



P-ISSN: 2349-8528
 E-ISSN: 2321-4902
 IJCS 2017; 5(4): 1141-1146
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 Received: 25-05-2017
 Accepted: 26-06-2017

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Deterioration in sesame (*Sesamum indicum* L.) seeds under natural and accelerated ageing

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Abstract

Laboratory studies were conducted at the Department of Seed Science and Technology, TNAU, Coimbatore to evaluate the deterioration in sesame seed under natural and accelerated ageing. Freshly harvested seeds of sesame (VRI 2) were obtained from Soil and Water Management Research Institute (Kattuthotam) were kept under accelerated ageing condition by exposing the seeds to 40°C temperature and 100 per cent relative humidity. Simultaneously, the seeds were packed in cloth bag and kept under ambient condition for natural ageing for 10 months in order to assess the physical, physiological and biochemical changes taking place during storage. However, reduction of Germination percent was coincided with the reduction of seed quality parameters. As a conclusion, three days of accelerated ageing was equivalent to ten Months of natural storage at the time maintained the Germination above Indian Minimum Seed Certification Standards.

Keywords: sesame seeds, seed storage, natural and accelerated ageing

Introduction

Sesame (*Sesamum indicum* L.) is an important oil seed crop and good source of edible oil which is often considered as the "queen" of vegetable oils. The seed contains 50 - 60 per cent oil with high variability between varieties. In India, it is cultivated over an area of 1.78 million hectares, with a production of 0.72 million tonnes and the productivity is 426 kg / ha. In Tamil Nadu, it is cultivated over an area of 0.57 lakh hectares with a production of 0.34 lakh tones and the productivity is 596 kg / ha (Source: www.indiastat.com, 2012-2013 and 2013- 2014). Quality seed is the key input for realizing potential productivity by increasing the yield upto 15-20 per cent. Seeds of oil seed crops are vulnerable to deterioration and considered as poor storer compared to pulses and cereals under ambient storage. Harrington (1972) [14] suggested that within the normal range of moisture and temperature for stored seeds, each one per cent reduction in seed moisture or each 10°F reduction in temperature doubles the storage life of the seeds. Using such "rules of thumb" and assuming that the effects are additive, it can be assumed that seed vigour would deteriorate 500 times more rapidly at 40°C and 18 per cent moisture content than it would have been at 20°C and 8 per cent moisture. Thus, accelerated ageing has been developed as a self-ageing technique. The accelerated ageing test has been used to estimate seed vigour and deterioration during storage (Delouche and Baskin, 1973) [8]. During seed ageing, physiological changes viz., delayed germination, reduced seedling growth, dry weight, vigour and biochemical changes viz., reduced activity of enzymes like catalase, peroxidase, superoxide dismutase and α -amylase were the notable events which reduce the quality of seeds (Raja, 2003 and Ramanadane, 2003) [24, 25]. Several researchers have studied the pattern of seed deterioration in relation to various physiological, biochemical and anatomical changes under ageing. The study on physical, physiological, biochemical and histological changes will be helpful for better understanding the process of deterioration under both accelerated and natural ageing conditions.

Materials and methods

Genetically pure and freshly harvested seeds sesame (VRI 2) were obtained from Soil and Water Management Research Institute (Kattuthotam) were kept under accelerated ageing condition by exposing the seeds to 40°C temperature and 100 per cent relative humidity. Simultaneously, the seeds were packed in cloth bag and kept under ambient condition for natural ageing for 10 months. The experiment was designed in Completely Randomized

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Design (CRD) with four replications. For accelerated ageing, the samples were drawn at daily interval and for natural ageing, samples were drawn at monthly intervals and analyzed for physical, physiological, biochemical and histological observations were recorded *viz.*, seed moisture content, germination per cent, root length, shoot length, dry matter production, vigour index, electrical conductivity, α -amylase, catalase, peroxidase activity, oil content and free amino acid were made. The storability period was calculated based on the time taken to reach the germination as per the IMSCS. In order to assess the pattern of deterioration, the accelerated ageing was continued when seed reaches the germination of about 50 per cent. The data collected from the various experiments were analysed statistically adopting the procedure described by Rangaswamy (2002) [26], wherever necessary, the per cent values were transformed to angular (arcsine) values before analysis. The critical differences (CD) were calculated at 5 per cent probability level. The data were tested for statistical significance. If the F test expressed is non-significant of variant it was indicated by the letter NS.

