Turkish Journal of Field Crops, 2011, 16(2):100-104

FATTY ACIDS AND TOCOPHEROL CONTENT IN SUNFLOWER SEEDS AFFECTED BY ACCELERATED AGEING AND PRIMING WITH ANTIOXIDANT SOLUTIONS

Ivana DRAGANIĆ[1*], Slavoljub LEKIĆ[1], Tatjana BRANKOVIĆ[2], Goran TODOROVIĆ[3]

[1] *Department of Crop Science, Faculty of Agriculture, Belgrade University, Zemun, Serbia*
[2] *IHTM Company for Technological Development, Laboratory for Analysis and Development, Belgrade, Serbia*
[3] *Institute for Medicinal Plant Research "Dr Josif Pančić", Belgrade, Serbia*
**Corresponding author: ivanadraganic@agrif.bg.ac.rs*

Received: 26.09.2011

ABSTRACT

Effects of accelerated ageing on the contents of both, fatty acids and tocopherol in sunflower seeds, and the influence of priming with antioxidant solutions on the tocopherol content were observed in the present study. Accelerated ageing did not affect contents of linoleic (C18:2), oleic (C18:1), stearic C18:0) and palmitic (C16:0) acids. It was determined that the contents of α-, β- and γ-tocopherol decreased in seeds subjected to accelerated ageing and in seeds primed prior to accelerated ageing. Priming with antioxidant solutions prior to accelerated ageing differently affected the contents of α-, β- and γ-tocopherol in seeds. Furthermore, seeds primed with a solution of a lower concentration of tocopherol and simultaneously primed with a solution of all three antioxidant substances (ascorbic acid, tocopherol and glutathione) had a higher content of α-tocopherol after accelerated ageing in relation to seeds which were not primed. Seeds primed with solutions of ascorbic acid, tocopherol (higher concentration) and glutathione had a lower content of α-tocopherol than seeds which were not primed prior to accelerated ageing. The total content of β- and γ-tocopherol in primed seeds were significantly lower ($p \geq 0.05$) than in seeds which were not primed prior to accelerated ageing, except in seeds simultaneously primed with the solution of all three antioxidant substances.

Key words: **accelerated ageing, antioxidants, fatty acids, seed enhancement, tocopherol**

INTRODUCTION

Seeds reach their maximum vigour at the stage of their physiological maturity after which vigour, faster or slower, starts to decline (Harrington, 1972). A decline of vigour is attributed to changes in almost all physiological processes in seeds, while research literature often mentions lipid peroxidation as an important factor of seed deterioration.

The decline in seed vigour results in the decrease in the fatty acids content in seeds of *Helianthus annuus* L. (Gidrol et al., 1989), *Acer platanoides* L. (Pukacka and Kuiper, 1988), *Phaseolus vulgaris* L. and *Zea mays* L. (Lin and Pearce, 1990). However, some researchers confirmed that accelerated ageing did not result in a lower content of fatty acids, as in case of *Glycine max*. L. seeds (Priestley and Leopold, 1979).

Complex systems of plant antioxidant defence located in seeds (Leprince et al., 1993) are divided into enzymic and nonenzymic antioxidants (Bartosz, 1997). Antioxidant nonenzymic substances include tocopherol, ascorbic acid and glutathione. Ascorbic acid and glutathione are principal water soluble antioxidants, while tocopherol is the most important liposoluble antioxidant (Noctor and Foyer, 1998). Vitamin E encompasses the group of biologically active hormone derivates: tocopherols (α-, β-, γ- and δ-) and tocotrienols (α-, β-, γ- and δ-) and it is considered the most important nonenzymic inhibitor of lipid peroxidation *in vivo* (Halliwell and Gutteridge, 2007). Sunflower seeds contain α-tocopherol that makes over 90% of total tocopherols, while the content of β- and γ-tocopherol range below 2% of the total tocopherol content (Demurin, 1993).

