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Effect of Accelerated Aging Process on Seed Quality and Biochemical Changes in Sweet Pepper (*Capsicum annuum* Linn.) Seeds

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Abstract: Seed deterioration during storage is a complex physiological and biochemical process leading to loss of germination ability. This study focuses on seed germination and biochemical changes during an artificially accelerated aging process in sweet pepper (*Capsicum annuum* Linn.) seeds. The sweet pepper seeds were incubated at 42°C and 100% relative humidity for 0, 5, 10, 15, 20, 25 and 30 days. The results showed that germination ability in terms of percentage of Emergence Radical (ER%) decreased and exhibited significantly different levels ($p<0.01$) with in all different accelerated aging times. The critical period for rapid decrease in percentage of ER is 20 days of aging time. The electrolytes leaked K^+, Na^+, Ca^{2+} and Mg^{2+}, including Electrical Conductivity (EC) were determined in 24 h soaked seed solution with distilled water. The EC, K^+, Na^+, Ca^{2+} and Mg^{2+} concentrations were decreased during 5 to 10 days of aging time and increased in soaked seed solution during 10-30 days of aging time. The decrease in germination ability was well correlated with increase in membrane deterioration, as assayed by electrical conductivity and electrolyte leakage in soaked seed. Malondialdehyde (MDA) was the major product of lipid peroxidation which its concentration was rapidly increased in sweet pepper seed from 0 to 75 mg g^{-1} within 10 days of accelerated aging time. This phenomenon was associated with an increase in total antioxidant activity when aging was carried on 0-10 days. The peroxidation reaction of fatty acid in the cell impact in germination ability and some biochemical parameters related to membrane deterioration and loss of membrane integrity.

Key words: Sweet pepper seed, accelerated aging, emergence radical, electrolyte leakage, peroxidation product, malondialdehyde, antioxidant

INTRODUCTION

Sweet peppers are bell-shape vegetables that are the source of nutritional benefits for health. The value of both sweet pepper and sweet pepper seed has high cost that are 4-7 and 400-700 $ kg^{-1}, respectively. Seed quality could be evaluated by testing seed germination and seed germination index in both laboratory scale and green house. On the other hand, the biochemical changes during seed deterioration such as chromosome aberrations and damage to the DNA, changes in the synthesis of RNA and protein, changes in enzymes, differences in respiratory activity caused by ATP production and membrane alteration are not completely understood (Kerter *et al*., 1997). Many researchers have reviewed the membrane alteration because the cell membrane was the first part of the cells to interact with the environments and suggested that lipid oxidation of cell membrane might underlie loss of seed viability (Kaloyereas, 1958; Spano *et al*., 2006). Many research analyzed phospholipid changes and raised the possibility that membrane peroxidation was associated with aging (Randhir and Shetty, 2005; Kerter *et al*., 1997). A Reactive Oxygen Species (ROS) is a major cause of lipid peroxidation on unsaturated fatty acids of cell membranes (Niakan and Saberi, 2009). These changes are associated with decreases in unsaturated fatty acids and induced more membrane damage and electrolyte leakage (Van-Pijlen *et al*., 1995).

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The hydroxyl radical is a very strong oxidant and can initiate radical chain reaction with organic molecules, particularly with polyunsaturated fatty acids in membrane lipids. The peroxidation of an unsaturated fatty acid is represented in Fig. 1. Initiation of the reaction sequence involves the reaction of the hydroxyl radical with the methylene group (-CH_2-) adjacent to a double bond of unsaturated fatty acid (RH) to give lipid free radical (R•) and peroxy radical (H•). The lipid free radical reacts with molecular oxygen to give a lipid peroxy radical (ROO•) which may attack another lipid and in so doing produce a lipid hydroperoxide (ROOH) as well as initiate a new lipid radical (R•) and hence produce an autocatalytic reaction. The lipid hydroperoxide is further decomposed to wide varieties of peroxidation products the main one of which is Malondialdehyde (MDA) (Forman and Fisher, 1981).

