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Physiological, enzymatic, and microstructural analyses of sunflower seeds during storage

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Abstract

This study aims to investigate changes in sunflower seed (cv. BRS 122) physiology, health, structural quality, and oil quantity and distribution, under storage at different temperatures and packaging conditions. Germination, seedling emergence, accelerated aging, electrical conductivity, potassium leaching, and seed health tests were performed before storage and at 4, 8, and 12 months of storage. In addition, the water content, oil content, and enzymatic activities were determined. The seed response to storage varied with time, packaging material, and temperature. The physiological quality of the sunflower seeds was best preserved at 10°C in Kraft paper bags packaging material. *Aspergillu*s sp. and *Penicillium* sp. occurred regardless of storage conditions. In addition, the seed oil concentrations decreased with time regardless of storage conditions. However, the fatty acid profile remained the same. At four months, the superoxide dismutase, catalase, alcohol dehydrogenase, and malate dismutase activities decreased regardless of storage conditions. This decrease was more obvious in the vacuum-sealed seeds. In addition, the cellular components of the sunflower seeds deteriorated during the storage period.

Keywords: Fatty acids; *Helianthus annuus*; isoenzymes; oil; ultrastructure.
Abbreviations: ADH_dehydrogenase alcohol; C_control; CAT_catalase; cv - cultivar; M_months; MDH_malate dismutase; P_ paper; SOD_superoxide dismutase; V_vacuum.

Introduction

Sunflower is becoming a more important crop for use in oil production, human food, animal seed, and biodiesel production. The use of high-quality seeds is important for establishing sunflower crops and increasing their yields. Deterioration of seeds during storage can cause significant declines in seedling vigor and crop yield .Although a positive correlation exists between crop yield and seed oil content, obtaining and preserving the quality of sunflower seeds during storage becomes difficult, according Anandhan et al. (2010) because sunflower anthesis is not uniform from the margins to the center of inflorescence. Thus, the seeds are harvested at different maturation stages, which results in non-uniform seed lots (Munshi et al., 2003). In addition, adversities during harvest, drying, and processing operations must be considered. According to Rondanini et al. (2003) brief periods of heat stress produced sunflower grain with a lower weight, a greater percentage of pericarp, a lower oil content and an altered fatty acid composition, and hence, of lower commercial quality. Therefore, information regarding the behavior of sunflower seeds and their deterioration during storage is important for ensuring crop quality and success. Seed storage serves multiple purposes, from regulating seed trade and maintaining genetic resources in seed banks to annually supplying seeds for species that are irregularly used in production (Santos and Paula, 2007). In addition, a method for preserving seed viability that maintains reasonable seed vigor between harvest and planting is important for controlling seed quality. However, during the storage period, a series of naturally occurring biochemical and physiological events gradually reduced seed quality and result in viability loss (Ellis, 1991). These deteriorative changes result from several factors. The most important factors include environmental humidity, air temperature, pathogen incidence, the location and severity of mechanical damage, the initial seed physiological conditions, and the cultivar's genetic traits. These deterioration factors operate together and can be responsible for behavioral differences between lots that are stored under the same conditions (Carvalho and Vilela, 2006). One manifestation of deterioration during storage is the disruption of the membrane system due to the attack of free radicals on the chemical constituents of the seed (Roveri José et al., 2010). In addition, deterioration can occur when the seed mass is heated. This heat occurs when increased moisture levels result in there respiration of a seed and its associated microorganisms. Deterioration cannot be avoided. However, the degree of damage can be controlled with adequate storage.

According to Fanan et al. (2009), seeds from oilseed plants have a lower storage potential than starchy plants due to the lower chemical stability of lipids relative to starch. A moderate increase in temperature from respiration is sufficient for lipid decomposition and increased deterioration. According to Abdellahand Ishag (2012), three types of oil and fat deterioration occur during storage, including (1) oxidation, which occurs more rapidly at high temperatures and requires oxygen; (2) hydrolysis, in which the degradation of fats into fatty acids can be promoted by the growth of microorganisms in the presence of water and fatty acids; and (3) contamination, which result from chemical material residues, rain, sea water, and other contaminants.In sunflower seeds, reduced vigor and viability during accelerated aging is associated with reduced antioxidant enzyme potential and increased lipid peroxidation (Torres et al., 1997). According Corbineau et al., 2002; Kibinza et al., 2006) at the cellular level, seed ageing is associated with various alterations including loss of membrane integrity, reduced energy metabolism, impairment of RNA and protein synthesis, and DNA degradation. The characteristics of the chemical composition of oil crops seed are related to specific processes occurring in the sunflower seed during storage. Although several studies have addressed this problem, the mechanisms by which seeds lose their viability during storage remain unclear. Therefore, it is important to obtain fast and reliable deterioration results and to improve the methods for evaluating this process during the storage period. Physiological and enzymatic analyses and the assessment of different temperatures and packaging materials for preserving oilseed quality during storage along with ultramicroscopic analysis of the seed tissues could be used to address this problem. Thus, the influences of storage conditions (temperature, packaging, and time) on changes in sunflower seed (cv. BRS 122) physiology, health, and structural quality were assessed along with their oil quantities and profiles during storage.