Results and Discussion

From the present investigation, control seeds recorded 5.0 per cent moisture content and it was found to be increased and reached 11.8 per cent on 9th day of accelerated ageing. In case of natural ageing, the moisture content increased and attained

9.2 per cent in 10 months stored seeds. The increase in moisture content due to the increase in relative humidity of the storage environment. This result was in agreement with Bharathi (1991) [5] in maize and Sawant *et al.* (2012) [29] in wheat which showed increasing trend of moisture content with respect to the storage period.

Deterioration in seed quality was associated with decrease in seedling quality parameters become evident with advancement of time in storage under both accelerated and natural ageing. With respect to sesame, the initial germination was recorded as 91 per cent and reached 81 per cent on 3rd day of accelerated ageing. After 9 days of accelerated ageing it was declined and showed as 44 per cent. The germination of 81 per cent was recorded which is above IMSCS (80 per cent) upto 10 months of storage. Ghassemi *et al.* (2010) [11] reported that decrease in germination per cent and other indices can be related to physiological and biochemical changes during seed ageing. Decrease in plasma membrane sustainability, changes in molecular structure of nucleic acids, decrease in enzyme activities and increase in time for germination were happened while ageing (Justice and Bass, 1979). The decline in germination over the period of storage might be due to depletion of food reserves coupled with decline in synthetic activity as reported by Pal and Basu (1988) [18] and Ravichandran (1994) [27] in paddy.



Plate 1: Physiological quality of control (fresh seeds), accelerated aged and natural aged seeds of sesame

The accelerated and natural ageing resulted significant reduction of seedling length. The root and shoot length were reduced from 0 to 9 days [(15.1cm to 5.6 cm) and (6.6 cm to 3.5 cm)]. At the same time, a decline was also observed in root length (15.1 cm to 9.6 cm) and shoot length (6.6 cm to

4.1cm) from 0 to 10 months of storage under natural ageing. Similar results of decrease in seedling length was also reported by Roy *et al.* (1994) [28] in chickpea, Kalpana and Madhav Rao (1995) [15] in pigeon pea, Preze and Arguello (1995) [22] in peanuts and Basra *et al.* (2003) [3] in cotton.

Table 1: Physical and physiological changes due to accelerated ageing of sesame seeds

Accelerated ageing (days)	Moisture content (%)	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g 10 seedlings ⁻¹)	Vigour index
0 (control)	5.0	91 (72.54)	15.1	6.6	0.025	1975
1	5.5	88 (69.73)	14.9	6.3	0.024	1866
2	5.7	85 (67.21)	13.7	5.4	0.023	1624
3	6.0	81 (64.15)	11.9	4.6	0.022	1336
4	6.1	75 (60.00)	10.6	4.5	0.021	1132
5	8.2	68 (55.55)	9.8	4.4	0.019	966
6	8.7	67 (54.94)	8.9	4.2	0.018	878
7	10.0	64 (53.13)	7.8	4.1	0.016	762
8	10.7	59 (50.18)	6.9	3.9	0.014	637
9	11.8	44 (41.55)	5.6	3.5	0.012	400
Mean	7.8	72 (58.05)	10.5	4.8	0.019	1158
SEd	0.15	1.07	0.20	0.10	0.0002	17.19
CD (P=0.05)	0.31	2.18	0.41	0.21	0.0005	35.12

(Figures in parenthesis indicate arcsine values)