Ascorbic acid and glutathione regenerate tocopherol and due to it, tocopherol breaks lipid oxidation several times before it is degraded. (Fryer, 1992). Tocopherol breaks a propagation chain of lipid oxidation by reduction of radical intermediates (Vollhardt and Schore, 2004). One molecule each of the α-, β-, γ-, δ-tocopherol is capable of protecting 220, 120, 100, 30 and 20 molecules of polyunsaturated fatty acid from oxidation, respectively (Fukuzawa et al., 1982). Results obtained by many researchers showed that the germination loss was related to the decreased tocopherol (especially α-tocopherol) content, such as in seeds of the species *Sesamum indicum* L., *Gossypium* spp. and *Ricinus communis* L. (Sharma, 1977), *Glycine max.* L. (Senaratna et al., 1988), *Pinus sylvestris* L. (Tammela et al., 2005) and *Suaeda maritima* L. (Seal et al., 2010a), while studies carried on the mutant of *Arabidopsis* confirmed the effect of tocopherol on seed longevity (Sattler et al., 2004). Short-term priming with 1% aqueous solution of tocopherol during seed storage had a beneficial effect on germination of pine, onion

and okra seeds (Socrates et al., 1961). Zalewski et al. (2000), testing seeds of *Vicia faba* L. var. *minor* primed with α-tocopherol solution, gained similar results.

Some researchers pointed out that there was no correlation between a germination loss and a tocopherol content in ageing seeds - for instance, seeds of *Glycine max.* L. (Priestley et al., 1980), *Triticum* spp. (Fielding and Goldsworthy, 1980) and *Vernonia galamensis* (Cass.) Less. (Seal et al., 2010b).

Sunflower seeds were most often primed with the polyethylene glycol solution. Bailly et al. (2002) determined that polyethylene glycol neutralised damages of seeds exposed to accelerated ageing, because it supported the activation of antioxidant systems of catalase and glutathione reductase during seed imbibition and during the initial stages of the seedling development. Moreover, sunflower seed priming neutralised damages caused by accelerated ageing and decreased the level of lipid peroxidation in aged seeds (Bailly et al., 1998).

Effects of accelerated ageing on the content of both, fatty acids and tocopherol in sunflower seeds, and the influence of priming with antioxidant solutions on the tocopherol content were observed in the present study.

MATERIAL AND METHODS

The hybrid seeds (moisture of 6.2%) of the F_1 generation of French high-oil sunflower Alvaro were used in the experiment. Seeds were treated with the pesticide METALAXYL-1 and stored at the temperature of 18°C and relative humidity of 75%. The experiment was carried out in 2010.

The most important fatty acids, as well as, their participation in seeds of the initial material (control) and in seeds after accelerated ageing were identified. The contents of α-, β- and γ-tocopherol (mg kg^{-1} raw weight) were estimated in seeds of the initial material (control), seeds exposed to accelerated ageing and seeds primed with solutions of antioxidant substances and then subjected to accelerated ageing.

Germination of observed seeds (control) amounted to 85.8%, while this value declined to 73.8% after accelerated ageing. Furthermore, germination of seeds primed with different solutions of antioxidant substances varied from 69.4% to 77.0%.

Seed priming. Prior to accelerated ageing, seeds were primed with aqueous solutions of antioxidant substances - ascorbic acid, tocopherol and glutathione. Seed priming was preformed with solutions of the following concentrations (seven combinations, for each combination 4×100 seeds were used): 1) ascorbic acid (A) - 0.5% and 2.5%; 2) tocopherol (T) - 0.3% and 0.9%; 3) glutathione (G) - 0.05% and 0.1%; 4) ascorbic acid - 0.5%, tocopherol 0.3% and glutathione - 0.05% (A+T+G).

Tocopherol used in the experiment was in a powder form (1g = 500 IU) dl-α-tocopheryl acetate (DSM Nutritional Products). Ascorbic acid and glutathione were manufactured by the company SERVA Electrophoresis.

Seed priming with the aqueous solution of antioxidants encompassed the following steps: 1) dissolving of antioxidant substances in distilled water; 2) two-hour seed priming with the obtained aqueous solution; 3) taking seeds out of the solution and drying at the temperature of 28ºC for five hours.