Almost all organisms are well protected against free radical damage by antioxidant. When the mechanism of antioxidant protection becomes unbalanced by the deterioration of cell, oxidation can occur which result in accumulation of free radical. The antioxidant is important to find compounds that prevent oxidation (Khanahmadi *et al*., 2010).

Seeds storage at ambient condition is a problem, particularly, the high temperature and high humidity in tropical regions. The rate of deterioration of seeds depends on storage condition such as temperature, atmospheric moisture and oxygen concentration (Walters *et al*., 2005). High temperature and moisture content increase the respiration of seeds, causing seeds to deteriorate more rapidly (Goela *et al*., 2003).

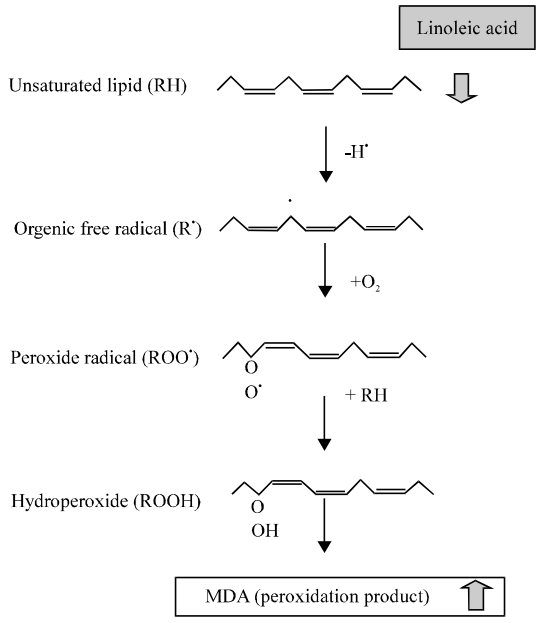

Fig. 1: Peroxidation reaction of polyunsaturated fatty acids resulting in MDA production

However, seed deterioration under natural condition takes a long time to affect the seed qualities, the accelerated aging was brought about relatively rapidly by subjecting seeds to elevated temperatures (40-50°C) at high relative humidity (up to 100%). The artificial accelerated aging is useful for lowering viability quickly for experimental purposes. Hence, in the present study, we investigated the seed germination, electrolyte leakage, fatty acid concentration, peroxidation products and axtioxidant activity in different artificially accelerated aging time (different seed quality levels). Any association among those parameters would pave the way to understanding membrane alteration impact on seeds deterioration during storage.

MATERIALS AND METHODS

Study area: Germination test was performed at Seed Processing Plant Laboratory, Faculty of Agriculture, Khon Kaen University, Thailand. All biochemical determinations were analyzed at Department of Biotechnology, Faculty of Technology, Khon Kean University, Thailand. This research study was done from January 2008 to September 2010.

Sweet pepper seed preparation

Seed material: Hybrid sweet pepper seeds TPP004 (*Capsicum annuum* L.) were purchased from AG Universal Company, Khon Kaen, Thailand.

Accelerated aging of sweet pepper seeds: Seeds were artificially aged in an accelerated aging chamber by incubating the seeds in closed plastic boxes with 100% relative humidity (R.H.) at 42°C for 0, 5, 10, 15, 20, 25 and 30 days. The seeds with different aging times were dried at 32°C for 3 h (Kerter *et al*., 1997; Van-Pijle *et al*., 1995; Walters *et al*., 2005) and were kept for determinations at 15°C.

