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Evaluation of Sesame, Peanut and Canola Seed Quality Using Accelerated Ageing

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ABSTRACT

Accelerated ageing (AA) has been used for predicting seed storability because it is known to reduce seed viability and vigor in many crop species. This study was undertaken to evaluate the effect of AA on germinability, physiological, chemical and biochemical characteristics in the seed of three sesame (*Sesamum indicum* L.) cultivars ('Giza 32', 'Toshki 1' and 'Shandaweel 3'), three peanut (*Arachis hypogaea* L.) cultivars ('Giza 5', 'Giza 6' and 'Ismailia') and three canola (*Brassica napus* L.) cultivars ('Pactol', 'Sirw 4' and 'Sakha 1'). AA was achieved by incubating seed at 45°C and 100% relative humidity in a closed chamber for three days. AA decreased seed germination, which was well correlated with increased accumulation of the total content of polyphenoloxidase, peroxidase and catalase enzymes. The decline in seed germination during AA was also accompanied by an increase in electrical conductivity of solute leakage, free fat acidity, lipid peroxidation, percentage of crude protein and carbohydrates, while the percentage oil decreased. The results of SDS-PAGE showed changes in the protein-banding pattern under AA. So, the results suggest that oilseed deterioration during AA is closely related to a decrease in activities of enzymatic systems due to lipid peroxidation and an increase in free fat acidity.

Keywords: *Arachis hypogaea* L., *Brassica napus* L., chemical changes, deterioration, chemical changes, *Sesamum indicum* L., SDS-PAGE
Abbreviations: AA, accelerated ageing; **EC**, electrical conductivity

INTRODUCTION

Crop production depends heavily on planting of high quality seeds. Rapid and uniform emergence is of utmost importance because it is the foundation on which stand establishment is based and potential yield is determined. Absolute longevity depends on initial seed quality, which is controlled by genetic factors such as seed structure and composition, maturity, dormancy, purity, initial viability and vigor (Justice and Bass 1978; Powell and Matthews 2005) and post-harvest treatments such as forced drying, cleaning and proper storage.

Oil seeds are sensitive to harsh environmental conditions. It is hypothesized that their oil content readily oxidizes and deteriorates seed health in storage (Wilson and McDonald 1986). These oxidative reactions occur through non-enzymatic auto-oxidation (Vertucci and Leopold 1986). These deteriorative changes decline germinability (McDonald 1976) and vigor (Copeland and McDonald 1995).

Low vigor (aged) seeds could be obtained through accelerated ageing (AA) (McDonald 1995; Tekrony 1995, 2005). AA is recognized as an accurate indicator of seed vigor and storability; it correlates with field emergence (Egli and Tekrony 1996). The seeds that deteriorate rapidly under AA generally show a marked reduction in germinability (McDonald 1999; Hampton *et al*. 2004; McDonough *et al*. 2004). The deleterious effects of AA on germination processes are associated with damage to membranes, nucleic acid and protein levels (Fujikura and Karssen 1995). AA also results in an increase in lipid peroxidation, a decrease in activities of antioxidants and several enzymes involved in scavenging free radicals and peroxide (Hsu and Sung 1997; Bailly *et al*. 1998). This is true for rice (Ray *et al*. 1990; Farooq *et al*. 2006), soybean (Trawatha *et al*. 1995), sunflower (Halder *et al*. 1983; Kausar *et al*. 2009), lettuce (Hannan and Hill 1991), cotton (Basra *et al*. 2003) and onion (Hyatt and Tekrony 2008).

This study was conducted to evaluate the underlying physiological, chemical and biochemical factors for AA in three oil seed crops (sesame, peanut and canola).

MATERIALS AND METHODS

Plant material

Seed samples of three sesame cultivars ('Giza 32', 'Toshki 1' and 'Shandaweel 3'), three peanut cultivars ('Giza 5', 'Giza 6' and 'Ismailia') and three canola cultivars ('Pactol', 'Sirw 4' and 'Sakha 1') were provided by the Oil Crops Research Department, Field Crops Research Institute, Agricultural Research Center (ARC).

Accelerated ageing test

A sub-sample of about 100 g of each sample was drawn at random and placed in one layer deep on the upper surface of a stainless steel wire mesh screen, which was fixed into a plastic box containing 50 ml of distilled water, allowing an air space of about 2.5 cm between the water surface and the bottom surface of the steel wire mesh screen. Seeds were aged at uniform 40°C and 100% relative humidity, both maintained for 3 days. Seeds were removed and three replicates, each of 50 seeds, as well as the control, were planted directly in three layers of germination paper towels as for the standard ISTA (2004) germination test.

