) (g)

Screening for moulds. Screening was done by the standard blotter method (Anonymous 1996). About 400 seeds were plated on blotters well soaked in water, incubated at 22°C for 7 days under 12 h alternating cycles of light and darkness. After incubation, each seed was examined for the growth of fungi under different magnifications of a stereomicroscope. The identification of fungi was based on habit characters of fruiting bodies, spores and conidia observed under a compound microscope.

Screening for insects. Insect count was obtained by counting the number of adults (both dead and live) in 100 g of seed material using ethyl ether as anaesthesia and sieving out insects from the seed sample (Tripathy *et al*. 2001).

Extraction of total lipids. Total lipids were extracted by the method adopted by Bhattacharya and Raha (2002): lipids from redgram and greengram seeds were extracted with hexane (60–80°C) in the Soxhlet extractor for eight to twelve hours and oil content was expressed as dry weight basis.

Triglyceride composition. Triglyceride content was determined by using the method of Fletcher (1968). Tripalmitin (Sigma Chemicals, Perth, Australia) was used as a standard.

Phospholipid composition. Phospholipid content was determined by the method described by Stewart (1980) using phosphatidyl choline as a standard.

Free fatty acid content. Free fatty acids were determined by the method of Lisker *et al*. (1985). Estimation was done titrimetrically, and the amount of free fatty acids was quantified using the standard graph of oleic acid.

Peroxide value. The peroxide value was determined by the method described by Stine *et al*. (1953). Ferrous iron was used as a standard.

Fatty acid composition. Fatty acids were determined using boron fluoride methanol as described

Table 1. Atmospheric temperature and relative humidity in laboratory during storage period

Month	Temperature (°C)			Relative humidity (%)		
	maximum	minimum	mean	maximum	minimum	mean
June	29.8	18.9	25.3	98	39	76.4
July	31.2	18.7	24.6	96	41	79.8
August	29.6	18.6	24.2	96	42	76.4
September	32.1	19.2	25.6	95	29	80.2
October	30.8	19.0	29.2	96	24	81.6
November	28.1	16.7	23.8	97	34	77.5
December	29.5	12.4	21.6	92	25	74.8

by Morrison and Smith (1964). Fatty acid methyl esters were analysed using a Shimadzu 14-B gas chromatograph fitted with silica capillary column (30 m × 0.24 mm, Konick, Spain), while the injection port and detector temperature was maintained at 230°C and 240°C, respectively. The column temperature was at 220°C with nitrogen as a carrier with the flow rate of 1 ml/minute. Individual fatty acids were identified by comparison with fatty acid methyl esters procured from Nechek prep. USA.

Statistical analysis. Statistical analysis of all the parameters was performed with the SPSS 10.0 software package (SPSS, Chicago, USA). Tukey's multiple comparison tests were used to compare the differences between the means by least significant differences at a 5% level of probability.

RESULTS

In 2004 (June–December) the maximum mean temperature was recorded in the month of September (32.1°C) followed by July (31.2°C), October (30.8°C), June (29.8°C), August (29.6°C), December (29.5°C), and November (28.1°C). The mean relative humidity was maximum in the month of October (81.6%) followed by September (80.2%), July (79.8%) and November (77.5%), whereas June and August (76.4%) recorded the same percentage of relative humidity during storage (Table 1).

Moisture content in the samples was found to be the highest in varieties PUSA BISAKI (10.3%) followed by TTB-7, ICP-8863, CHINA MOONG, BRG-1, and KDM-1. At the end of three-month storage all the samples recorded an increase in the percentage of moisture content and the highest percentage was found in varieties TTB-7 and PUSABISAKI (13.3%) followed by BRG-1, ICP-8863, KDM-1 and CHINA MOONG. After six-month storage the maximum moisture content was found in the variety TTB-7 followed by PUSABISAKI, CHINA MOONG, BRG-1, ICP-8863, and KDM-1(Table 2).