Seed germination and biochemical determination

Germination test in laboratory: Three replicates of one hundred seeds from each aging condition were incubated on wet paper towels at 24±1°C. Seed Emergence Radical (ER), which was 0.5 cm radical protrusion, was counted from germinate seed on 1-14 days. Percentage of seed germination was calculated as described in International Seed Testing Association (Boonsiri *et al*., 2007) and showed in equation:

$$\text{Percentage of seed emergence radicle} = \frac{\text{No. of germinated seeds}}{\text{Total of seed used in germination}} \times 100$$

Determination of Electrical Conductivity (EC), potassium ion (K^+), sodium ion (Na^+), calcium ion (Ca^{2+}) and Magnesium ion (Mg^{2+}) leakage from aged seeds: Three replicates of one hundred seeds from each aging condition were soaked in the bottle containing 50 mL deionized water and kept in the incubator at 20°C for 12 h. Soaked solution were used to determine the electrical conductivity and electrolyte leakage.

Determination of Electrical Conductivity (EC) from aged seeds: Soaked solution was used to determine the electrical conductivity by a microprocessor conductometer model LF3000, WTW, Germany. The electrical conductivity units were expressed in m sec/cm/g of seed.

Determination of potassium ion (K^+), sodium ion (Na^+), calcium ion (Ca^{2+}) and Magnesium ion (Mg^{2+}) leakage from aged seeds: Soaked solution was filtered by a membrane filter then 2 mL of 98% nitric acid and 0.5 mL 5% strontium chloride were added. The potassium (K), sodium (Na), calcium (Ca) and magnesium (Mg) were analyzed by Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer model AAS-3110, USA). Electrolyte concentrations in soaked solution were expressed in milligram per gram of seed. Percentage of electrolyte leakage from soaked seeds was calculated as percentage of electrolyte leakage in soaked solution from total electrolyte contained in seeds.

Determinations of fatty acids in aged seeds by Gas Chromatography (GC)

Lipid extraction: One gram of aged seed was crushed to fine powder in a grinder and was put into a cellulose thimble tube. Lipid was extracted from the sample using an automatic lipid extractor (Buchi SW T2, model B-811, Switzerland). Soxhlet standard extraction program was set as described in lipid extractor Buchi SW T2.

Preparation of standard solutions: Stock standard solutions of palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid were prepared at a concentration of 100 mg L^{-1} in methylene chloride. Calibration curve of palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid were prepared from the stock standard in range 1.0-5.0, 1.0-5.0, 1.5-6.0, 1.5-6.0 and 1.5-6.0 mg L^{-1}, respectively. All working standard solutions were prepared in fatty acid methyl ester before injecting into GC.

Fatty Acid Methyl Ester (FAMEs) preparation: Eighty milligrams of samples or standards were mixed with 4.0 mL 0.5 N methanolic sodium hydroxide in a 50.0 mL test tube and incubated at 95°C for 6 min in a water bath. The solution was cooled at room temperature and then 5.0 mL of 14% boron trifluoride (BF_3) in methanol was added and incubated at 95°C for 6 min in the water bath. After the solution was cooled at room temperature, 10.0 mL of deionized water were added into the test tube. The FAMEs was extracted by 4.0 mL n-hexane. The n-hexane layer was separated and passed through 0.1 g anhydrous sodium sulfate to eliminate water from n-hexane (Boonsiri *et al*., 2007; Walters *et al*., 2005).

Gas chromatography analysis: The fatty acid methyl ester was analyzed by Gas Chromatography (GC) followed the published methods of Ganzera *et al*. (1999) with slight modifications. GC analysis was performed on a Varian, model GC 3600 and an auto-sampler-8200, Palo Alto, CA, USA using a FUSED SILICA capillary column 0.25 mm ID, 100 m in length, 0.2 μm film thickness; SUPULCO (Col: 32901-04, USA), coupled to a flame ionization detector. The temperature of injector and detector were adjusted to 240 and 260°C, respectively. Ten microliters of each sample was injected on the column at the split rate of 1:50 with a helium carrier gas at a flow rate of 2.0 mL min^{-1}.

The separation temperature began by holding at 100°C for 5 min. Then, the temperature was raised from 100 to 175°C at 8°C min^{-1}, holding at 175°C for 10 min before rising from 175 to 215°C at 10°C min^{-1}, holding at 215°C for 10 min before rising to 220°C at 4°C min^{-1} with a final holding at 220°C for 10 min (total running time of 56.62 min). Gas chromatograph peaks were identified from the calibration curve of pure standard fatty acid methyl esters with respect to retention times (Orhan *et al*., 2002).