Electrical conductivity

Twenty-five seeds per replication (three replicates) were weighed and soaked in 25 ml of deionized water at 20°C for 24 hrs. Electrical conductivity of seed leachates was measured using an elec-

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trical conductivity (EC) meter (ORION Cat. No. 012210, Thermo Electron Co.). The mean values were expressed in μS cm^{-1} g^{-1} seed weight.

SDS-protein electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was carried out according to Laemmli (1970). Protein bands were visualized by staining the gel with 0.25% Coomassie Brilliant Blue R-250. Protein band sizes were determined by comparisons with a high molecular weight protein marker. Chemicals for electrophoresis were purchased from BDH Laboratory Supplies, UK and Riedel-de Haë, Germany.

Extraction and estimation of enzymes

Seeds from each genotype imbibed for 18-h were used for the preparation of enzyme extract. 200 mg of tissue was ground in a chilled mortar and homogenized with 10 ml of 0.1 mol/L phosphate buffer (pH 7.8) containing 0.2 g polyvinylpyrolidone (PVP-40), 10 mmol/L α-mercaptoethanol, 10 mmol/L KCl, 1 mmol/L $MgCl_2$ and 1 mmol/L EDTA. The homogenate was centrifuged at 15,000 × *g* for 15 min at 4°C twice. The resultant supernatant was filtered and used for the enzyme assay. Peroxidase (PER; E.C. 1.11.1.11) activity was assayed essentially according to the method of Allam and Hollis (1972). The reaction mixture contained 0.3 ml of enzyme extract, 0.3 ml 0.05 M pyrogallol, 0.1 ml 1.0% H_2O_2 and distilled water to bring cuvette contents to 3.0 ml. The reaction mixture was incubated at 25°C for 15 min. The change in absorbance was recorded at 470 nm (Milton Roy Spectronic 1201) at an interval of 15 sec for 2 min. Catalase (CAT; E.C. 1.11.1.6) activity was estimated by the method of Aebi (1984). The reaction mixture contained 0.4 ml enzyme extract, 0.5 ml of 0.1 M sodium phosphate buffer 7.6 and complete with distilled water up to 10 ml. The reaction mixture incubated at 25°C for 15 min. The change in absorbance was read at 240 nm immediately after the addition of the enzyme extract at an interval of 15 sec for 2 min. Polyphenol oxidase (POX; E.C. 1.14.18.1) activity was determined according to the method described by Maxwell and Bateman (1967). The reaction mixture contained 0.2 ml enzyme extract, 1.0 ml of 0.2 M sodium phosphate buffer 7.0 and 1.0 ml 10^{-3} M catechol and complete with distilled water up to 6.0 ml. The reaction mixture was incubated at 30°C for 30 min. The change in absorbance was recorded at 420 nm at an interval of 15 sec for 2 min.

Chemical composition analysis

Protein, carbohydrates, and oils percentages were determined according to the procedures outlined by AOAC (2000). Percentages of protein were obtained by multiplying the obtained nitrogen percentages by a factor of 6.25 as stated by Sadasiuam and Manickam (1996). Oil percentage was determined using a Soxhlet apparatus for 12 hrs according to the AOAC methods and using petroleum ether of boiling point 40-60°C. Carbohydrate percentages were determined according to Agrawal and Dadlani (1987). Free fatty acidity (FFA), acid value (AV), and peroxide value (PV) were determined according to the official methods reported by AOAC (2000).

Data analysis

All data were statistically analyzed by the analysis of variance according to Snedcor and Cochran (1981). Differences among means were tested by L.S.D at $P = 0.05$. The resulted protein-banding patterns were analyzed in comparison to the protein marker using a computer program (Bio-1D).