Results and Discussion

The seed moisture during storage at different temperatures only varied by 1% (6.9 to 7.9) when stored in the paper packaging (Table 1). In the vacuum-sealed packaging, less moisture variation occurred following 12 months of storage. These results were expected because the water content varies based on air humidity when the seeds are stored in permeable packaging, such as paper, which is hygroscopic (Baudet, 2003). For seed germination analysis (Figure 1), a linear trend of reduced quality occurred regardless of the packaging material or storage temperature, except for the seeds that were stored in paper at 25°C, which decreased dramatically after four months of storage. These results correspond with the results of Grisi et al. (2007) and Abreu (2010), who concluded that the physiological quality of seeds from the Helio 250 and Helio 251 sunflower hybrids was negatively affected by increasing storage time following four months of conventional warehouse or cold chamber storage conditions.
Based on the seedling emergence results under controlled conditions (Figures 2 A and B), seed quality was affected following four months of storage in both packaging materials. However, the seeds that were packaged in paper resulted in a greater emergence percentage at all three temperatures than the seeds packaged in vacuum-sealed plastic at 25 and 30°C (Table 2).These results indicated that paper packaging could maintain sunflower seed viability for up to four months of storage. The accelerated aging test results indicated that germination decreased with time for all storage conditions (Figure 3). However, the seeds that were stored at 10°C in both packaging materials maintained greater germination rates than the seeds that were stored at 25 and 30°C. According to Sattler et al. (2004), lipid oxidation is an important factor for determining seed longevity. Some studies have shown an inverse relationship between various lipid oxidation products and seed longevity during the deterioration process. Abdellahand Ishag (2012) reported that lipid oxidation (which occurs more rapidly at higher temperatures) requires oxygen, which explains the low deterioration speed in the vacuum-sealed seeds and the greater deterioration speeds at higher temperatures (Figures 3B, D, and F). However, a lack of oxygen does not fully explain the deterioration process, which suggests that other factors, such as membrane integrity, antioxidant levels, nucleic acid and protein damage, and recovery enzyme activities (such as catalase, superoxide dismutase, and glutathione reductase) determine seed longevity. The seeds that were packaged in paper and in vacuum-sealed plastic packages at 10°C maintained their physiological properties better until the end of the storage period relative to the seeds stored at 25 and 30°C based on the accelerated aging test. Although other authors have reported that vacuum-sealed package preserve seed quality relative to conventional storage conditions (Camargo; Carvalho, 2008; Santos, 2010; 2011; Abreu et al., 2011a). This study indicated that the quality of the vacuum-sealed seeds that were stored at 25 and 30°C degraded with time. The electrical conductivity test, which assessed the selective permeability of the cell membranes, indicated that no differences occurred between the packaging type sor storage temperatures (Figure 4). In addition, Abreu (2011a) found no differences between packaging types regardless of the storage environment. However, Abreu (2011a) reported that the electrical conductivity values increased in the sunflower seeds with storage time, which corresponded to the results of this study. Several authors (Fessel et al., 2006; Panobianco et al., 2007) have agreed that electrical conductivity test results can be influenced by storage temperature. In addition, these studies have suggested that seed deterioration at low temperatures is potentially related to the loss of cell membrane integrity because membrane damage did not occur at the same intensity when stored at 10°C relative to storage at 20, 25 or 30°C. As expected, potassium leaching followed the same trend that was observed in the electrical conductivity assessment. However, ions were intentionally released by the seeds after four months of storage (Figure 4B). Based on these results, inferior seed vigor occurred after eight months of storage. This finding was confirmed by the electrical conductivity, seedling emergence, germination, and accelerated aging results. The amount of oil in the seeds significantly decreased after four months of storage, regardless of the storage conditions (Figure 5).In addition, Abreu et al. (2011) observed the same results regarding the seed oil contents of the Helio 250 hybrid during 12 months of storage. Similar results were obtained by Balesevic-Tubic et al. (2007). These authors found observed lipid autoxidation and increasing free fatty acids concentrations with storage time and indicated that these changes were responsible for rapid seed deterioration in oilseed plants, such as sunflowers (Balesevic-Tubic et al., 2007). In other hand (Beratlief and Iliescu, 1997), Martini and Anon (2005) evaluated the quality and chemical composition of the oil in the sunflower seeds that were stored at different temperatures and for different periods. These authors found that the amount of extracted oil was not significantly different between storage conditions. Similar results were obtained by Balesevic-Tubic et al. (2007). The

Table 1.Water content (%) of the sunflower seeds (BRS 122) as a function of storage duration and conditions.

Duration (months of storage)	Packaging	Storage temperature (°C)		
		10	25	30
0	Paper	7.8	7.8	7.8
	Vacuum-sealed	7.8	7.8	7.8
4	Paper	7.6	7.4	7.4
	Vacuum-sealed	7.7	7.9	7.9
8	Paper	7.3	7.1	7.2
	Vacuum-sealed	7.8	7.6	7.6
12	Paper	6.9	7.0	6.8
	Vacuum-sealed	7.0	7.3	7.1