Determination of malondialdehyde (MDA), total peroxide and antioxidant activity: One hundred milligrams of seeds were hand-homogenized using a mortar and pestle with 4.0 mL of 5% (w/v) Trichloroacetic Acid (TCA) at 4°C to precipitate proteins and then centrifuged at 14,000 rpm for 20 min. The supernatants were separated and used for MDA and total peroxide determination (Yeh *et al*., 2005).

MDA determination: MDA was determined by adding 0.8 mL of 20% (w/v) trichloroacetic acid in 0.5% (w/v) thiobarbituric acid to 0.2 mL of extracted sample (supernatant) and 3.0 mL of distilled water. The reaction was carried out at 95°C for 30 min and then terminated by soaking in ice-cold water and monitored by spectrophotometer at 532 nm (Heath and Packer, 1968). Stock standard solutions of MDA were prepared at a concentration of 100 mg L^{-1}. The MDA was determined from the calibration curve in the ranges 2-10 mg L^{-1}.

Total peroxide determination: Total peroxide was determined by adding 0.26 mL of reaction medium containing 10 mM ferrous ammonium sulfate, 2.5 mM potassium thiocyanate, 50% (w/v) TCA to 10 μL of extracted sample (supernatant) and 3.7 mL of distilled water. The reaction was monitored by the spectrophotometer at 480 nm. Stock standard solutions of MDA were prepared at a concentration of 1,000 mg L^{-1}. The total peroxide was determined from the calibration curve in the ranges of 100-500 mg L^{-1} (Sagisaka, 1976).

Antioxidant activity determination: Antioxidant activity was determined as the percentage scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radicals (Khanahmadi *et al*., 2010; Blois, 1958). Antioxidant compounds can reduce the stable free radical DPPH and change the color of the solution from violet to pale yellow, resulting in a reduction of absorbance at 517 nm. The total antioxidant activity was determined by adding 0.1 mL of sample solution, 1.9 mL of deionized water and 2.0 mL of 0.1 mM of DPPH. The reaction was carried out at room temperature for 30 min and monitored by the spectrophotometer at 517 nm. Stock standard solutions of gallic acid were prepared at a concentration of 100 mg L^{-1}. The total antioxidant was determined from the calibration curve of gallic acid in range of 10-50 mg L^{-1}. Antioxidant units were expressed as milligram of gallic acid equivalent per milliliter (mg GE mL^{-1}).

Statistical analysis: All treatments were determined by three replicates of biological sample. Data were statistically analyzed by ANOVA and the significance of the differences between means at $p<0.001$ was estimated by Duncan's new multiple range test (DMRT).

RESULTS AND DISCUSSION

Seed germination and biochemical changes during accelerated aging process

Effect of accelerated aging on seed germination: Seed germination was determined in term of percentage of seed Emergence Radical (ER). The seven levels of seed quality were 99±1.00, 96±1.00, 85±1.00, 81±1.52, 70±2.00, 20±1.00 and 0±0.00% ER when seeds were accelerated aging on 0, 5, 10, 15, 20, 25 and 30 days, respectively (Table 1). Those seven levels exhibited significant difference at $p<0.001$ of seed quality. The explanation for seed deterioration is that high temperature and moisture content reduce the seed quality and these parameters are the factors to predict the life spans of seeds (Roberts *et al*., 1973). Moreover, the reactions involved in seed aging are controlled by the thermodynamic status of water (Walters, 1998). Finally, high seed moisture and high temperature further accelerate the seed deterioration (Joao-Abba and Lovato, 1999) support present finding. Seed deterioration results in delayed germination, slower seedling growth rates, abnormal growth, decreased tolerance to adverse conditions and finally loss of germination ability. At this point, germination may be delayed until repair is completed, or the damage may be too extensive for repair to be effective.