RESULTS

Sesame

All three sesame cultivars had high germination (83.33-92%; **Table 1**), highest in 'Shandaweel 3' (92%) and lowest in 'Giza 32' (83.33%). The EC of seed leachates was different among cultivars and it ranged from 23.33 to 52.33 μS cm^{-1} g^{-1} (**Table 1**). Data of chemical composition analysis are presented in **Table 2**. Protein percentage ranged from 20.4 ('Toshki 1') to 26.57% ('Giza 32'). Carbohydrate percentage ranged from 11.03 ('Shandaweel 3') to 14.09% ('Toshki 1'). Oil percentage ranged from 45.53 ('Giza 32') to 57.33% ('Shandaweel 3'). The AV ranged from 0.87 ('Shandaweel 3') to 1.37 ('Giza 32'). The FFAs ranged from 0.42 ('Shandaweel 3') to 0.70 ('Giza 32'). The PV ranged from 1.60 ('Shandaweel 3') to 2.32 ('Giza 32'). POX content ranged from 2.04 ('Giza 32') to 2.38 ('Toshki 1'). PER content ranged from 3.99 ('Shandaweel 3') to 13.88 ('Giza 32'). CAT content ranged from 4.67 ('Shandaweel 3') to 8.33 ('Toshki 1').

AA had a significant effect on germination percentage, EC, enzyme contents, protein, carbohydrate and oil percentages, AV, PV and FFAs. AA reduced the germination percentage, while solute leakage of seeds (EC) increased with AA (**Table 1**). The EC significantly differed among cultivars. Maximum EC (95.06 μS cm^{-1} g^{-1}) was recorded for the aged seeds of 'Shandaweel 3', while the minimum EC (36.94 μS cm^{-1} g^{-1}) was observed in the aged seeds of 'Giza 32'. Protein and carbohydrate percentages increased in the accelerated aged seeds, while the oil percentage decreased (**Table 2**). AA increased the total content of POX, PER and CAT enzymes. The AV, PV and FFAs of accelerated aged seeds under investigation increased (**Table 2**). The SDS-

Table 1 Influence of accelerated ageing on germination, electrical conductivity and enzymes content of three sesame cultivars.

	Germination (%)	EC (μS cm^{-1} g^{-1})	POX (mg/g fresh weight)	Catalase (mg/g fresh weight)	Peroxidase (mg/g fresh weight)
Cultivars					
Giza 32 (V1)	83.33	23.22	2.04	0.05	13.88
Toshki 1 (V2)	86.67	27.11	2.38	0.08	5.54
Shandweel 3 (V3)	92.00	52.33	2.31	0.05	4.00
L.S.D. 0.05%	7.59	1.68	0.06	0.007	0.32
Treatments					
Control (C)	92.22	10.17	2.27	0.04	4.64
Ageing (A)	82.44	58.67	3.22	0.08	10.98
L.S.D. 0.05%	7.59	1.68	0.06	0.007	0.32
Interaction					
V1 x C	90.67	9.50	2.03	0.05	4.25
V1 x A	76.00	36.94	3.04	0.10	23.52
V2 x C	92.00	11.40	2.62	0.03	5.46
V2 x A	80.33	44.03	4.01	0.14	7.63
V3 x C	96.00	9.60	2.17	0.04	4.20
V3 x A	84.00	95.06	3.85	0.10	6.80
L.S.D. 0.05%	10.73	2.38	0.08	0.01	0.46
C.V.	6.75	3.80	1.94	0.09	3.22

POX: polyphenol oxidase

Table 2 Influence of accelerated ageing on chemical composition of three sesame cultivars.

	Protein (%)	Carbohydrates (%)	Oil (%)	PV (%)	AV (%)	FFA (%)
Cultivars						
Giza 32 (V1)	26.57	12.89	45.53	2.32	1.37	0.70
Toshki 1 (V2)	20.40	14.09	46.06	1.84	0.90	0.47
Shandwee 3 (V3)	25.41	11.03	57.33	1.60	0.87	0.42
L.S.D. 0.05%	0.99	0.85	1.45	0.06	0.07	0.04
Treatments						
Control (C)	19.64	11.38	54.09	1.53	0.91	0.58
Ageing (A)	28.62	13.96	45.20	2.67	1.18	0.97
L.S.D. 0.05%	0.99	0.85	1.45	0.06	0.07	0.04
Interaction						
V1 x C	21.74	11.60	53.50	1.80	1.13	0.60
V1 x A	31.40	14.19	37.56	2.73	2.60	1.20
V2 x C	17.99	11.79	54.62	1.61	0.80	0.50
V2 x A	22.82	16.39	37.54	2.20	1.89	0.95
V3 x C	19.19	10.77	54.14	1.08	0.80	0.45
V3 x A	31.64	12.53	39.53	2.13	2.01	0.91
L.S.D. 0.05%	1.41	1.21	2.05	0.08	0.19	0.06
C.V.	3.22	5.23	2.27	2.06	5.15	6.62

AV: acid value, FFA: Free fatty acids, PV: peroxide value

Table 3 Influence of accelerated ageing on SDS-PAGE of total proteins extracted from the seeds of the three sesame cultivars.