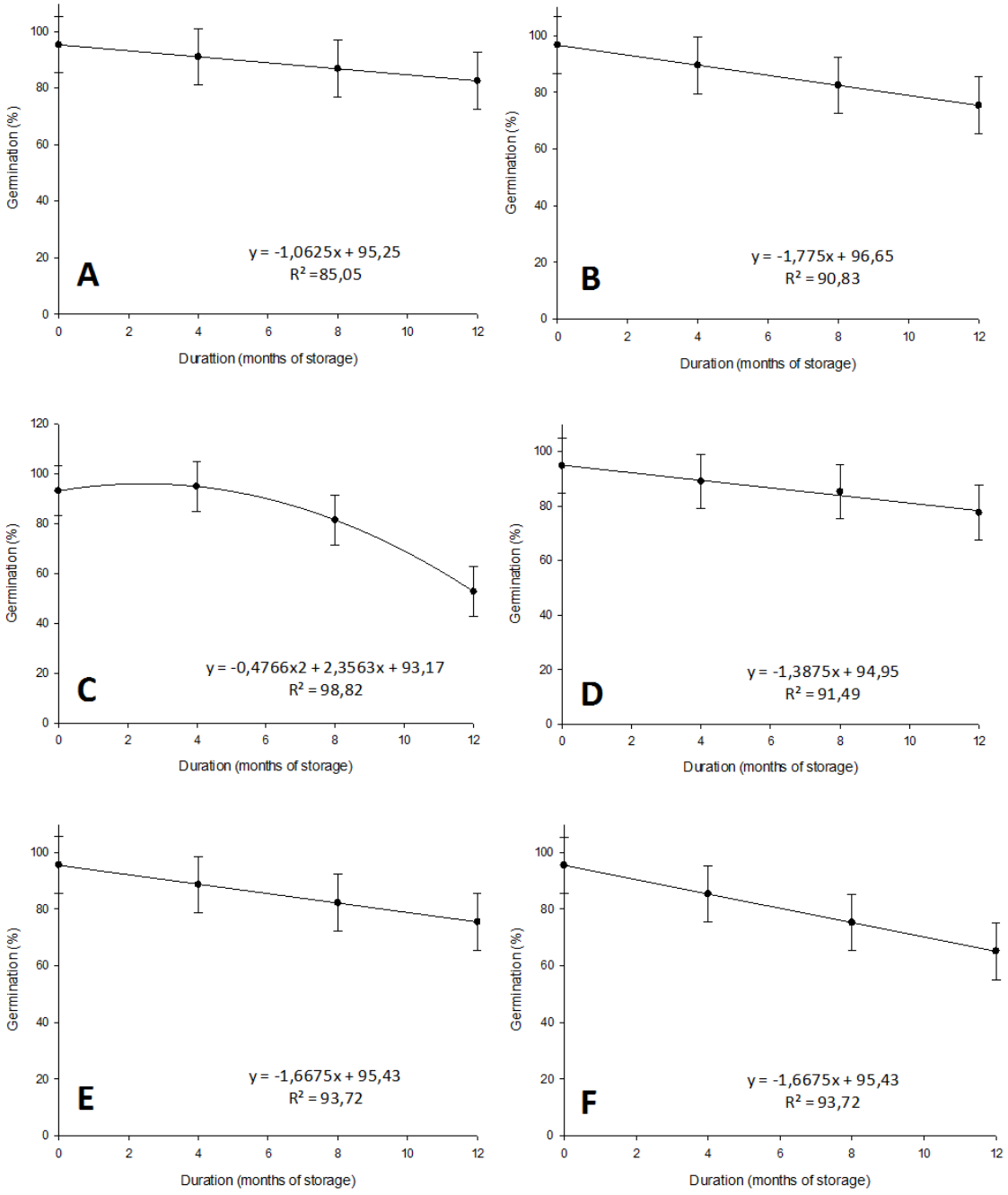

Fig 1. Germination (%) of sunflower seeds (cv. BRS 122) as a function of storage duration (0, 4, 8, and 12 months), temperature and packaging conditions. (A) paper 10°C (B) vacuum-sealed 10°C (C) paper 25°C (D) vacuum-sealed 25°C (E) paper 30°C and (F) vacuum-sealed 30°C.

fatty acid profiles (Table 3) indicated that the sunflower (cv. BRS 122) oil composition was similar to that in other species. In addition, linoleic (C18:2n6), oleic (C19:1n9), palmitic (C16:0) and stearic acids (C18:0) were prevalent and varied over a specific range (Osorio et al., 1994; Queiroga and Duran, 2010; Rodriguez et al., 2011). However, the column used in this study was able to separate trace concentrations of the following acids: eicosapentaenoic (C20:5), arachidonic (C20:4), omega 3 and omega 6.There are no reports in the literature that explain why detection or non-detection of these acids occurs in oil samples from the same species. However, when this acid is present in low concentrations, a lack of detection may result from the amounts of time in which the samples remained in the analysis column. These results were compared with the co-chromatography standard based on previous data. In addition, these sunflower seeds were found to exhibit trace concentrations of these omega acids (Dietary fat, 2011; Rail, 2011). The results of this study can be compared to the results that were obtained by Queiroga and Duran (2010). These authors found linoleic, oleic, palmitic, and stearic fatty acids in the sunflower seeds. Similarly, Abreu et al. (2011 b, c) investigated the fatty acid profiles in seeds of two different sunflower hybrids (Helium 250 and 251) with high and low oil contents. These sunflower seeds were stored in different packaging materials and environments for twelve months. In addition, the same acids that are reported in this study were identified. Changes in the fatty acid composition during storage were also observed by Crapiste et al. (1999). These authors found that the unsaturated fatty acid concentrations continuously decreased with storage. The decreasing linoleic acid concentrations were more pronounced at higher temperatures. The decreasing linoleic acid concentrations resulted in an increased conversion rate ofoleic acid to linoleic acid and suggested the preferential use of linoleic acid (18:2) in oxidation reactions. In addition, these authors stated that the oxidation rate strongly depends on the oxygen concentration and temperature during storage, and that oxidation is relatively low or absent at low temperatures with limited oxygen availability. Similar results were observed in this study after 12 months of seed storage in paper at 10 and 25°C, but not in vacuun condictions. In the seed health test, a higher percentage of contamination by *Aspergillus* and *Penicillium* sp. fungi occurred over time, regardless of the storage conditions. However, the *Alternaria* and *Fusarium* sp. fungi were also identified. Relative to the initial percentage of fungi observed at time zero, the *Aspergillus* and *Penicillium* sp. populations in the seeds that were packaged in paper at 25 and 30°C and in vacuum-sealed plastic at 30°C increased after eight months of storage. These results occurred because the ideal growth conditions of the fungi occur at seed temperatures of between 25 and 35ºC (Dhingra, 1985). These results correspond with those obtained by Abreu (2010), who observed a greater percentage of contamination by *Aspergillus* and *Penicillium* sp. under all conditions and for all sunflower hybrids (Helio 250 and Helio 251) over 12 months. In addition, Aguiar et al. (2001) studied the physical, physiological, and health qualities of different-sized sunflower seeds that were stored at 17°C for six months and identified *Alternaria* and *Penicillium* sp. in the seeds.Similarly, Gomes et al. (2008) discovered a high incidence of fungi (*Aspergillus*) in of the seeds of 12 sunflower genotypes during storage at 10°C and at 50% R.H. over 18 months. Contamination by *Aspergillus* sp. usually occurs in sunflower seeds during storage (Menten et al., 2005; Goulart, 2007). The initial effects of contamination are defined by a weakened embryo and are followed by embryo death. The seed isoenzyme systems for alcohol dehydrogenase (ADH), superoxide dismutase (SOD), catalase (CAT), and malate dehydrogenase (MDH) are shown in Figure 6. Reduced superoxide dismutase (SOD) activities (Figure 6) began four months after storage. These results are consistent with those found by Abreu (2010), who found that SOD decreased four months after sunflower seed storage (Helio 250 and Helio251) regardless of the storage conditions. Previously, Balesevic-Tubic et al. (2007) observed reduced superoxide dismutase activitie sduring the natural aging of sunflower seeds. In addition, these results were more dramatic in seeds that were subjected to accelerated aging. However, the SOD activity in the seeds is expected to decrease during storage because aging stimulates lipid peroxidation and reduces peroxide-removing enzyme activities, such as peroxidase and superoxide dismutase (Bailly et al., 1996). The catalase concentrations in the seeds decreased (Figure 6 B) during the months of storage regardless of temperature. According Kibinza et al., 2006 the activities of detoxifying enzymes, superoxide dismutase (SOD), catalase (CAT), and glutathione reductase (GR), decreased rapidly, thus leading to ROS accumulation in sunflower seed. In addition, Bailly et al. (1996) observed reduced enzyme activities associated with loss of sunflower seed viability. Furthermore, these results have been reported for cotton seeds by Goel et al. (2003). Because the catalase enzyme is involved in removing hydrogen peroxide, these results indicate that additional cell deterioration in the seeds occurs with storage time due to increased respiration. The alcohol dehydrogenase enzyme (Figure 6 D) remained active in the seeds that were stored in paper packaging until four months of storage, regardless of temperature. This activity may be attributed to the better physiological conditions of the seeds during this period, as found in the seedling emergence test. However, the enzyme concentration decreased with time, regardless of the storage conditions (except in the seeds that were packaged by vacuum-sealing and stored at10 and 30°C).However, these results are supported by the results that were observed in the seeds that were packaged in paper after four months of storage. Similarly, Santos (2010) found greater ADH activity in castor seeds (cv.IAC-226) that were stored in vacuum-sealed in a cold chamber after 8 to 12 months of storage. Abreu (2010) found greater ADH activities in Helio 251 sunflower seeds following 4 and8 months of storage in a cold chamber. No changes in the malate dehydrogenase enzyme (MDH) (Figure 6 D) concentrations were associated with the reduced physiological quality of the seed. Similar results were observed by Santos (2010) in IAC castor cultivars (80 and 226) that were subjected to different storage conditions over one year. According to Scandálios (1974), MDH is associated with a large number of organelles that may cause the stability of enzymes during storage. This trait makes it difficult to evaluate MDH as a secure marker for deteriorative changes in seeds during storage (Camargo and Carvalho, 2008; Goodman and Stuber, 1987). Although no significant damage was found in the seeds at four months of storage (based on the germination and emergence tests), the seed quality was compromised with time following four months. With ultrastructural analyses (Figures 7, 8, and 9), it was possible to test oil reduction and cellular degradation with increasing storage time, especially at eight months of storage. The reduced cell volume potentially resulted in cell rupture, which allowed the cytoplasmic components to leach and contribute to the high electrical conductivity and potassium leaching values. Following four months of storage at 10°C, no significant difference in the presence of lipid bodies was