Table 1: Effect of accelerated aging process on percent emergence radicle (ER) of sweet pepper seed at different aging time

Time of aging (days)	Emergence radicle (%)
0	99±1.00^{a}
5	96±1.00^{b}
10	85±1.00^{c}
15	81±1.52^{d}
20	70±2.00^{e}
25	20±1.00^{f}
30	0±0.00^{g}

Values are Mean±SD of three replications; Means within a column not showing the same letter were significantly different at $p<0.001$ by DMRT ($n = 7$)

The percentage of ER was grouped according to the rate of deterioration which was 0-20 and 20-30 days of accelerated aging. First group (during 0-20 days) was slowly decreased and the second group (during 20-30 days) was rapidly decreased. The zero percentage of ER occurred after 30 days of accelerated aging time. Change in germination rate could be explained in the mean of the biological change in cells to protect cell damage. The slow reduction in germination rate of the first group may be due to the impairment of metabolic processes caused by membrane aberrations and the need for repair mechanisms to take place in order to compensate for the accumulated damage. However, after 20 days of aging seeds, cells cannot tolerate the severe biochemical changes that why seed germination decreases very quickly. At this point, repair mechanisms in the cell had low ability to recover any biochemical changes in the cell. The 70% ER (20 days of accelerated aging time) is the critical point. It seems like that the percentage of ER lower than this point the defense mechanism system could not compensate seed deterioration.

Effect of accelerated aging process on electrolyte leakage in sweet pepper seed: Membrane deterioration of the seed was assayed by electrolyte leakage from soaked seed in deionized water. The EC, K^{+}, Na^{+}, Ca^{2+} and Mg^{2+} concentrations were decreased during 5 to 10 days of aging time and increased in soaked seed solution during 10-30 days of aging time. At 30 days of accelerated aging time, K^{+}, Na^{+}, Ca^{2+} and Mg^{2+} concentration were 2556±0.10, 852±0.02, 284±0.02 and 95±0.00 μg g^{-1} of seed, respectively (Table 2).

Table 2: Effect of accelerated aging process on electrolyte leakage from sweet pepper seed at different aging time in

Time of aging (days)	Electrical conductivity ((ms cm^{-1}) g^{-1} of seed)	Electrolyte leakage (μg g^{-1} of seed)			
		K^+	Na^+	Ca^{2+}	Mg^{2+}
0	154±5.89^e	1332±1.34^e	444±0.44^d	148±0.15^e	49±0.05^c
5	144±2.57^d	1218±0.10^f	406±0.03^d	145±0.01^e	45±0.00^c
10	127±3.81^d	1382±0.10^e	461±0.01^d	154±0.01^d	51±0.00^c
15	144±9.86^d	1403±0.10^d	468±0.01^d	156±0.01^d	52±0.00^c
20	157±21.60^c	1661±0.08^c	554±0.02^c	185±0.01^c	62±0.00^b
25	191±6.84^b	2013±0.09^b	671±0.02^b	224±0.01^b	75±0.00^b
30	222±23.70^a	2556±0.10^a	852±0.02^a	284±0.02^a	95±0.00^a

Values are Mean±SD of three replications, Means within a column not showing the same letter(s) were significantly different at $p<0.001$ by DMRT ($n = 7$)

Table 3: Effect of accelerated aging process on percentage of electrolyte leakage from sweet pepper seed at different aging time in

Time of aging (days)	Electrolyte leakage (%)			
	K^+	Na^+	Ca^{2+}	Mg^{2+}
0	39±0.23^g	55±0.06^b	24±0.22^e	2±0.03^c
5	43±0.39^f	47±0.11^d	22±0.12^f	2±0.05^c
10	46±0.67^e	47±0.22^d	26±0.20^e	2±0.18^c
15	53±0.11^d	52±0.16^c	25±0.12^d	2±0.12^c
20	54±0.23^c	51±0.12^c	26±0.32^e	3±0.09^b
25	58±0.55^b	56±0.23^b	28±0.17^b	3±0.06^b
30	62±0.20^a	64±0.12^a	34±0.35^a	4±0.19^a