The initial evaluation of fresh greengram samples revealed a minimum incidence of storage fungi

Table 2. Moisture content (%) in redgram and greengram during storage

Samples and varieties	Control	Three months stored	Six months stored
Redgram			
TTB-7	10.1± 0.1	13.3 ± 0.2	15.7 ± 0.1
BRG-1	9.8 ± 0.2	12.9 ± 0.2	13.4 ± 0.2
ICP-8863	9.9 ± 0.2	12.8 ± 0.2	13.3 ± 0.1
Greengram			
PUSA BISAKI	10.3 ± 0.1	13.3 ± 0.1	15.5 ± 0.1
KDM-1	9.7 ± 0.2	12.8 ± 0.1	13.1 ± 0.2
CHINA MOONG	9.8 ± 0.2	12.7 ± 0.2	14.2 ± 0.2

Values are mean ± SEM of three values

Table 3. Storage mycoflora incidence (%) in green gram and red gram samples during storage

Fungi infected	KDM-1			CHINA MOONG			PUSA BISAKI		
	control	three months	six months	control	three months	six months	control	three months	six months
Greengram									
Aspergillus flavus	1	8	16	–	10	12	–	12	20
Aspergillus niger	–	9	14	1	9	17	–	7	15
Aspergillus candidus	–	–	–	–	–	1	–	2	3
Aspergillus tamari		–	–		–	–	–	–	2
Aspergillus terreus	–	2	4	–	1	4	–	1	1
Aspergillus flavipes	–	–	–	–	–	–	–	–	1
Aspergillus versicolor		2	3		3	4		3	4
Redgram									
Aspergillus flavus	2	9	14	1	7	13	2	6	11
Aspergillus niger	–	33	45	2	27	41	–	19	27
Aspergillus candidus	–	2	4	–	3	5	–	3	4
Aspergillus tamari	–	1	3	–	2	3	–	2	4
Aspergillus terreus	–	1	2	–	2	3	–	1	2

Data based on four replicates of 100 seeds of each variety

A. flavus 1% and *A. niger* 1%. In samples stored for three months a gradual increase of the storage fungal spectrum was observed. *Aspergillus flavus* recorded the highest incidence ranging from 8% to 12% followed by *A. niger* 70% to 9%, *A. versicolor* 2% to 3%, *A. candidus* 2%. At the end of six-month storage the density of storage fungi of *Aspergillus* species increased, when the incidence of *A. flavus* ranged from 12% to 20%, followed by *A. niger* 14–7%, *A. candidus* 1–3%, *A.tamarii* 2%, *A. versicolor* 3–4%, *A. terreus* 1–4% and *A. flavipes* 1% (Table 3).

The incidence of storage fungi found at a lower frequency among *A. flavus* incidence ranged from 1% to 2% followed by *A. niger* 2% in fresh samples of redgram samples. Mycofloral analyses of samples stored for 3 months revealed *A. niger* found predominantly ranging from 19% to 33%, followed by *A. flavus* 6–9%, *A. tamarii* 1–2%, *A. candidus* 2–3%, and *A. terreus* 1–2%. At the end of 6-month storage, the incidence of storage fungi of the species *Aspergillu*s completely dominated. *A. niger* incidence ranged from 27% to 45% followed by *A. flavus* 11–14%, *A. candidus* 4–5%, *A .tamarii* 3–4%, and *A. terreus* 2–3% (Table 3).

The initial screening results did not record any incidence of storage insects. At the end of three-month storage, the varieties TTB-7 and PUSA BISAKI were found to be infested by the insects

Table 4. Incidence of storage insects in redgram and greengram samples

Samples/varieties	Total number of insects/100g sample (%)			Insect infested
	control	three months stored	six months stored	
Greengram				
KDM-1	–	–	03	
CHINA MOONG	–	–	02	*Callosobruchus chinensis*
PUSA BISAKI	–	9	97	
Redgram				
BRG-1	–	–	02	
TTB-7	–	25	70	*Callosobruchus chinensis*
ICP-8863	–	–	03	

Data based on total number of insects (dead and live) for 100g seed samples

Callosobruchus chinensis at 25% and 9%, respectively, whereas no incidence was found in the other varieties. At the end of six-month storage the variety PUSA BISAKI (97%) recorded a maximum population of insects followed by TTB-7 (70%), CHINA MOONG, ICP-8863 (3%), BRG-1 and CHINA MOONG (2%) (Table 4).