Table 2. Mean seedling emergence (%) in sunflower seeds (cv. BRS 122) under different storage conditions (temperature and packaging). UFLA, Lavras, MG, Brazil, 2011.

Packaging	Temperature (°C)		
	10	25	30
Paper	85 aA	85 aA	84 aA
Vacuum-sealed	85 aA	82 bB	80 bC
SD= 3.18			

Means followed by the same letter (uppercase in rows and lowercase in columns) do not differ according to the Scott Knott test at a 5% probability level.

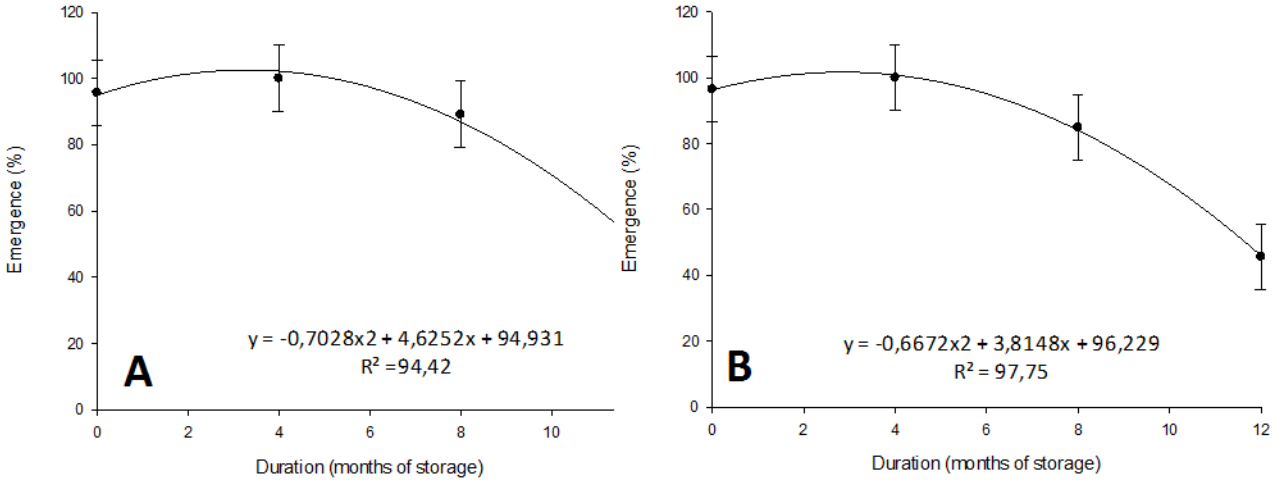

Fig 2. Emergence (%) of sunflower seeds (cv. BRS 122) as a function of storage duration (0, 4, 8, and 12 months) for packaging types. A) paper, and B) vacuum-sealed.

Table 3. Percentage composition of the main fatty acids in sunflower seeds (BRS 122) as a function of analyzed duration with different storage temperatures (10, 25, and 30°C) and packaging materials (paper and vacuum-sealed plastic).