Values are Mean±SD of three replications, Means within a column not showing the same letter(s) were significantly different at $p<0.001$ by DMRT ($n = 7$)

Table 3 showed the percentage of K^+, Na^+, Ca^{2+} and Mg^{2+} leakage from the soaked seed. The percentage of K^+ and Ca^{2+} leakage from seed were increased from 0-30 days of accelerated aging time. The percentage of Na^+ leakage from aged seed decreased after 5 and 10 days of aging time and then increased during the aging time from 15-30 days. However, Mg^{2+} leakage from soaked seed was not substantially changed at all accelerated aging times. Differences in pattern of the percentage of K^+, Na^+, Ca^{2+} and Mg^{2+} leakage from soaked seed was proposed that the cell membrane damage in different seed qualities impacted the low ability of transporting protein in cell membrane to control the electrolyte transportation. Although electrolyte leakage is apparently a measure of the loss of semipermeability of the plasma membrane, it might be possible that phase transition of membrane fatty acids from fluid liquid-crystalline to a solid-gel state is the primary event that affects the transport protein (Boonsiri *et al*., 2007). After the primary event, membrane lipids further degraded at the phospholipid bilayer by enzymes such as lipidperoxygenase (LOX) or the sequencial reaction of lipid peroxidation by ROS (Torres and Andrews, 2006) and cause a loss in membrane integrity. However, our results support the possibility that electrolyte leakage from soaked seed cause from the impairment of transporting protein on membrane.

The deterioration of membranes which probably involves lipid peroxidation and associates free radical oxidative stresses, leads to membrane leakage. The leakage of ions, amino acids and sugars is a clear sign of membrane deterioration that results in greatly increased permeability (Spano *et al*., 2006). This free radical induced non-enzymeatic peroxidation has the potential to damage the membrane. It is also the major cause of electrolyte leakage and the decrease in seed germination (Kaewnareea *et al*., 2008). However, present results revealed that transporting protein instead of lipid bilayer permeability caused an increase in electrolyte leakage from different qualities of soaked seed.

Effect of accelerated aging time on fatty acid concentration in sweet pepper seed: Table 4 showed the changing of five fatty acids (saturated fatty acids; palmitic acid and stearic acid and unsaturated fatty acids; oleic acid, linoleic acid and linolenic acid) during accelerated aging of seeds. The results revealed that five fatty acids were changed in similar pattern when the aging time was increased. The fatty acid concentrations were not substantially changed from 0 to 10 days of aging time, were rapidly increased from 10 to 15 days of aging time and were decreased after 15 days of aging time. The increased fatty acid during 10-15 days of aging seed exhibited the ability of the cell to survive through lipid accumulation. The decreased fatty acid after 15 days of aging seed was due to the increase in respiration in cell during process to prevent cell damage (Torres and Andrews, 2006) and the lipid accumulation mechanism in the cell was shut down by severe deterioration of seed. Another explanation is that the lipid peroxidation induced by ROS caused membrane degradation during accelerated aging of sweet pepper seed which might decrease fatty acid concentration in seed.

Effect of accelerated aging process on peroxidation product in sweet pepper seed: Total peroxidation product and MDA during aging process were determined and the results showed in Table 5. At the initial step of aging time (5-10 days), the total peroxide and MDA rapidly increased and the total peroxide concentrations on days 5 and days 10 of aging time were 22±0.56 and 76±0.72 mg g^{-1} of seed, respectively whereas MDA concentration on days 5 and days 10 of aging time were 19±0.30 and 75±0.41 mg g^{-1} of

Table 4: Effect of accelerated aging process on fatty acid profile at different aging time in sweet pepper seed