When subjecting the sesame seeds to accelerated ageing, dry matter production and vigour index were drastically reduced from 0 to 9 days from 0.025 g 10 seedlings⁻¹ to 0.012 g 10 seedlings⁻¹ and 1975 to 400, respectively. A gradual decline from 0 to 10 months was observed with dry matter production (0.014 g 10 seedlings⁻¹) and vigour index (1109). Deshpande and Mahadevappa (1994) [10] observed decrease in

germinability and seedling vigour as the accelerated and natural ageing prolonged in rice. Verma *et al.* (1999) [34] also found that seedling vigour decreased with increase in age of seeds in rapeseed and mustard. Atici *et al.* (2007) [11] reported that aged seeds showed decreased vigour and produced weak seedlings that are unable to survive once reintroduced into a habitat.

Table 2: Physical and physiological changes due to natural ageing of sesame seeds

Natural ageing (months)	Moisture content (%)	Germination (%)	Root length (cm)	Shoot length (cm)	Dry matter production (g 10 seedlings ⁻¹)	Vigour index
0 (Control)	5.0	91 (72.54)	15.1	6.6	0.025	1975
1	5.3	90 (71.56)	14.7	6.5	0.024	1908
2	5.7	90 (71.56)	13.9	5.8	0.023	1773
3	6.1	90 (71.56)	13.4	5.6	0.022	1710
4	6.8	89 (70.63)	13.1	5.3	0.022	1638
5	7.1	88 (69.73)	12.7	4.9	0.021	1549
6	7.2	86 (68.02)	11.7	4.7	0.019	1410
7	7.7	84 (66.42)	11.1	4.5	0.019	1310
8	8.2	83 (65.65)	10.5	4.4	0.017	1237
9	8.7	82 (64.89)	10.2	4.2	0.016	1181
10	9.2	81 (64.15)	9.6	4.1	0.014	1109
Mean	7.0	87 (68.86)	12.4	5.1	0.020	1527
SEd	0.12	1.29	0.18	0.10	0.0003	20.38
CD (P=0.05)	0.25	2.62	0.38	0.21	0.0007	41.48

(Figures in parenthesis indicate arcsine values)

The electrical conductivity of seed leachate increased with increase in period of accelerated and natural ageing. The electrical conductivity was increased from 20.8 $\mu\text{S cm}^{-1}$ to 30.1 $\mu\text{S cm}^{-1}$ and eventually it reached 61.7 $\mu\text{S cm}^{-1}$ on 10th day of accelerated ageing. Simultaneously, in natural ageing the electrical conductivity on 10th month was 35.4 $\mu\text{S cm}^{-1}$.

Gupta *et al.* (2005) [13] reported that electrical conductivity increased after the seeds were subjected to accelerated ageing because of membrane deterioration and metabolic changes in the seed. Loss of seed vigour and viability is associated with deterioration of membrane properties (Priestley, 1986) [23].

Table 3: Biochemical changes due to accelerated ageing of sesame seeds

Accelerated ageing (days)	Electrical conductivity ($\mu\text{S cm}^{-1}$)	α -amylase (mg maltose min ⁻¹)	Catalase (m mol H ₂ O ₂ reduced min ⁻¹ g ⁻¹ seed)	Peroxidase (m mol tetra guaiacol formed min ⁻¹ g ⁻¹ seed)	Oil content (%)	Free fatty acid (%)
0 (control)	20.8	19.34	0.549	0.497	47.3	0.33
1	22.3	19.29	0.469	0.475	45.3	0.35
2	24.9	18.94	0.350	0.427	44.2	0.38
3	30.1	16.21	0.340	0.401	40.5	0.42
4	35.6	15.66	0.325	0.391	38.6	0.44
5	40.4	14.02	0.289	0.378	36.3	0.49
6	43.7	11.83	0.274	0.371	32.2	0.52
7	48.8	10.44	0.263	0.259	29.4	0.56
8	55.5	9.89	0.253	0.239	24.8	0.61
9	61.7	8.56	0.242	0.219	21.7	0.64
Mean	38.4	14.42	0.335	0.366	36.0	0.47
SEd	0.51	0.13	0.004	0.001	0.40	0.006
CD (P=0.05)	1.04	0.27	0.009	0.003	0.81	0.013