Treatments	Fatty acids %			
	Palmitic (C16:0)	Stearic (C18:0)	Oleic (C19:1n9)	Linoleic (C18:2n6)
Control	4.60	2.61	30.40	62.40
P10°C 4 M	4.87	3.03	31.07	61.01
P25°C 4 M	4.84	2.70	29.16	63.27
P30°C 4 M	4.84	2.73	30.12	63.02
V10°C 4 M	4.83	2.93	33.06	56.98
V25°C 4 M	3.98	2.08	29.59	64.33
V30°C 4 M	2.89	1.97	24.39	53.34
P10°C 8 M	5.30	3.02	29.44	60.40
P25°C 8 M	4.48	2.14	29.38	66.13
P30°C 8 M	4.48	2.34	30.16	62.99
V10°C 8 M	4.30	1.97	26.52	67.19
V25°C 8 M	4.35	2.31	21.72	41.63
V30°C 8 M	5.16	2.95	30.18	61.69
P10°C 12 M	3.61	2.13	22.13	43.45
P25°C 12 M	3.82	2.07	23.50	43.98
P30°C 12 M	4.61	2.81	29.84	61.37
V10°C 12 M	4.30	2.43	27.34	65.44
V25°C 12 M	5.66	3.15	32.85	57.35
V30°C 12 M	3.83	1.81	30.11	64.23

P=paper, V=vacuum, M=months

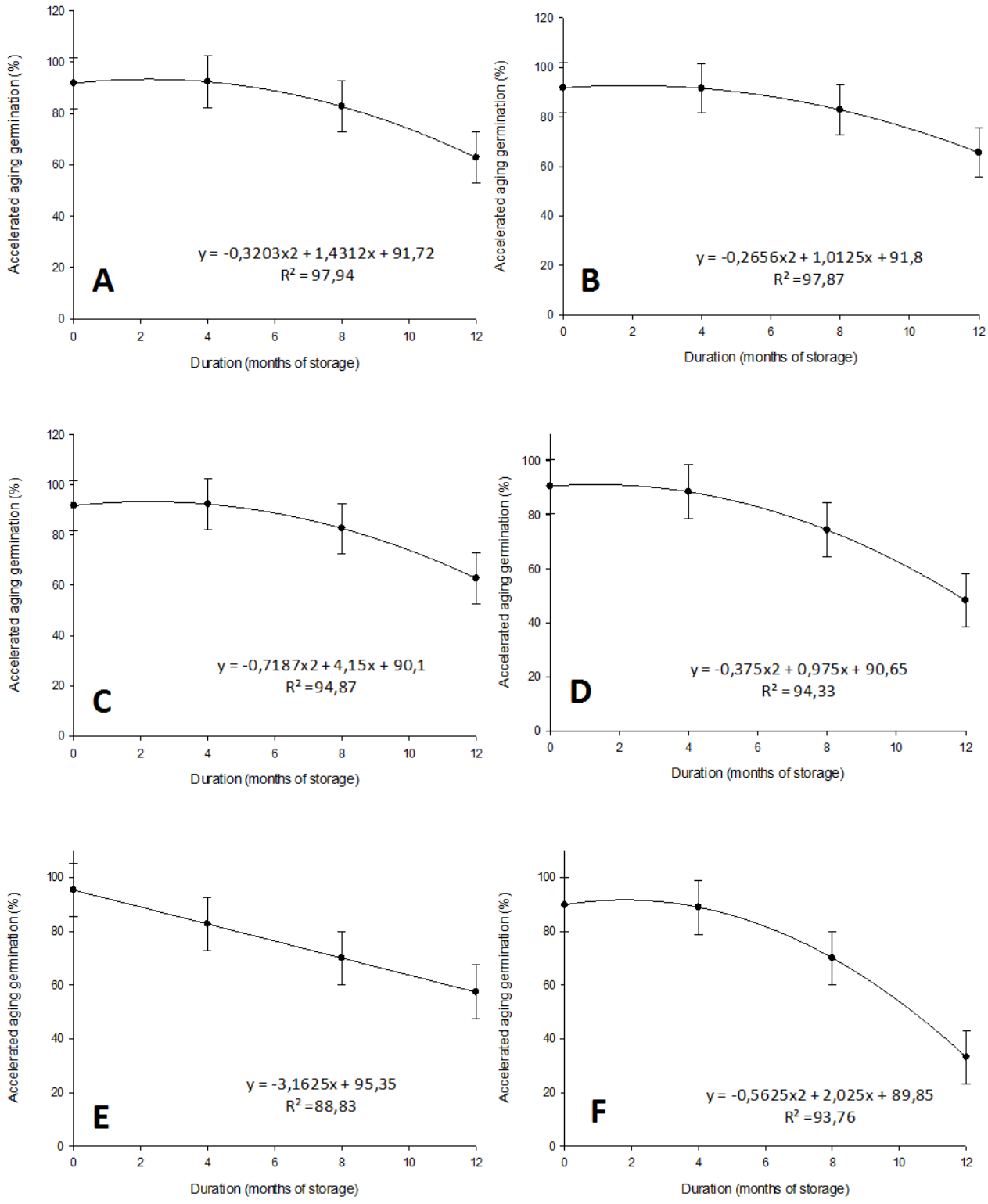

Fig 3. Accelerated aging(%) of sunflower seeds (cv. BRS 122) as a function of duration and different temperatures (10, 25, and 30°C) and packaging materials (paper and vacuum-sealed plastic). (A) paper 10°C (B) vacuum-sealed 10°C (C) paper 25°C (D) vacuum 25°C (E) paper 30°C and (F) vacuum-sealed 30°C.