Content of fatty acids in seed. Oils were extracted by petroleum ether after Soxhlet. Fatty acids were converted into methyl esters by the transmethylation procedure, because they were neutral (acid number was below 2). Methyl esters were then analysed by gas chromatography in order to identify fatty acids and to determine their relative relationship. The content of fatty acids was estimated in both, the initial material (control) and the seeds exposed to accelerated ageing.

Content of tocopherols in seed. The tocopherol content in seeds was determined by reversed-phase liquid chromatography with UV detection. The mobile phase consisted of acetonitrile and methanol in a 97 to 3 ratio at a flow rate of 0.7 ml min^{-1}. The tocopherol content was determined by the calibration curve method and was expressed over the α-tocopherol content and the content of β- and γ-tocopherol. The fluorescence detector was set at 295 nm. The tocopherol isolation was done by the optimised saponification procedure (Branković, 2005). For each replication we used 1.0 g of homogenised seed sample. After filtering through membrane syringe filter, the solution was injected into the LC system. The tocopherol content was estimated in the initial material (control), the seeds exposed to accelerated ageing and also in the seeds primed before accelerated ageing.

Accelerated ageing of seed. Seeds were exposed to the temperature of 40ºC and air humidity of 95-100% for 72 hours.

Statistical analysis. Results obtained by studying fatty acid contents were tested by the Wilcoxon signed-rank test. Descriptive and analytical statistics were performed with the statistic package SPSS 10.0 for Windows. Significance of differences between mean values of observed factors (α-, β- and γ-tocopherol) was tested by the application of the analysis of variance (ANOVA). All estimates of significance were derived from F and LSD tests at the 5% probability level.

RESULTS AND DISCUSSION

Fatty acids in seed. Results of Wilcoxon signed-rank test ($p = 0.875$, $p \geq 0.05$) applied to the values presented in Table 1 point out that there was no statistical significant difference between the fatty acid contents prior and after accelerated ageing of seeds.

α-tocopherol in seeds. A single factor analysis of variance (ANOVA) indicates statistically very significant differences ($p \leq 0.01$) in α-tocopherol contents between the control and all other applied treatments (Table 2).

LSD test results point out to several facts. Firstly, the content of α-tocopherol in seeds of the initial material (control) significantly differed ($p \leq 0.05$) from the content of

α-tocopherol in seeds subjected to accelerated ageing and in seeds primed with antioxidant solutions (all applied combinations) (Table 3). Secondly, the content of α-tocopherol in seeds primed with tocopherol solutions (lower

Table 1. Content (%) of studied fatty acids of the initial material and after accelerated ageing of seeds

Fatty acid	Initial material (control)	After accelerated ageing
Linoleic (C18:2)	59.3	58.3
Oleic (C18:1)	29.9	31.0
Palmitic (C16:0)	6.1	6.5
Stearic (C18:0)	4.3	4.1
Linolenic (C18:3)	*	*
Eicosanoic (C20:0)	*	*
Docosanoic (C22:0)	*	*

* In traces

Table 2. Analysis of variance for the contents of α-, β- and γ-tocopherols (mg kg^{-1})

Source of variation	Df	Mean squares (MS)	
		α-tocopherol (mg kg^{-1})	β- and γ-tocopherol (mg kg^{-1})
Replication	1	5.05	0.26
Treatments	8	2265.97**	171.59**
Error	8	0.45	0.03

** $p \leq 1\%$

concentration) and seeds simultaneously primed with all three antioxidants was significantly higher ($p \leq 0.05$) than the content of α-tocopherol in seeds after accelerated ageing (Table 3). Thirdly, the content of α-tocopherol in seeds primed with solutions of ascorbic acid, tocopherol (higher concentration) and glutathione prior to accelerated ageing was statistically significantly lower ($p \leq 0.05$) than the content of α-tocopherol in seeds after accelerated ageing (Table 3).

Table 3. α-tocopherol content (mg kg^{-1})

α-tocopherol	Replication		Average
	I	II	
Control	330.8	331.8	331.3 a
Accelerated ageing	271.9	273.7	272.8 d
Ascorbic acid 0.5%	264.2	263.8	264.0 f
Ascorbic acid 2.5%	260.4	262.2	261.3 g
Tocopherol 0.3%	275.2	274.6	274.9 c
Tocopherol 0.9%	265.9	266.3	266.1 e
Glutathione 0.1%	256.5	256.3	256.4 h
Glutahione 0.05%	262.5	263.5	263.0 f
A+T+G	280.0	281.6	280.8 b

$LSD_{0.05}$ = 1.55 mg kg-1

β- and γ-tocopherol in seed. A single factor analysis of variance (ANOVA) indicates that there were statistically very significant differences ($p \leq 0.01$) in the total content of β- and γ-tocopherol between the control and all other applied treatments (Table 2).