Band No.	MW (kDa)	Cultivars					
		Giza 32		Toshki 1		Shandweel 3	
		Control	AA	Control	AA	Control	AA
1	619.75	+	+	+	+	+	+
2	585.28	+	-	+	-	+	+
3	542.19	+	-	+	+	-	-
4	539.73	+	-	-	-	-	-
5	518.80	-	-	-	-	+	-
6	513.87	-	+	-	-	-	-
7	499.10	+	-	-	-	-	-
8	454.77	+	+	-	-	+	+
9	256.44	+	+	+	+	+	+
10	219.41	+	+	+	+	+	+
11	136.24	+	+	+	+	+	+
12	130.88	+	+	-	-	+	+
13	119.60	+	+	+	+	+	+
14	112.63	+	+	+	-	+	+
15	104.57	+	+	-	+	+	+
16	90.08	+	+	+	+	+	+
17	69.15	+	+	+	+	+	+
18	55.73	+	+	+	+	+	+
19	41.24	+	+	+	+	+	+
Total		17	14	12	11	15	14

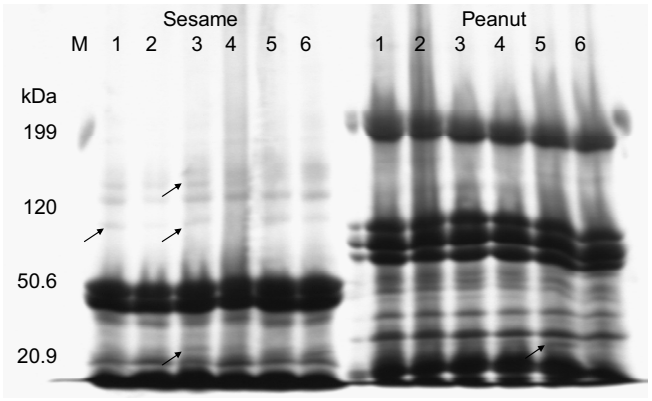

Fig. 1 SDS-PAGE of total proteins of the three sesame and three peanut cultivars under unaged and aged conditions. Left lanes 1-6 represent the sesame cultivars 'Giza 32', 'Toshki 1' and 'Shandweel 3', right lanes 1-6 represent the peanut cultivars 'Giza 5', 'Giza 6' and 'Ismailia', respectively. M refers to HMW protein marker.

PAGE for total proteins of the three cultivars (control and accelerated) is illustrated in **Table 3** and **Fig. 1**. A maximum of 19 bands were detected in the proteome of un-aged and aged cultivars with a molecular weight ranging from 41.2 to 619.8 kDa. Nine common bands (monomorphic) were detected, while 10 polymorphic bands were observed. Some abundant proteins were marked and they varied in the cultivars. Some proteins were absent as a result of AA. New proteins appeared in the aged seed. For example, bands 2, 3, 4, 5, 7 and 14 existed in the control only, while bands 6 and 15 appeared in the aged seed only.

Peanut

All three peanut cultivars had high germination (77-86.67%; **Table 4**), highest in 'Ismailia' (86.67%) and lowest in 'Giza 6' (77%). The EC of seed leachates varied among cultivars and ranged from 8.47 to 13.97 μS cm^{-1} g^{-1} (**Table 4**). Data of chemical composition analysis are presented in **Table 5**. Protein percentage ranged from 31.54 ('Giza 6') to 34.54% ('Ismailia'). Carbohydrate percentage ranged from 14.94 ('Giza 5') to 17.36% ('Giza 6'). Oil percentage ranged from 42.64 ('Giza 5') to 48.37% ('Ismailia'). The AV ranged from 0.50 ('Ismailia') to 0.73 ('Giza 6'). The FFAs ranged from 0.25 ('Ismailia') to 0.39 ('Giza 6'). The PV ranged from 1.65 ('Giza 5') to 2.65 ('Ismailia'). POX content ranged from 2.97 ('Giza 5') to 3.13 ('Ismailia'). PER content ranged from 15.40 ('Giza 5') to 19.68 ('Giza 6'). CAT content ranged from 1.10 ('Giza 6' and 'Ismailia') to 1.12 ('Giza 5').