Variation in the lipid composition of redgram varieties was determined in both control and stored samples of redgram and greengram varieties. The total lipid content was significantly ($P \leq 0.05$) reduced in TTB-7 variety from 1.64 g to 1.55 g and 1.5 g under storage. Whereas in BRG-1 variety it decreased from 1.94 g to 1.74 g and in ICP-8863 it was reduced from 1.54 g to 1.31 g in stored samples. There was a significant reduction in triglyceride content in stored varieties of redgram, the maximum decrease was in var. TTB-7 from 1.57g to 1.41g and 1.25 g in three and six months respectively, however in var. BRG-1 it was reduced from 1.84 g to 1.64 g and 1.60 g during storage. In ICP-8863 variety a significant difference between the control (1.47 g) and samples stored for three months (1.24 g) was recorded whereas there was no significant difference between samples stored for three and six months (Table 5).

Table 5. Lipid composition of redgram and greengram varieties during storage

Samples and duration of storage	Total fat	Triglycerides	Phospholipids	Free fatty acids	Peroxide value (meq)
	(g/100g)				
Redgram					
TTB-7					
Control	1.64 ± 0.003^{c}	1.57 ± 0.01^{c}	0.06 ± 0.008^{a}	0.01 ± 0.0008^{a}	2.1 ± 0.02^{a}
3M	1.55 ± 0.006^{b}	1.41 ± 0.03^{b}	0.11 ± 0.00^{b}	0.02 ± 0.0003^{b}	3.9 ± 0.01^{b}
6M	1.50 ± 0.01^{a}	1.25 ± 0.03^{a}	0.21 ± 0.005^{c}	0.03 ± 0.0001^{b}	4.4 ± 0.03^{c}
BRG-1					
Control	1.94 ± 0.003^{b}	1.84 ± 0.01^{b}	0.08 ± 0.005^{a}	0.01 ± 0.0001^{a}	2.8 ± 0.03^{a}
3M	1.77 ± 0.003^{a}	1.64 ± 0.04^{a}	0.11 ± 0.003^{ab}	0.02 ± 0.0003^{b}	3.1 ± 0.01^{b}
6M	1.75 ± 0.003^{a}	1.6 ± 0.02^{a}	0.12 ± 0.0^{b}	0.03 ± 0.0001^{b}	3.4 ± 0.01^{c}
ICP-8863					
Control	1.55 ± 0.005^{b}	1.47 ± 0.01^{b}	0.06 ± 0.006^{a}	0.01 ± 0.0001^{a}	2.4 ± 0.02^{a}
3M	1.34 ± 0.005^{a}	1.24 ± 0.02^{a}	0.08 ± 0.002^{a}	0.02 ± 0.0003^{b}	2.6 ± 0.02^{b}
6M	1.31 ± 0.003^{a}	1.20 ± 0.02^{a}	0.09 ± 0.005^{b}	0.02 ± 0.0001^{b}	3.7 ± 0.02^{c}
Greengram					
PUSA BISAKI					
Control	1.52 ± 0.003^{c}	1.46 ± 0.01^{b}	0.06 ± 0.006^{a}	0.002 ± 0.0003^{a}	2.6 ± 0.03^{a}
3M	1.25 ± 0.003^{b}	1.15 ± 0.02^{b}	0.09 ± 0.0002^{b}	0.003 ± 0.0008^{b}	3.1 ± 0.02^{b}
6M	1.24 ± 0.003^{a}	1.07 ± 0.02^{a}	0.14 ± 0.005^{c}	0.01 ± 0.0008^{c}	3.6 ± 0.05^{c}
KDM-1					
Control	1.21 ± 0.008^{c}	1.14 ± 0.02^{a}	0.06 ± 0.008^{a}	0.003 ± 0.0003^{a}	1.4 ± 0.03^{a}
3M	1.17 ± 0.005^{b}	1.08 ± 0.04^{b}	0.09 ± 0.006^{ab}	0.006 ± 0.0003^{b}	2.8 ± 0.02^{b}
6M	1.1 ± 0.008^{a}	0.98 ± 0.04^{a}	0.1 ± 0.008^{b}	0.01 ± 0.0008^{c}	3.7 ± 0.01^{c}
CHINA MOONG					
Control	1.35 ± 0.003^{b}	1.29 ± 0.01^{b}	0.06 ± 0.008^{a}	0.003 ± 0.0003^{a}	1.4 ± 0.03^{a}
3M	1.25 ± 0.003^{ab}	1.15 ± 0.03^{ab}	0.09 ± 0.003^{ab}	0.005 ± 0.0001^{b}	2.4 ± 0.01^{b}
6M	1.24 ± 0.005^{a}	1.12 ± 0.01^{a}	0.1 ± 0.003^{b}	0.01 ± 0.0005^{c}	2.8 ± 0.02^{c}