Based on results obtained by the LSD test, the following three facts should be emphasised. Firstly, the contents of β- and γ-tocopherol in seeds of the initial material (control) significantly differed ($p \leq 0.05$) from the total content of β- and γ-tocopherol in seeds subjected to accelerated ageing and also in seeds primed prior to accelerated ageing (Table 4). Secondly, differences in the total content of β- and γ-tocopherol between seeds exposed to accelerated ageing and seeds simultaneously primed with three antioxidants prior to accelerated ageing were not statistically significant ($p \geq 0.05$). Thirdly, the total content of β- and γ-tocopherol in seeds primed and then subjected to accelerated ageing was significantly lower ($p \leq 0.05$) than in seeds that were exposed to accelerated ageing without previous priming (Table 4).

Table 4. Total content of β- and γ-tocopherol (mg kg^{-1})

β- and γ-tocopherol	Replication		Average
	I	II	
Control	11.7	11.3	11.5 a
Accelerated ageing	7.2	7.0	7.1 b
Ascorbic acid 0.5%	5.8	6.0	5.9 f
Ascorbic acid 2.5%	6.5	6.7	6.6 cd
Tocopherol 0.3%	6.4	6.6	6.5 de
Tocopherol 0.9%	6.2	6.0	6.1 ef
Glutathione 0.1%	6.6	6.2	6.4 de
Glutathione 0.05%	6.4	6.6	6.5 de
A+T+G	7.0	7.0	7.0 bc

$LSD_{0.05}$ = 0.425 mg/kg

Although tested sunflower seeds were rapidly loosing germination under conditions of accelerated ageing, the content of fatty acids in seeds did not changed significantly. Same results were presented by Priestley and Leopold (1983) for *Glycine max.* L. seeds. Corbineau et al. (2002) determined that accelerated ageing of sunflower seeds resulted in the decrease of seed germination and in an insignificant increase of the content of palmitic (C16:0), stearic (C18:0) and oleic (C18:1) acids, while linoleic acid (C18:2) maintained its original concentration. These results point out to the fact that enzymes, engaged in biosynthesis of lipids, were not affected by high temperatures, but that high temperatures stimulatingly affected enzymes (Corbineau et al., 2002). However, some researchers determined that accelerated ageing of *Prunus dulcis* Mill. seeds (Zacheo et al., 1998) and natural ageing of *Pinus sylvestris* L. seeds (Tammela et al., 2005) led to a decrease in the content of unsaturated fatty acids.

Obtained results indicate that the contents of α-, β- and γ-tocopherol decreased in seeds subjected to accelerated ageing and seeds primed prior to accelerated ageing. The results on the decrease of the contents of α-, β- and γ-tocopherol after seeds were subjected to high temperatures and humidity supported the view of Senaratna et al. (1988) that tocopherol, under stress conditions, was used for seed protection against effects of free radicals. Certain researches pointed out that the decrease in the α-tocopherol content in *Fagus sylvatica* L. seeds (Ratajczak and Pukacka, 2005; Pukacka and Ratajczak,

2007) and *Acer platanoides* L. seeds (Pukacka, 1991) was a result of ageing. On the other hand, Merritt et al. (2003) did not establish a correlation between seed viability, during natural ageing, and the α-tocopherol content in seeds of several Australian plant species. Furthermore, Ponquett et al. (1992) did not determine a correlation between seed longevity and the tocopherol level in eight observed species.