AA had a significant effect on germination percentage, EC, enzyme contents, protein percentage, carbohydrate percentage, oil percentage, AV, PV and FFAs. AA reduced the germination percentage, while solute leakage of seeds (EC) increased with AA (**Table 4**). The EC significantly differed among the cultivars. Maximum EC (13.97 μS cm^{-1} g^{-1}) was recorded for the aged seeds of 'Giza 6', while the minimum EC (8.47 μS cm^{-1} g^{-1}) was observed from the aged seeds of 'Giza 5'. Protein and carbohydrate percentages increased for the accelerated aged seeds, while the oil percentage decreased (**Table 5**). The artificial ageing increased the total content of enzymes POX, PER and CAT. The AV, PV and FFAs of accelerated aged seeds under investigation increased (**Table 4**). The SDS-PAGE for total proteins of the three cultivars (control and accelerated) is illustrated in **Table 6** and **Fig. 1**. A maximum of 14 bands were detected in the proteome of un-aged and aged cultivars with molecular weight ranging from 40.7 to 616.06 kDa. Five common bands (monomorphic) were detected, while 9 polymor-

Table 4 Influence of accelerated ageing on germination, electrical conductivity and enzymes content of three peanut cultivars.

	Germination (%)	EC (µS cm^{-1} g^{-1})	POX (mg/g fresh weight)	Catalase (mg/g fresh weight)	Peroxidase (mg/g fresh weight)
Cultivars					
Giza 5 (V1)	81.83	8.47	2.97	1.12	15.40
Giza 6 (V2)	77.00	13.97	3.07	1.10	19.68
Ismailia (V3)	86.67	9.67	3.13	1.10	16.06
L.S.D. 0.05%	7.67	0.56	0.04	0.04	0.54
Treatments					
Control (C)	84.23	5.86	1.39	0.09	8.10
Ageing (A)	75.45	15.58	4.73	2.17	22.66
L.S.D. 0.05%	7.67	0.56	0.04	0.04	0.54
Interaction					
V1 x C	86.67	9.81	1.31	0.05	11.39
V1 x A	75.00	12.14	4.64	2.19	19.41
V2 x C	80.00	7.50	1.63	0.04	4.72
V2 x A	70.00	20.45	4.52	2.17	24.63
V3 x C	86.00	5.26	1.23	0.06	8.18
V3 x A	77.00	14.14	5.03	2.16	23.94
L.S.D. 0.05%	8.85	0.79	0.06	0.06	0.77
C.V.	5.89	4.05	1.14	3.44	2.74

POX: polyphenol oxidase

Table 5 Influence of accelerated ageing on chemical composition of three peanut cultivars.

	Protein (%)	Carbohydrates (%)	Oil (%)	PV (%)	AV (%)	FFA (%)
Cultivars						
Giza 5 (V1)	33.41	14.94	42.64	1.65	0.65	0.28
Giza 6 (V2)	31.54	17.36	44.28	1.83	0.73	0.39
Ismailia (V3)	34.54	14.95	48.37	2.65	0.50	0.25
L.S.D. 0.05%	**0.80**	**0.43**	**0.46**	0.12	**0.04**	**0.01**
Treatments						
Control (C)	26.93	13.66	45.84	1.53	0.52	0.27
Ageing (A)	39.40	17.84	40.35	2.55	0.73	0.34
L.S.D. 0.05%	**0.80**	**0.43**	**0.46**	0.12	**0.04**	**0.01**
Interaction						
V1 x C	25.98	11.91	46.39	1.01	0.57	0.28
V1 x A	40.84	17.97	38.90	2.23	0.89	0.35
V2 x C	27.62	15.89	45.82	1.51	0.56	0.30
V2 x A	35.47	18.83	42.74	2.15	0.90	0.47
V3 x C	27.20	13.18	45.31	2.07	0.56	0.22
V3 x A	41.89	16.72	43.02	3.23	0.92	0.28
L.S.D. 0.05**%**	**1.14**	**0.60**	**0.65**	0.16	**0.06**	**0.02**
C.V.	**1.88**	**2.11**	**0.79**	4.45	**5.57**	**0.003**

AV: acid value, FFA: Free fatty acids, PV: peroxide value

Table 6 Influence of accelerated ageing on SDS-PAGE of total proteins extracted from the seeds of the three peanut cultivars.