Time of aging (days)	Fatty acid (μg g^{-1})				
	Stearic acid	Palmitic acid	Oleic acid	Linoleic acid	Linolenic acid
0	112±0.01^{e}	644±0.06^{b}	178±0.02^{b}	1128±0.08^{b}	4±0.00^{b}
5	134±0.06^{b}	657±0.07^{b}	178±0.02^{b}	1147±0.14^{b}	6±0.00^{a}
10	91±0.01^{d}	559±0.08^{c}	154±0.79^{c}	970±0.13^{c}	3±0.00^{c}
15	144±0.02^{a}	910±0.11^{a}	242±0.03^{a}	1538±0.20^{a}	6±0.00^{a}
20	57±0.00^{g}	345±0.01^{d}	99±0.52^{d}	624±0.02^{d}	0±0.00^{d}
25	36±0.00^{f}	229±0.25^{e}	66±0.00^{e}	411±0.02^{e}	0±0.00^{d}
30	22±0.00^{g}	144±0.02^{f}	41±0.00^{f}	258±0.03^{f}	0±0.00^{d}

Values are Mean±SD of three replications, Means within a column not showing the same letter(s) were significantly different at p<0.001 by DMRT (n = 7)

Table 5: Effect of accelerated aging process on total peroxide, MDA content and total antioxidant at different aging time in sweet pepper seed

Time of aging (days)	Total peroxide (mg g^{-1})	MDA content (mg g^{-1})	Total antioxidant (μg g^{-1})
0	0±0.00^{e}	0±0.00^{f}	90±0.1.53^{a}
5	22±0.56^{d}	19±0.30^{e}	104±1.53^{b}
10	76±0.72^{c}	75±0.41^{d}	123±0.58^{d}
15	85±0.58^{b}	87±0.53^{c}	117±1.00^{c}
20	94±0.27^{a}	92±0.34^{b}	115±2.00^{c}
25	96±0.51^{a}	96±0.32^{a}	115±1.00^{c}
30	96±0.80^{a}	96±0.57^{a}	114±2.00^{c}

Values are Mean±SD of three replications, Means within a column not showing the same letter(s) were significantly different at p<0.001 by DMRT (n = 7)

seed, respectively. During 10-20 days of aging time, the total peroxide and MDA slowly increased and total peroxide and MDA concentration were in range 76±0.72-94±0.27 and 75±0.41-92±0.34 mg g^{-1} of seed, respectively. There was no substantial increase from 20 to 30 days of aging time and total peroxide and MDA concentration were about 94±0.27, 96±0.80 and 92±0.34 - 96±0.57 mg g^{-1} of seed, respectively. From these results it could be concluded that deteriorated seed exhibited high amount of peroxidation products including MDA. The similar concentrations of MDA and total peroxide at all aging times implied that lipid in seed was the target of peroxidation and MDA was a major product of lipid peroxidation in deteriorated seed.

The increase in total peroxide particularly on MDA could be explained as shown in Fig. 1. Peroxidation products are the result from peroxidation of unsaturated fatty acids. The lipid hydroperoxides decompose readily in the presence of metal ions, generating new free radical species and ultimately leading to highly complex mixtures of olefins, alkanes, alcohols, malondialdehyde and carbonyl compounds. The MDA is the end product of lipid peroxidation and hydrogen peroxide is the product of oxidation which is the intermediate in a cell. The damage to unsaturated fatty acids during seed deterioration is caused by lipid peroxidation and increase peroxidation products, particularly, the MDA content (Spano *et al*., 2006). The decrease in unsaturated fatty acids and increase in MDA supported the hypothesis that degradation of fatty acids in deteriorated sweet pepper seed during accelerated aging process.