CONCLUSION

This study shows that seed priming with antioxidant solutions prior to accelerated ageing differently affected the content of α-, β- and γ-tocopherol in seeds. Firstly, seeds primed with the solution of a lower concentration of tocopherol and also simultaneously primed with three antioxidants had a higher content of α-tocopherol after accelerated ageing in relation to seeds which were not primed. Secondly, seeds primed with solutions of ascorbic acid, tocopherol (higher concentration) and glutathione had a lower content of α-tocopherol in relation to seeds which were not primed prior to accelerated ageing. Thirdly, the total content of β- and γ- tocopherol in primed seeds was significantly lower ($p \leq 0.05$) than in seeds which were not primed prior to accelerated ageing, except in the seeds which were simultaneously primed with all three antioxidants.

Gained results indicate that only seed priming with α-tocopherol had a positive effect on the content of α-tocopherol in seeds which were exposed to accelerated ageing, but it did not affect the contents of β- and γ-tocopherol, which is probably a result of their greater reactivity. Nevertheless, a seed treatment with natural antioxidants (α-tocopherol) could not neutralise an adverse effect of accelerated ageing on the content of α-tocopherol in observed sunflower seeds. Finally, results obtained in this study showed that seed priming with α-tocopherol solution could have potential to maintaining seed vigour during a prolong storage in seed banks.

ACKNOWLEDGMENTS

This study was supported by the Ministry of Science and Technological Development—of the Republic of Serbia (Project number 143020). We wish to thank Prof. Slađana Šobajić for her critical advice and assistance with the fatty acids analysis.

LITERATURE CITED

Bailly, C., A. Benamar, F. Corbineau, D. Côme, 1998. Free radical scavenging as affected by accelerated ageing and subsequent priming in sunflower seeds. Physiol. Plant. 104: 646-652.

Bailly, C., R. Bogatek-Leszczynska, D. Côme, F.Corbineau, 2002. Changes in activities of antioxidant enzymes and lipoxygenaze during growth of sunflower seedlings from seeds of different vigour. Seed Sci. Res. 12: 47-55.

Bartosz, G., 1997. Oxidative stress in plants. Acta Physiol. Plant. 19: 47-64.

Branković, T., 2005. Isolation of tocopherols from vegetable oils using optimized saponification procedure in aim to determine them by liquid chromatography. Master thesis. Belgrade (in serbian).

Corbineau, F., C. Gay-Mathieu, D. Vinel, D. Côme, 2002. Decrease in sunflower (*Helianthus annuus*) seed viability caused by high temperature as related to energy metabolism, membrane damage and lipid composition. Physiol. Plant. 116: 489–496.

Demurin, Y., 1993. Genetic variability of tocopherol composition in sunflower seeds. Helia. 16: 59-62.

Fielding, J.L., A. Goldsworthy, 1980. Tocopherol levels and ageing in wheat grains. Ann. Bot. 46: 453-456.

Fryer, M.J., 1992. The antioxidant effects of thylakoid vitamin E (α-tocopherol). Plant Cell Environ. 15: 381-392.

Fukuzawa, K., A. Tokumura, S. Ouchi, H. Tsukatani, 1982. Antioxidant activities of tocopherols on Fe^{2+}-ascorbate-induced lipid peroxidation in lechitin liposomes. Lipids. 17: 511-513.

Gidrol, X., H. Serghini, A. Noubhani, B. Mocouot, P. Mazliak, 1989. Biochemical changes induced by accelerated aging in sunflower seeds. I. Lipid peroxidation and membrane damage. Physiol. Plant. 76: 591–597.

Halliwell, B.H., J.M.C. Gutteridge, 2007. Free radicals in biology and medicine. Oxford, UK: Oxford University Press.

Harrington, J.F., 1972. Seed storage and longetivity. In: Kozlowski, T.T (edr), Seed Biology. Vol. III. Academic Press, New York, London, pp. 145-245.

Leprince, O., G.A.F. Hendry, B.D. McKersie, 1993. The mechanisms of dessication tolerance in developing seeds. Seed Sci. Res. 3: 231-246.

Lin, S.S., R.S. Pearce, 1990. Changes in lipids of bean seeds (*Phaseolus vulgaris*) and corn caryopses (*Zea mays*) aged in contrasting environments. *Ann. Bot.* 65: 451-456.