Values are mean ± SEM of three values; Mean values with different superscripts are significantly different from each other (≤ 0.05); meq= milliequivalents

Phospholipid contents were determined in all the redgram samples during storage, and a significant increase in all the stored varieties of redgram was recorded. In TTB-7 variety phospholipid content increased from 0.06 g to 0.11 g and 0.21 g in the stored samples, in BRG-1 it varied from 0.08 g to 0.11 g and 0.12 g, whereas in ICP-8863 it increased from 0.06 g to 0.08 g and 0.09 g during storage (Table 5).

The free fatty acids and peroxide value determined in redgram varieties during storage show a significant increase in the fatty acid level of var. TTB-7 from 0.01 g to 0.02 g and 0.03 g, in BRG-1 it increased from 0.01 g to 0.02 g and 0.03 g in the control, 3- and 6-month storage whereas in var. ICP-8863 it increased from 0.01 g to 0.02 g and 0.03 g. The peroxide value significantly increased in all the varieties of redgram, in TTB-7 it increased from 2.14 meq to 4.4 meq, in BRG-1 it increased from 2.8 meq to 3.4 meq, and in ICP-8863 from 2.4 meq to 3.71 meq (Table 5).

Variations in the lipid composition of greengram varieties during storage were determined. Total lipids in greengram varieties showed a significant reduction ($P \leq 0.05$) in samples stored for three and six months when compared to the control. The total lipids of PUSA BISAKI were depleted from 1.52 g to 1.25 g and 1.24 g, in KDM-1 they varied from 1.21 g to 1.17 g and 1.1 g, whereas in CHINA MOONG they decreased from 1.35 g to 1.25 g and 1.24 g during storage. The total triglyceride content was significantly reduced in all greengram varieties, in PUSA BISAKI it was from 1.46 g to 1.15 g and 1.07 g, however in KDM-1 it decreased from 1.14 g to 1.08 g and 0.98 g and in CHINA MOONG it was reduced from 1.29 g to 1.15 g and 1.12 g in stored samples (Table 6).

The phospholipid level was significantly increased in all stored varieties of greengram samples, in PUSABISAKI it increased from 0.06 g to 0.09 g and 0.14 g during storage, in var. KDM-1 from 0.06 g to 0.09 g and 0.10 g and in CHINA MOONG it increased from 0.06 g to 0.09 g and 0.10 g in the control and in samples stored for three and six months, respectively (Table 7).

Total free fatty acids and peroxide values of stored greengram varieties were determined in greengram and redgram during storage. Among all the samples a significant increase of free fatty acid content was recorded in var. PUSA BISAKI from 0.002 g to 0.01 g and 0.03 g, whereas in KDM-1 it increased from 0.003 g to 0.006 g and 0.01 g and