The α -amylase activity was decreased with increasing period of storage both under accelerated and natural ageing. It was reduced to 8.56 mg maltose min⁻¹ from 19.34 mg maltose min⁻¹ over the period of 9 days whereas in natural ageing it was 16.29 mg maltose min⁻¹ by 10th month of storage. It might be due to the accumulation of aldehyde compounds especially methyl jasmonate which act as potential inhibitor of α -amylase by arresting the biosynthesis of gibberellic acid which stimulates α -amylase synthesis and its secretion from aleurone layers in cereals (Norastehnia *et al.* 2007).

The catalase activity of sesame seeds was initially measured as 0.549 m mol H₂O₂ reduced min⁻¹ g⁻¹ seed and reduced to 0.340 m mol H₂O₂ reduced min⁻¹ g⁻¹ seed on 3rd day of

accelerated ageing and finally it was reached 0.242 m mol H₂O₂ reduced min⁻¹ g⁻¹ seed on 9th day of accelerated ageing. In case of natural ageing, declining trend was observed and the value was 0.397 m mol H₂O₂ reduced min⁻¹ g⁻¹ seed during 10th month of natural ageing. The peroxidase activity followed the similar trend of results as that of catalase activity under both accelerated and natural ageing. Gidrol *et al.* (1989) reported that the decreased activity of catalase and peroxidase was due to accumulation of H₂O₂ and this accumulation of hydrogen peroxide itself was detrimental to seeds. Cakmak *et al.* (2009) observed a decrease in the activities of catalase and peroxidase enzymes, but an increase in activity of superoxide dismutase in the old dry seeds of legumes during storage for

40 years. Similar results were reported by Bhanuprakash *et al.* (2010) in bell pepper, Demirkaya *et al.* (2010) in onion seeds,

Scialabba *et al.* (2002) in radish and Pallavi *et al.* (2003) sunflower.

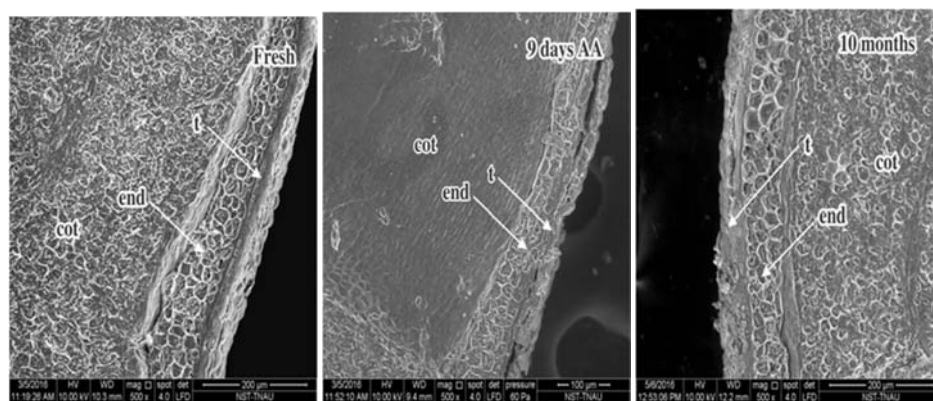
Table 4: Biochemical changes due to natural ageing of sesame seeds

Natural ageing (months)	Electrical conductivity ($\mu\text{S cm}^{-1}$)	α -amylase (mg maltose min^{-1})	Catalase (m mol H_2O_2 reduced $\text{min}^{-1} \text{g}^{-1}$ seed)	Peroxidase (m mol tetraguaiacol formed $\text{min}^{-1} \text{g}^{-1}$ seed)	Oil content (%)	Free fatty acid (%)
0 (control)	20.8	19.34	0.549	0.497	47.3	0.33
2	23.4	19.06	0.526	0.478	45.1	0.34
4	24.8	18.99	0.498	0.466	44.4	0.36
6	31.3	17.46	0.438	0.459	42.4	0.38
8	33.5	16.54	0.411	0.421	41.2	0.40
10	35.4	16.29	0.397	0.411	40.7	0.42
Mean	28.0	17.94	0.470	0.455	43.5	0.37
SEd	0.47	0.29	0.007	0.004	0.38	0.007
CD (P=0.05)	0.96	0.62	0.016	0.008	0.81	0.016