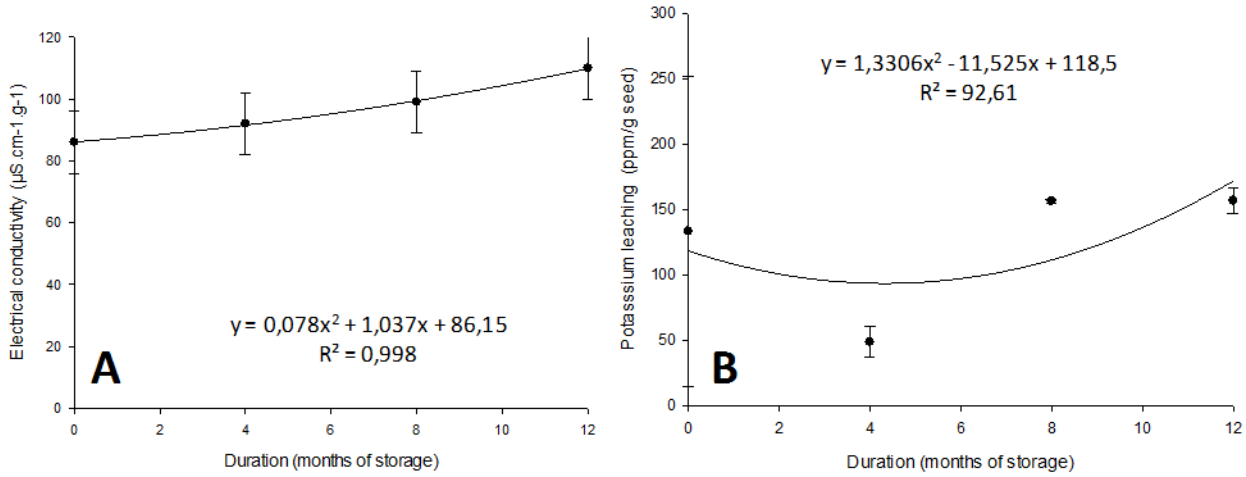

Fig 4. Electrical conductivity ($\mu S.cm^{-1}.g^{-1}$) of sunflower seeds (cv. BRS 122) as a function of storage duration and different packaging types (paper and vacuum-sealed plastic) (A). Potassium leaching (ppm/g seeds) of sunflower seeds (cv. BRS 122) as a function of storage duration, temperature (10, 25, and 30°C) and packaging type (paper and vacuum-sealed plastic) (B).

observed between the seeds that were packaged in paper or vacuum-sealed plastic (Figures 7 B and C). However, following eight months of storage at10°C, reduced oil contents, cell dryness, and cellular disorganization were observed in the seeds that were packaged in the vacuum-sealed plastic (Figures 7D and E).These results were intensified in the seeds that were stored for one year (Figures 7 F and G). At 25°C, the oil content decreased (Figure 8).However, the cells in the seeds that were packaged in the vacuum-sealed plastic following 4, 8, and 12 months of storage were dry. In the seeds stored at 30°C, cell disintegration and dryness generally increased (Figure 9). Based on the ultrastructural analysis, it was generally observed that the disintegration of the cellular components occurred. This finding was mainly observed from the electrical conductivity and potassium leaching results during the storage period. According to Taiz and Zaiger (2004), the majority of plant cells are interconnected by plasmodesmata that form a cytoplasmic continuum. These cells are responsible for solute transport. Under these experimental conditions, the ultrastructural analysis of the endosperm cells visually confirmed that cell degradation in sunflower seeds occurs during storage.

Materials and Methods

This study was conducted in the Central Seed Laboratory (*Laboratório Central de Sementes*) in the Department of Agriculture at the Federal University of Lavras (*Universidade Federal de Lavras* - UFLA), Brazil.

Plant material

Sunflower seeds (cv. BRS 122) were harvested and packaged in either Kraft paper bags trifoliate or vacuum-sealed plastic before storing at three different temperatures (10, 25, and 30°C). The vacuum-sealed packaging was performed with a vacuum pump that was set to produce a pressure of 0.1 atm.

Seed quality evaluation

Seed moisture and physiological quality were evaluated with germination tests every 4 months (over a total of 12 months of storage) according Brasil, 2009). For these germination tests, the temperature of 25°C was used with 8 replicates of 25 seeds. Emergence was controlled at a temperature of 25°C with a saturation capacity of 60% for the 2:1 mixture (soil and sand) (Nakagawa, 1999). Accelerated aging was conducted in a germination box at 42°C for 60 hours (Adamo et al., 1984). In addition, a mass electrical conductivity test was performed with a Digimed® conductivity meter (Model CD21) by soaking 50 seeds for 24 hours in 75 mL of deionized water (as recommended by Vieira and Krzyzanowski (1999). Potassium leaching was evaluated by following the method described by Dias et al. (1997). Seed health quality of seeds was evaluated by blotter test method at temperature of 25 C, incubated during 7 days as described in the Rules for Seed Analysis (Brasil, 2009). To extract oil from the seeds, the methyl esters of the fatty acids were obtained by using the method described by Hartman and Lago (1973). The lipids were extracted from the samples with a chloroform and methanol (2:1) solution after homogenization. The lipid extract was saponified with 0.5 M NaOH in methanol and subjected to esterification in an ammonium chloride, methanol, and sulfuric acid solution in a boiling water bath. After esterification, the methyl esters were extracted with hexane before evaporating under nitrogen (N_2) and storing for subsequent injection into the chromatograph. The esters were resuspended in hexane and 1 µL of the solution was manually injected into the machine. The fatty acid profile was obtained with gas chromatography by using a Shimadzu GC-17A V3 gas chromatograph that was equipped with a flame ionization detector and a DB-Wax polyethylene glycol capillary column (30 m long, 0.25 mm internal diameter, and 0.25 µm thick). Nitrogen was used as a carrier gas at a flow rate of 2.74 mL min^{-1}. The following conditions were used for chromatographic analysis: initial column temperature of 60°C (1 minute); a 30°C increase per minute until 180°C (5 minutes); a 3°C increase until 230°C (14 minutes); a split ratio of 1:20; an injector temperature of 230°C, and a detector temperature of 250ºC. The fatty acids were identified by comparing their retention times with the Pufa-2 standard (Animal Source, from Supelco) and were quantified by using area normalization. Enzyme analyses were performed by following the method described by Alfenas (2006).