Effect of accelerated aging process on antioxidant activity in sweet pepper seed: Antioxidants could prevent seed deterioration by reducing free radicals from ROS within the cell. Antioxidant activity was determined by spectrophotometer as the microgram scavenging of 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radicals (Khanahmadi *et al*., 2010; Blois, 1958). The total antioxidant in aged seed were 90±1.53, 104±1.53, 123±0.58, 117±1.00, 115±2.00, 115±1.00 and 114±2.00 μg g^{-1} of seed when seeds were accelerated aging on 0, 5, 10, 15, 20, 25 and 30 days of aging time, respectively, as showed in Table 5. The increased total antioxidant during 0-10 days of aging seed was evidence of the ability of the cell to survive and the antioxidant system in the cell had the ability to prevent the damaging by ROS. The decreased total antioxidant after 10 days of aging seed could be explained by the failure of the defense mechanism in terms of antioxidant system in the cell. The increasing in total antioxidant at 5-10 days of aging time implied that cells had the capability to produce the antioxidant to protect themselves against the damaging effects of ROS, such as free oxygen, superoxide, peroxyl and hydroxyl. When seed exhibited severe deterioration (15-25 days of aging time), an imbalance between antioxidants and ROS would create and lead to cellular damage.

Relationship of seed germination, potassium ion (K^+) and MDA content during accelerated aging process of sweet pepper seed: The seed germination, K^+ and MDA content of sweet pepper seed in different accelerated aging time were shown in Fig. 2. The results revealed that seed quality in terms of ER was decreased whereas both K^+ and MDA content were increased 0-30 days of aging time. The opposite correlation between K^+, MDA content and seed quality which was not reported elsewhere, could be possible, used as a marker of sweet pepper seed quality. Before 20 days of aging time, the %ER slowly decreased, K^+ was slowly leaked from seed and MDA content substantially increase and after 20 days of aging seed, the %ER decrease very quickly, K^+ substantially leaked

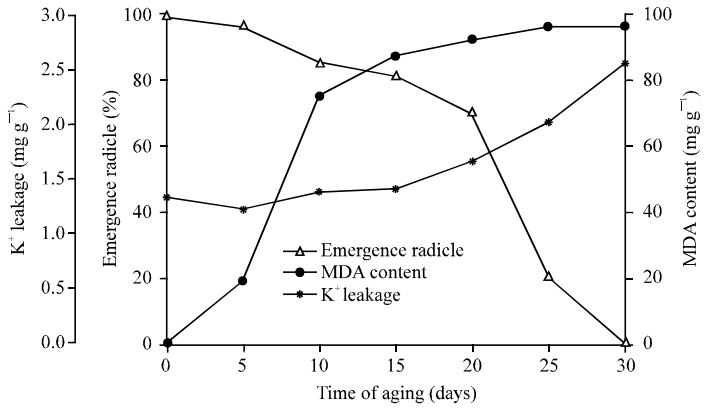

Fig. 2: Relationship of percent emergence radical, potassium ion leakage from seed and MDA content during accelerated aging process of sweet pepper seed

from seed and MDA content slowly increase that were interesting to identify these correlation more serious. The experimental design would be performed to test the significance this conclusion.

CONCLUSION

The accelerated aging process is a technique for deteriorating seed under stress conditions. The seed deterioration during storage could be determined by changes in seed quality and biochemical response to seed quality. Seed germination (% ER) decreased progressively with artificially accelerated aging. One possible explanation for the seed deterioration is lipid peroxidation and cell membrane damage. In this study the seed germination correlated well with increased electrolyte leakage, thus reflecting a loss in membrane integrity. Our results indicate an inability of transporting protein to maintain their activities, resulting in loss of germinability. The determination of MDA is a convenient method of quantifying the extent of lipid peroxidation. Present results showed the increase in MDA level and total peroxidation product with accelerated aging time. These results taken in conjunction with reduced germinability indicate that increased lipid peroxidation might explain the loss of viability of sweet pepper seed. The presence of antioxidants helps in preventing the lipid peroxidation due to free radical formation. The results suggest that sweet pepper seed deterioration during accelerated aging is closely related to membrane damage from lipid peroxidation that impact to decrease the ability of transporting protein.

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