Band No.	MW (kDa)	Cultivars					
		Giza 5		Giza 6		Ismailia	
		Control	AA	Control	AA	Control	AA
1	616.06	-	+	-	-	-	-
2	579.12	-	-	-	-	+	+
3	544.65	-	+	-	-	+	+
4	533.57	+	-	+	+	-	-
5	504.02	+	+	+	+	+	+
6	452.31	+	+	+	-	+	+
7	245.38	-	-	-	-	+	-
8	142.49	-	-	-	-	+	-
9	121.75	+	+	+	+	+	+
10	104.57	+	+	+	+	+	+
11	80.96	+	+	+	+	+	+
12	70.76	+	+	-	-	-	-
13	55.73	+	+	-	+	+	+
14	40.70	+	+	+	+	+	+
Total		9	10	7	7	11	9

phic bands were observed. Some abundant proteins were marked and they varied in the cultivars. Some proteins were completely lost as a result of AA. New proteins appeared in the aged seed. For example, bands 3 and 13 existed in the aged seed only, while bands 4, 7 and 8 appeared in the control only.

Canola

All three cultivars of canola had high germination of 89.33 – 92.33% (**Table 7**). 'Sakha 1' scored a high germination percentage (92.33%), while 'Sirw 4' gave a lower germination percentage (89.33%). The electrical conductivity (EC) of seed leachates was vary among cultivars and ranged from 72.41 µS cm^{-1} g^{-1} to 113.20 µS cm^{-1} g^{-1} (**Table 7**). Data of chemical composition analysis are presented in **Table 8**. Protein percentage ranged from 24.35 ('Sirw 4') to 26.83% ('Sakha 1'). Carbohydrate percentage ranged from 12.98 ('Sirw 4') to 14.13% ('Pactol'). Oil percentage ranged from 40.67 ('Sakha 1') to 45.13% ('Pactol'). The AV ranged from 0.57 ('Pactol') to 0.84 ('Sirw 4'). The FFAs ranged from 0.27 ('Pactol') to 0.42 ('Sirw 4'). The PV ranged from 1.33 ('Sakha 1') to 1.92 ('Pactol'). POX content ranged from 0.14 ('Pactol') to 0.86 ('Sakha 1'). PER content ranged from 11.42 ('Sirw 4') to 20.85 ('Pactol'). CAT content ranged from 0.05 ('Sirw 4' and 'Sakha 1') to 0.06 ('Pactol').

It is clear that AA had a significant effect on germination percentage, EC, enzyme contents, protein percentage, carbohydrate percentage, oil percentage, AV, PV and FFAs. AA reduced the germination percentage, while solute

Table 7 Influence of accelerated ageing on germination, electrical conductivity and enzymes content of three canola cultivars.

	Germination (%)	EC (μS cm^{-1} g^{-1})	POX (mg/g fresh weight)	Catalase (mg/g fresh weight)	Peroxidase (mg/g fresh weight)
Cultivars					
Pactol (V1)	91.67	72.41	0.14	0.06	20.85
Sirw 4 (V2)	89.33	113.2	0.21	0.05	11.42
Sakha 1 (V3)	92.33	98.50	0.86	0.05	14.39
L.S.D. 0.05%	4.30	5.93	0.02	0.006	0.83
Treatments					
Control (C)	100.00	34.67	0.33	0.04	11.86
Ageing (A)	82.22	156.70	0.49	0.07	19.25
L.S.D. 0.05%	4.30	5.93	0.02	0.006	0.83
Interaction					
V1 x C	100.00	31.98	0.11	0.04	13.85
V1 x A	82.33	112.80	0.16	0.06	27.85
V2 x C	100.00	38.82	0.16	0.04	7.94
V2 x A	78.67	187.60	0.27	0.08	14.91
V3 x C	100.00	33.21	0.70	0.04	13.80
V3 x A	83.67	169.80	1.03	0.6	15.99
L.S.D. 0.05%	6.08	8.38	0.03	0.008	1.17
C.V.	3.67	4.82	0.03	0.008	4.14

POX: polyphenol oxidase

Table 8 Influence of accelerated ageing on chemical composition of three canola cultivars.