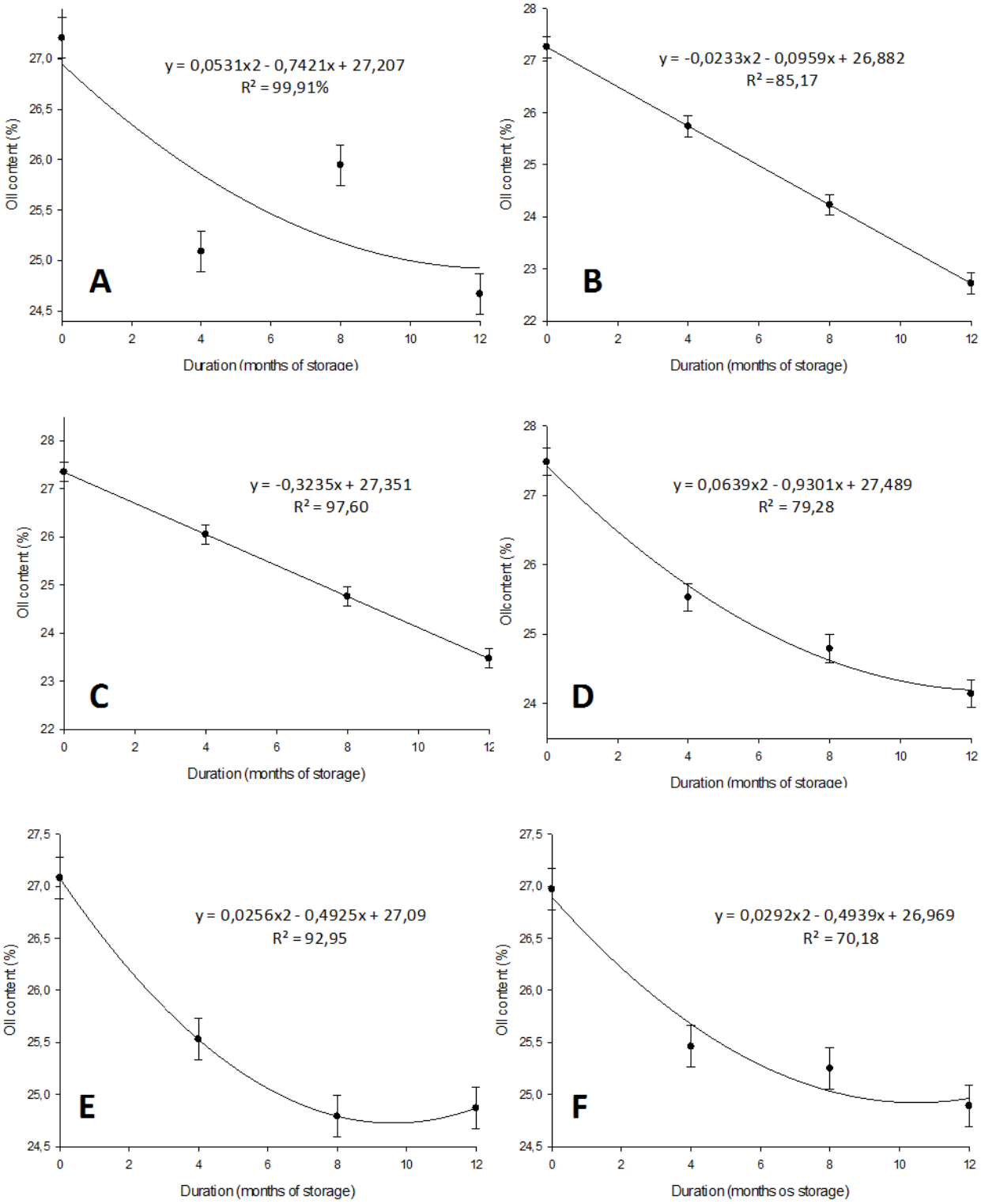

Fig 5. Oil content of sunflower seeds (cv. BRS 122) as a function of storage duration at different temperatures (10, 25, and 30°C) and in different packaging materials (paper and vacuum-sealed plastic). (A) paper 10°C, (B) vacuum-sealed 10°C, (C) paper 25°C, D) vacuum-sealed 25°C (E) paper 30°C, and (F) vacuum-sealed 30°C.

Statistical analysis

The work was carried out in a completely randomized experimental design, in a scheme of split plots, with four replications and carried out for two times. The data were subjected to regression analysis. Polynomial regression analysis was performed using the SISVAR software. (Ferreira, 2003). Each plot consisted of different temperature treatments (10°C, 25°C, and 30°C) and each sub-plot corresponded with a different packaging material (paper and

vacuum-sealed plastic) for a total of six storage conditions. The germination, electrical conductivity, potassium leaching, oil content, moisture, accelerated aging, and emergence test results were subjected to regression analyses. Based on these analyses, only the variables that significantly ($p < 0.05$) contributed to the regressions were used for constructing simple graphs that illustrated the effects of the variables.

All analyses were performed with the SISVAR program (Ferreira, 2003). The endosperm cells were analyzed with scanning electron microscopy techniques in triplicates. The seeds were transversely cut after freezing in liquid nitrogen. These sections were immersed in a fixative solution at room temperature or were refrigerated for 24 hours with the modified Karnovsky fixative (2.5% glutaraldehyde, and 2.5% formaldehyde in a 0.05 M Na cacodylate buffer at pH 7.2 and with 0.001 M CaCl2). The samples that were pre-fixed in aldehyde were washed three times for 10 minutes in a 0.05 M cacodylate buffer solution before immersing in a solution of 1% osmium tetroxide (OsO_4) in 0.05 M cacodylate buffer at pH 7.2 for one hour. After dehydrating, these materials were transported to the critical point apparatus before gold sputtering and visualizing under a microscope. All generated images were digitized and analyzed at different magnifications. Images were selected and processed with the Corel Draw X4 Photopaint software.

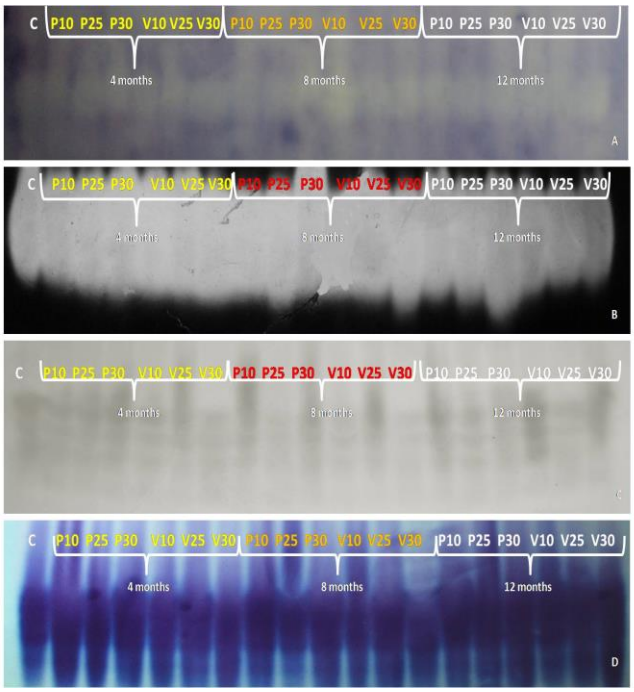

Fig 6. Isoenzyme patterns of sunflower seeds, (BRS 122) subjected to different storage durations (0, 4, 8, and 12 months) and conditions (P10°C, 25°C, and 30°C, V 10°C, 25°C, and 30°C) revealed for enzymes: (A) superoxide dismutase (SOD), (B) catalase (CAT), (C) dehydrogenase alcohol (ADH), and (D) malate dismutase (MDH). C=control, P=paper, V=vacuum.