Table 6. Fatty acid compositions in redgram varieties during storage

Samples and fatty acids	Control	Three months	Six months
Redgram			
TTB-7			
16:0	24.0 ± 1.0^{a}	23.1 ± 0.2^{a}	22.9 ± 0.6^{a}
18:0	4.6.0 ± 0.1^{a}	4.4 ± 0.2^{a}	4.3 ± 0.4^{a}
18: 1	8.3 ± 0.2^{a}	14.8 ± 0.2^{b}	21.6 ± 1.4^{c}
18: 2	56.2 ± 0.4^{c}	49.6 ± 0.4^{b}	45.2 ± 0.5^{a}
18: 3	6.9 ± 0.2^{b}	6.5± 0.2^{b}	4.7 ± 0.2^{a}
BRG-1			
16:0	25.4 ± 0.4^{a}	25.0 ± 0.2^{a}	24.3 ± 0.5^{a}
18:0	4.8 ± 0.2^{a}	4.5 ± 0.2^{a}	4.1 ± 0.2^{a}
18: 1	10.0 ± 0.2^{a}	19 ± 0.05^{b}	21.7 ± 0.3^{c}
18: 2	55.3 ± 0.4^{b}	46.0 ± 0.2^{a}	45.4 ± 0.2^{a}
18: 3	4.6 ± 0.2^{b}	3.9 ± 0.2$^{a\,b}$	3.4 ± 0.2^{a}
ICP-8863			
16:0	24.2 ± 0.28^{a}	23.9 ± 0.39^{a}	23.5 ± 0.2^{a}
18:0	5.4 ± 0.17^{b}	4.83 ± 0.08$^{a\,b}$	4.6 ± 0.2^{a}
18: 1	14.0 ± 0.29^{a}	15.1 ± 0.3^{a}	19.1 ± 0.3^{b}
18: 2	52.1 ± 0.23^{b}	51.9 ± 0.97^{b}	47.1 ± 0.9^{a}
18: 3	4.4 ± 0.29^{a}	4.3 ± 0.3^{a}	4 .0 ± 0.1^{a}
Greengram			
PUSA BISAKI	26.2 ± 0.5^{b}	24.9 ± 0.3^{b}	23.56 ± 0.7^{a}
16:0	7.4 ± 0.3^{b}	5.8 ± 0.3^{a}	5.46 ± 0.2^{a}
18:0	7.3 ± 0.2^{a}	11.9 ± 0.1^{b}	14.8 ± 0.2^{c}
18: 1	43.7 ± 0.2^{b}	42 .9 ± 0.1^{b}	37.4 ± 0.4^{a}
18: 2	15.1 ± 0.3^{b}	13.7 ± 0.4^{b}	11.0 ± 0.6^{a}
18: 3			-
KDM-1	26.0 ± 0.5^{b}	25.5 ± 0.3^{b}	23.9 ± 0.03^{a}
16:0	8.9 ± 0.2^{b}	7.9 ± 0.2^{ab}	6.7 ± 0.5^{a}
18:0	7.8 ± 0.7^{a}	10 ± 0.3^{b}	13.7 ± 0.4^{c}
18: 1	40.0 ± 0.5^{a}	39.7 ± 1.0^{a}	36.8 ± 0.5^{a}
18: 2	17.5 ± 0.4^{a}	16.9 ± 0.8^{a}	15.36 ± 1.0^{a}
18: 3			
CHINA MOONG	28.0 ± 0.4^{a}	27.1 ± 0.3^{a}	26.0 ± 0.6^{a}
16:0	6.8 ± 0.15^{a}	6.0 ± 0.2^{a}	5.3 ± 0.5^{a}
18:0	6.3 ± 0.2^{a}	9.5 ± 0.2^{b}	13.6 ± 0.4^{c}
18:1	26.2 ± 0.5^{b}	24.9 ± 0.3^{b}	23.56 ± 0.7^{a}
18:2	7.4 ± 0.3^{b}	5.8 ± 0.3^{a}	5.46 ± 0.2^{a}
18:3	7.3 ± 0.2^{a}	11.9 ± 0.1^{b}	14.8 ± 0.2^{c}

Values are mean ± SEM of three values; mean values with different superscripts are significantly different from each other ($P \leq 0.05$)

in CHINA MOONG from 0.003 g to 0.005 g and 0.01 g in the control and in samples stored for three and six months. Peroxide value increased in PUSA BISAKI from 2.6 meq to 3.1 meq and 3.6 meq, whereas in KDM-1 it increased from 1.4 meq to 2.8 meq and 3.7 meq and in CHINA MOONG it increased from 1.4 meq to 2.4 meq and 2.8 meq (Table 7).

The fatty acids detected by GLC analysis were palmitic, stearic, oleic, linoleic, and linolenic acids. In TTB-7 samples no significant difference in a reduction in stearic acid and palmitic acid was observed whereas linoleic and linolenic acids were significantly reduced during storage. In BRG-1 variety, a significant decrease in linoleic and linolenic acids was found out and in ICP-8863 a significant decrease in stearic acid and linoleic acid was determined whereas there was no significant reduction in palmitic acid and linolenic acid (Table 8).