Merritt, D.J., T. Senaratna, D.H. Touchell, K.W. Dixon, K. Sivasithamparam, 2003. Seed ageing of four Western Australian species in relation to storage environment and seed antioxidant activity. Seed Sci. Res. 13: 155-165.

Noctor, G., C.H. Foyer, 1998. Ascorbate and glutathion: keeping active oxygen species under control. Ann. Rev. Plant Physiol. Plant Mol. Bio. 49: 249-279.

Ponquett, R.T., M.T. Smith, G. Ross 1992. Lipid autoxidation and seed ageing: putative relationships between seed longevity and lipid stability. Seed Sci. Res. 2: 51-54.

Priestley, D.A., A.C. Leopold, 1979. Absence of lipid oxidation during accelerated aging of soybean seeds. Plant Physiol. 63: 726-729.

Priestley, D.A., A.C. Leopold, 1983. Lipid changes during natural aging of soybean seeds. Physiol. Plant. 59: 467–470.

Priestley, D.A., M.B. McBride, A.C. Leopold, 1980. Tocopherol and organic free radical levels in soybean seeds during natural and accelerated aging. Plant Physiol. 66: 715-719.

Pukacka, S., P.J.C. Kuiper, 1988. Phospholipid composition and fatty acid peroxidation during ageing of *Acer plantanoides* seeds. Physiol. Plant. 72: 89-93.

Pukacka, S., 1991. Changes in membrane lipid components and antioxidant levels during natural ageing of seeds of *Acer platanoides*. Physiol. Plant. 82: 306–310.

Pukacka, S., E. Ratajczak, 2007. Age-related biochemical changes during storage of beech (*Fagus sylvatica L.*) seeds. Seed Sci. Res. 17: 45-53.

Ratajczak, E., S. Pukacka, 2005. Decrease in beech (*Fagus sylvatica*) seed viability caused by temperature and humidity conditions as related to membrane damage and lipid composition. Acta Physiol. Plant. 27: 3-12.

Sattler, S.E., L.U. Gilliland, M. Magallanes-Lundback, M. Pollard, D. DellaPenna, 2004. Vitamin E is Essential for Seed Longevity and for Preventing Lipid Peroxidation during Germination. Plant Cell, 16: 1419-1432.

Seal, C.E., R. Zammit, P. Scott, T.J. Flowers, I. Kranner, 2010a. Glutathione half-cell reduction potential and α-tocopherol as viability markers during the prolonged storage of *Suaeda maritima* seeds. Seed Sci. Res. 20: 47-53.

Seal, C.E., R. Zammit, P. Scott, D.O. Nyamongo, M.I. Daws, I. Kranner, 2010b. Glutathione half-cell reduction potential as a

seed viability marker of the potential oilseed crop *Vernonia galamensis*. Ind. Crop. Prod. 32: 687-691.

Senaratna, T., J.F. Gusse, B.D. McKersie, 1988. Age-induced changes in cellular membranes of imbibed soybean seed axes. Physiol. Plant. 73: 85–91.

Sharma, K.D., 1977 Biochemical changes in stored oil seeds. Indian J. Agric. Res. 11: 137-141.

Socrates, A., C.A. Kaloyereas, W. Mann, J.C. Miller, 1961. Experiments in preserving and revitalizing pine, onion, and okra seeds. Economic Bot. 15: 213-217.

Tammela, P., P. Salo-Vänänen, I. Laakso, A. Hopia, H. Vuorela, M. Nygren, 2005. Tocopherols, tocotrienols and fatty acids as indicators of natural ageing in *Pinus sylvestris* seeds. Scand. J. For. Res. 20: 378-384.

Vollhardt, K.P., N.E. Schore, 2004. Organic Chemistry: structure and function. Fourth Edition. New York:W.H. Freeman and Company.

Zacheo, G., A.R. Cappello, L.M. Perrone, G.V. Gnoni, 1998. Analysis of factors influencing lipid oxidation of almond seeds during accelerated ageing. LWT – Food Sci. Technol. 31: 6-9.

Zalewski K., D. Widejko, R.J. Górecki, 2000. The influence of CO_2, temperature and α-tocopherol on phospholipid changes in embryonic axes of field bean seeds during storage. Acta Soc. Bot. Pol 69: 123-126.