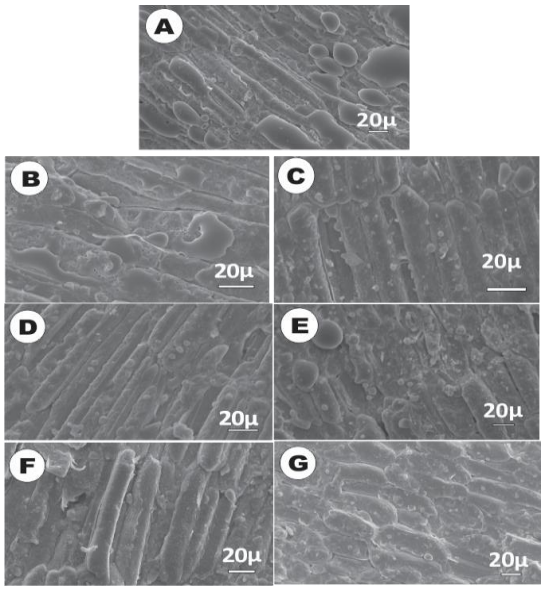

Fig 7. Scanning electron micrograph of sunflower seeds (BRS 122) packaged in paper and vacuum-sealed packaging and stored at 10°C. (A) before storage (control), (B) paper/4 months of storage, (C) vacuum-sealed/4 months of storage, (D) paper/8 months of storage, (E) vacuum-sealed/8 months of storage, (F) paper/12 months of storage, and (G) vacuum-sealed/8 months of storage.

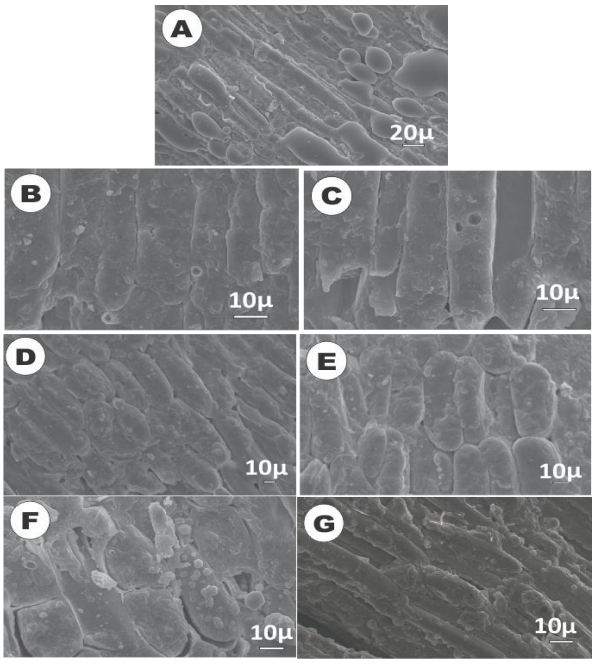

Fig 8. Scanning electron micrograph of sunflower seeds (BRS 122) packaged in paper and vacuum-sealed packaging and stored at 25°C. (A) before storage (control), (B) paper/4 months of storage, (C) vacuum-sealed/4 months of storage, (D) paper/8 months of storage, (E) vacuum-sealed/8 months of storage, (F) paper/12 months of storage and (G) vacuum-sealed/8 months of storage.

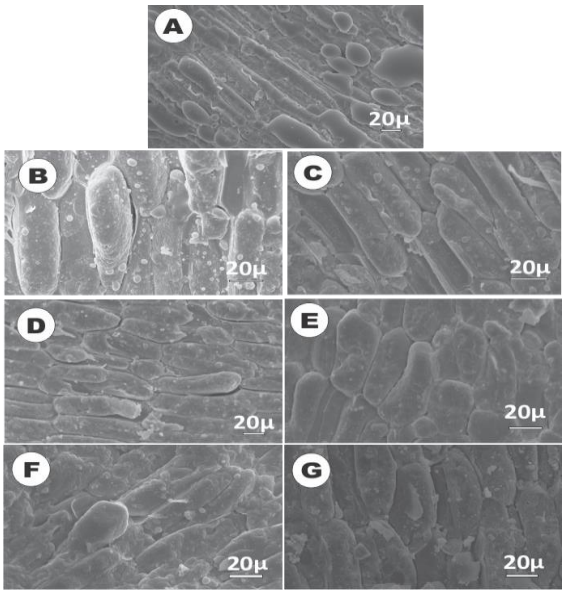

Fig 9. Scanning electron micrograph of sunflower seeds (BRS 122) packaged in paper and vacuum-sealed packaging and stored at 30°C. (A) before storage (control), (B) paper/4 months of storage, (C) vacuum-sealed/4 months of storage, (D) paper/8 months of storage, (E) vacuum-sealed/8 months of storage, (F) paper/12 months of storage and (G) vacuum-sealed/8 months of storage.

Conclusions

The physiological quality of the sunflower seeds is maintained for up to four months of storage , either Kraft paper bags trifoliate or vacuum-sealed plastic before storing at three different temperatures (10, 25, and 30°C). Following four months, the quality of the sunflower seeds is adversely affected. Seed deterioration can be observed by the reduced oil content, the disintegration of cellular components, and from the analysis of the dehydrogenase, superoxide dismutase, catalase and malate dehydrogenase enzymes. The vacuum condition affects negatively the quality of seed stored at temperature of 25 and 30^0C.

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