The fatty acid composition of greengram varieties during storage was analysed: among them, in PUSA BISAKI the contents of palmitic, stearic, linoleic and linolenic acid were significantly reduced during storage whereas the oleic acid content was significantly increased during six- month storage. In the KDM-1 variety fatty acid contents of palmitic and stearic acids were significantly reduced and a significant increase in oleic acid was found out. In var. CHINA MOONG, a significant reduction was observed only in linoleic acid followed by a significant increase in the oleic acid content (Table 9).

DISCUSSION

The study shows that significant changes can occur in redgram and greengram samples during six-month storage with respect to lipid composition. We can conclude from the present investigation that the fungi are mainly responsible for deterioration of grains by excessive moisture, which is in tune with earlier studies (Pitt & Hocking 2009). Earlier work showed that the lipase activity of storage fungi of *Aspergillus* sp. and *Fusarium* sp. increased the free fatty acid level (Kakde & Chavan 2011; kamimura *et al*. 2001; Burkert *et al*. 2004). A widely used chemical index of deterioration is the increase of fat acidity in stored seeds. It has been suggested that fat acidity arises from the action of seed lipases (Fsatnaught *et al*. 2006), but it may also arise from the action of fungi on seed constituents. The present study reveals that an increase in mould count positively correlates with an increase in fat acidity. There is often a fair close correlation between an increase in fat acidity and an increase in the mould count of stored seeds (Dhingra *et al*. 2009). Fungi would thus seem to play a more dominant role in the acidity increase than the seed lipases. The peroxide value increase may be due to a direct autocatalytic attack by atmospheric oxygen on lipids under very dry conditions. At higher moisture levels during storage, seed lipoxygenase activity might provide an alternative mechanism for the lipid attack by oxygen (Vick & Zimmerman 1976). The phospholipid level increase strongly suggests that these changes can occur independently of mould growth, the moisture uptake during storage probably accounts for the observed increase in phospholipid levels (Sharma *et al*. 2007). The increase in phospholipid levels during storage was reported in earlier investigations (Bernal Lugo & Leoplod 1992; Sharma *et al*. 2007). The phospholipid increase may also be due to a decrease in the other lipid constituents during storage. In the varieties infested by insects, TTB-7 of redgram and PUSA BISAKI of greengram, there was a maximum increase in free fatty acids and peroxide value, which are the indices of lipid deterioration. The feeding activities of insect larvae of the pulse beetle C*allosobruchus chinensis* could have encouraged interaction between endogenous lipases and their substrates, which could have led to an exposure of lipid components to a higher level of oxygen enhancing oxidation. Insect emergence holes allowed more atmospheric oxygen into the seed. Hydrolytic changes in the feeding of insect larvae (Ojimelukwe 2002) accelerate deteriorative changes in the lipids of redgram and greengram seeds, increase free fatty acid contents and peroxide values as was observed during the infestation of other legume seeds such as cowpea (Modgil & Metha 1996; Ojimelukwe *et al*. 1999; Patro *et al*. 2001); qualitative losses arising from the portions of seed eaten by insects and qualitative losses reflected in an increased deterioration of the nutritive value are both due to the infestation. Oxidative rancidity of fat can result in the development of toxic products, pigment discoloration, texture changes and loss in the nutritive value (Ojimelukwe 2002). The present experimental results are in par with the earlier investigations

and they shed more light on the fate of lipid constituents in detail during storage. According to a previous report (Pearce & Abdel Samad 1980), oleic acid is not susceptible to the attack of lipoxygenase when compared to other polyunsaturated fatty acids. This might be the reason for an increase in the percentage of oleic acid with respect to the total fatty acid percentage during storage.

The results of the study show that storage enhances the role of the lipolytic activity of grains, which results in a decrease of total lipids and triglycerides, and in an increase in phospholipids, free fatty acids and peroxide values. In the total fatty acid composition, palmitic, stearic, linoleic, linolenic acids were decreased while oleic acid was increased. The investigation clearly indicates the effect of storage on the activation and deactivation of seed lipases in biodeterioration of lipids during storage.

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Received for publication December 29, 2010
Accepted after corrections July 7, 2011

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