At initially, the oil content was estimated as 47.3 per cent. Thereafter, it was started to reduce and reached to 40.5 per cent on 3rd day of accelerated ageing correspondingly the value during 10th month of storage under natural ageing was 40.7 per cent. Further, it was reduced to 21.7 per cent on 9th day of accelerated ageing. An increasing trend was observed in case of free fatty acid. The free fatty acid content was same (0.42 per cent) in 3 days accelerated of aged seeds and 10 months stored seeds under natural ageing. It was increased from 3rd day onwards and observed as 0.64 per cent on 9th day of accelerated ageing. The results agreed with the findings of Narayanaswamy (2003) in groundnut, Simic *et al.* (2007) in sunflower, soybean and maize and Paramasivam and Balamurugan (2007) in groundnut. The decrease in oil content with increased free fatty acid content may be due to the

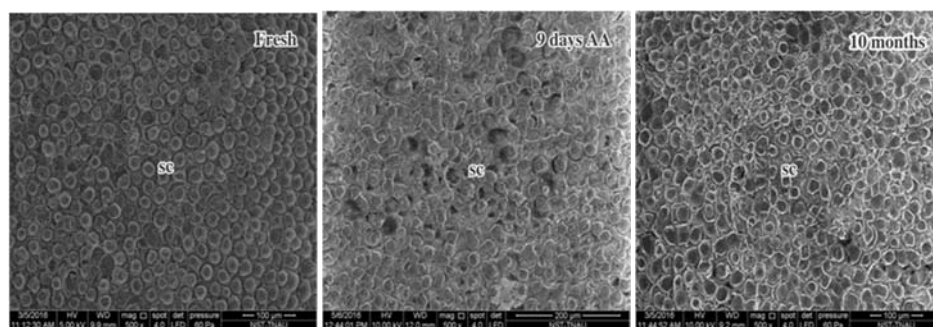
hydrolysis of storage lipids, coalescence of lipid bodies, subsequent formation of free radical that lead to lipid peroxidation (Balamurugan, 1993 and Paramasivam, 2005). The results of Sharma (1977) clearly pointed out the declining trends in total oil content and seed germination during storage of oilseed species.

The histological changes have been studied using Scanning Electron Microscope (SEM) for control, accelerated aged and naturally aged seeds. In sesame, the cell arrangement Pattern of seed coat surface, endosperm and cotyledon were found to be clear and intact in fresh seeds. Disintegration of these parts were observed in accelerated and naturally aged and disintegration was very deeper when period of storage was prolonged (9 days of accelerated ageing).



Control (fresh seeds) 9 days accelerated ageing 10 months natural ageing
t - testa, end - endosperm, cot - cotyledon

Plate 3: Longitudinal section of sesame seed viewed under Scanning Electron Microscope (SEM) - testa, endosperm and cotyledon



Control (fresh seeds) 9 days accelerated ageing 10 months natural ageing
sc – seed coat texture

Plate 4: Sesame seed viewed under Scanning Electron Microscope (SEM) - seed coat surface texture

The present results thus support the 'classical' conception of Briggs (1978) and Varner and Ho (1976) concerning the role of the aleurone layer in the production of α -amylase in barley. This result was also in harmony with the findings of Ramanadane and Ponnuswamy (2004) in rice.

Conclusion

It is summarized that, three days of accelerated ageing was equivalent to ten Months of natural storage at the time maintained the Germination above Indian Minimum Seed Certification Standards.

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