	Protein (%)	Carbohydrates (%)	Oil (%)	PV (%)	AV (%)	FFA (%)
Cultivars						
Pactol (V1)	26.40	14.13	45.13	1.92	0.57	0.27
Sirw 4 (V2)	24.35	12.98	44.87	1.69	0.84	0.42
Sakha 1 (V3)	26.83	13.95	40.67	1.33	0.77	0.38
L.S.D. 0.05%	**1.36**	**1.34**	**0.53**	**0.04**	**0.06**	**0.01**
Treatments						
Control (C)	22.58	12.15	47.88	1.28	0.67	0.33
Ageing (A)	29.14	15.22	39.24	2.01	0.98	0.40
L.S.D. 0.05%	**1.36**	**1.34**	**0.53**	**0.04**	**0.06**	**0.01**
Interaction						
V1 x C	21.37	11.40	49.27	1.63	0.50	0.22
V1 x A	31.43	16.85	40.99	2.20	0.83	0.33
V2 x C	22.04	12.47	48.12	1.09	0.80	0.40
V2 x A	26.66	14.49	41.62	2.28	1.18	0.45
V3 x C	24.33	12.59	46.23	1.11	0.70	0.33
V3 x A	29.33	15.31	35.10	2.01	0.98	0.45
L.S.D. 0.05%	**1.93**	**1.89**	**0.75**	**0.06**	**0.08**	**0.02**
C.V.	**4.10**	**7.60**	**0.94**	**2.14**	**5.46**	**0.003**

AV: acid value, FFA: Free fatty acids, PV: peroxide value

leakage of seeds (EC) increased with AA (**Table 7**). The EC significantly varied among the cultivars. Maximum EC (113.2 μS cm^{-1} g^{-1}) was recorded from the aged seeds of 'Sirw 4', while the minimum EC (72.41 μS cm^{-1} g^{-1}) was observed from the aged seeds of 'Pactol'. Protein and carbohydrate percentages increased for the accelerated aged seeds, while the oil percentage decreased (**Table 8**). The AA increased the total content of enzymes POX, PER and CAT. The AV, PV and FFAs of accelerated aged seeds under investigation increased (**Table 8**). The SDS-PAGE for total proteins of the three cultivars (control and accelerated) is illustrated in **Table 9** and **Fig. 2**. A maximum of 25 bands were detected in the proteome of un-aged and aged cultivars with molecular weight ranging from 8.17 to 405.66 kDa. Thirteen common bands (monomorphic) were detected, while 12 polymorphic bands were observed. Some abundant proteins were marked and they varied in the cultivars. Some proteins were absent as a result of AA. New proteins appeared in the aged seed. For example, bands 11, 13, 16 and 23 existed in the control only, while bands 1, 4, 6, 9, 10, 13, 14 and 17 appeared in the aged seed only.

DISCUSSION

The AA of seeds, induced by several days of exposure to high temperature and high humidity, is recognized as an indicator of seed vigor and storability (Bernal-Lugo and Leopold 1995; Hus *et al*. 2003; Torres 2005; Torres and Marcos-Filho 2005). In this study the decrease in germinability correlated well with increased electrolyte leakage (EC) (**Tables 1**, **4** and **7**), thus reflecting a loss in membrane integrity. This phenomenon may be indicative of an inability to maintain coherent membranes, resulting in losses of germinability (Chang and Sung 1998; McDonough *et al*. 2004; Meriaux *et al*. 2007). Enhanced lipid peroxidation mediated by free radicals and peroxides is considered to be one of the likely explanations for loss of seed viability during ageing (Sung 1996). One possible explanation for seed deterioration is lipid peroxidation, when membranes are perturbed (Ponquett *et al*. 1992; Chang and Sung 1998). Such alterations in the membranes of aged seeds would lead to electrolyte leakage during seed imbibition.

AA is in general, described in terms of its water content during storage. Under AA conditions oil seeds absorb more water than other seeds. This may be due to the denaturation of seed protein at high temperature and moisture levels (Krishnan *et al*. 2003). Krishnan *et al*. (2004) reported that soybean seed absorbed more moisture content than wheat seed at 45°C than at 35°C. Most workers reported that the leakage might be due to the loss of metabolic energy for membrane transport. Several workers have proposed that lipid within the membrane autoxidized or peroxidized cause membrane integrity ageing loss (McDonald 1976; Basra *et al*. 2003; Meriaux *et al*. 2007). Thus the first step in seed

Table 9 Influence of accelerated ageing on SDS-PAGE of total proteins extracted from the seeds of the three canola cultivars.

Band No.	MW (kDa)	Cultivars					
		Pactol		Sirw 4		Sakha 1	
		Control	AA	Control	AA	Control	AA
1	405.66	-	+	-	-	+	+
2	343.47	+	+	+	+	+	+
3	306.18	+	+	+	+	+	+
4	265.10	-	+	-	-	-	-
5	232.00	+	+	+	+	+	+
6	191.32	-	+	-	-	-	-
7	174.77	+	+	+	+	+	+
8	164.07	-	-	-	-	+	+
9	158.20	-	+	-	-	+	+
10	152.54	+	+	+	+	-	+
11	148.00	-	-	-	-	+	-
12	143.76	+	+	+	+	+	+
13	136.88	-	+	+	-	+	+
14	126.68	+	+	-	+	-	+
15	114.15	+	+	+	+	+	+
16	99.41	+	-	-	-	+	+
17	94.73	-	-	-	+	-	-
18	90.46	+	+	+	+	+	+
19	71.94	+	+	+	+	+	+
20	61.42	+	+	+	+	+	+
21	50.25	+	+	+	+	+	+
22	41.24	+	+	+	+	+	+
23	30.50	-	-	+	-	+	+
24	20.62	+	+	+	+	+	+
25	8.17	+	+	+	+	+	+
Total		16	20	16	16	20	21

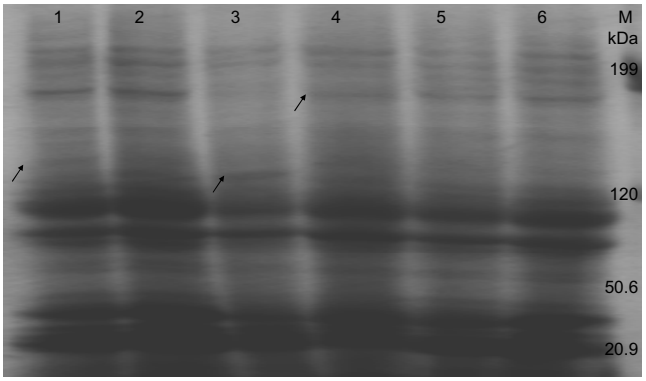

Fig. 2 SDS-PAGE of total proteins of the three canola cultivars under unaged and aged conditions. Lanes 1-6, 'Pactol', 'Sirw 4' and 'Sakha 1', respectively. M refers to HMW protein marker.

deterioration would be the loss of the membrane integrity, leading to an increase in its permeability and causing leakage of cellular solution during seed soaking (Wilson and McDonald 1986). Abdul-Baki and Anderson (1973) found that mitochondria of aged seeds are more fragile.

The AA increased the total content of POX, PER and CAT enzymes (**Tables 1**, **4** and **7**) and subsequently decreased the activities of these enzymes. Similar results were reported in peanut (Sung and Jeng 1994; Nautiyal *et al*. 1997), cotton (Basra *et al*. 2003) and sunflower (Bailly *et al*. 1998; Kausar *et al*. 2009). Cell membranes have large surface areas and a high proportion of unsaturated fatty acids, which makes the lipids particularly susceptible to peroxidative damage The AV, PV and FFAs increased with AA as compared to control (**Tables 2**, **5** and **8**). Another cause of seed viability and vigor loss during rapid ageing may be the increase in FFA level in the seeds. The hydrolysis of the ester linkage between fatty acyl chains and glycerol in seed triacylglycerols liberates fatty acids. FFA is used quite extensively as an index of seed quality. FFAs usually build up under high temperatures and high seed moisture conditions and are detrimental to normal cellular metabolism (Wilson and McDonald 1986; Basra *et al*. 2003; Meriaux *et al*. 2007). Priestley (1986) concluded that FFAs have deleterious effects on membranes, probably because they are detergents. Crowe *et al*. (1989) showed that addition of FFA increased the fusion of plant vesicles that led to an increase in membrane leakage. Generally, it can be concluded that both lipid peroxidation and FFA may contribute to seed deterioration through disruption of the membrane.

The SDS-PAGE studies provide clear differences between aged and un-aged seeds. The results showed a marked change in the banding pattern and intensity of protein (**Figs. 1-2**). This result was similar to those obtained by McDonald (1999) and Kausar *et al*. (2009), where they reported that the banding pattern of SDS-PAGE was differed between the aged and un-aged seeds through appearance and disappearance of some bands.

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