

Original Research

# Comparison of the Effect of Vitamins A and E on Aging Processes of Edible Vegetable Oils

E. Niedworok\*, A. Bielaszka

Department of Human Nutrition, Faculty of Public Health, Medical University of Silesia, 41-902 Bytom, Piekarska 18, Poland

Received: October 3, 2006

Accepted: July 16, 2007

## Abstract

One of the final products of aging processes of edible oils is malondialdehyde (MDA). The investigated oils (rapeseed oil, refined olive oil, grape seed oil) with the addition of vitamins were tested with accelerated aging test Schaal Oven. Every 24 hours the concentration of MDA was determined. The results showed that in rapeseed oil the highest concentration of MDA ( $1.59\mu\text{M}/\text{dm}^3$ ) was detected in the samples containing vitamin E and also in a mixture of vitamins A and E after 168 hours. The highest concentration of MDA in grape seed oil, about  $1.54\mu\text{M}/\text{dm}^3$ , was observed in the samples with vitamin A after 72 hours. For olive oil it was shown that the highest amount of MDA was detected to be  $1.29\mu\text{M}/\text{dm}^3$  in the samples with vitamin A after 168 hours.

**Keywords:** aging of oil, malondialdehyde (MDA), vegetable oil

## Introduction

Peroxidation of lipids is a binding process connected with the formation of aldehydes. One of them is MDA [1, 2]. In oils which in their own composition possess 100% of fat, aldehydes are responsible for a very rancid and specific, unpleasant taste during the aging process [3, 4]. It is known that the quantity of MDA is an intensity index of peroxidation process of polyunsaturated fatty acids (PUFAs) contained in food. The peroxidation processes of PUFAs can be limited by use of antioxidants such as vitamins A and E [5, 6]. A consequence is a decline of MDA quantity during the aging process. MDA shows large reactivity in relation to most important biologic relationships. It may initiate mutagenic processes leading to carcinogenicity, so it is cytotoxic confirmed in numerous scientific reports [1, 7]. In etiopathogenesis of these toxicities vitamin antioxidants also fulfill important parts protecting the human body before injurious activity of products peroxidation in these free spores and of peroxides.

Additionally, MDA is responsible for the aging process in an organism because it contributes to postpone lipofuscin in cells, considered to be responsible for biological age [7].

## Objectives

The aim of our studies was comparison influence of vitamins A and/or E on aging processes selected of edible vegetable oils: rapeseed oils, olive oil (refined), and grape seed oil.

## Material and Methods

Three edible vegetable oils – rapeseed oil, olive oil (refined) and grape seed oil – were used in this study. To define the aging process, the Schaal Oven test [8, 9] was used. Prepared clear glass jars were plated with  $15\text{ cm}^3$  of investigated oils with suitable quantities of vitamin A and/or E (Table 1). The samples were placed in an incubator, keeping a constant temperature at  $60^\circ\text{C}$ . Before inserting glass jars into the incubator,  $0.5\text{ cm}^3$  of oil was taken to measure the concentration of MDA at zero time ( $t_0$ ). This

---

\*Corresponding author; e-mail: travel1@poczta.onet.pl

was the control value of the test. The second measurement was executed 12 and 24 hours after placing samples in the incubator. The concentration of MDA in the samples was analyzed every 48 hours during the next 14 days. The experiment was repeated three times to obtain the mean values. For measurement of concentration of MDA in studied samples, the spectrophotocolorimetric method [Bioxytech LPO-586 (Oxis,USA)] adopted in our laboratory for determination of MDA in oils was used [10, 11]. In the adopted method one part of MDA reacts with two parts of reagent N-methyl-2-phenylindole at the temperature of 45°C to yield a stable chromophore with maximal absorbance  $A_{\max} = 586$  nm. Standard deviation (SD) for the method used was: 0.00355 and coefficient of variation 3.48% [3, 11]. The obtained results are shown in Table 2 as mean values of the three experiments. The statistical analysis of data was performed using t-Student test. Data were expressed as mean values and statistical differences were calculated by analysis of variance (ANOVA), using STATISTICA Stat Soft, Inc. 2005 (analysis software system), version 7.1.

Table 1. The amount of antioxidants added to the samples of oils.

Antioxidants	Added amounts (g)
Vitamin A	0.060
Vitamin E	0.040
Vitamin A+E	0.060 + 0.040

Table 2. Concentration of MDA in the oil samples ( $\mu\text{M}/\text{dm}^3$ )  $\pm$ SD after 0-312 hours (h) of experiment with the mean values of three experiments.

	Concentration of MDA ( $\mu\text{M}/\text{dm}^3$ ) at different time points of measurement							
	12h*	24h*	72h*	120h*	168h*	216h*	264h*	312h*
<b>Rapeseed oil</b>								
+ Vit.A	0.070 $\pm 0.002$	0.720 $\pm 0.030$	0.850 $\pm 0.030$	1.480 $\pm 0.050$	x	x	x	x
+ Vit.E	0.040 $\pm 0.001$	0.250 $\pm 0.009$	0.950 $\pm 0.03$	1.260 $\pm 0.04$	1.590 $\pm 0.05$	x	x	x
+ Vit. A + E	0.150 $\pm 0.005$	0.250 $\pm 0.008$	0.850 $\pm 0.030$	0.860 $\pm 0.030$	1.590 $\pm 0.060$	x	x	x
<b>Olive oil</b>								
+ Vit.A	0.080 $\pm 0.010$	0.110 $\pm 0.010$	0.980 $\pm 0.040$	1.170 $\pm 0.040$	1.290 $\pm 0.050$	x	x	x
+ Vit.E	0.040 $\pm 0.010$	0.440 $\pm 0.020$	0.870 $\pm 0.030$	1.030 $\pm 0.040$	1.150 $\pm 0.040$	1.170 $\pm 0.040$	1.260 $\pm 0.050$	x
+ Vit. A + E	0.060 $\pm 0.002$	0.080 $\pm 0.002$	0.250 $\pm 0.008$	1.060 $\pm 0.030$	1.070 $\pm 0.040$	1.190 $\pm 0.050$	x	x
<b>Grape seed oil</b>								
+ Vit.A	0.110 $\pm 0.010$	0.110 $\pm 0.010$	0.230 $\pm 0.010$	1.520 $\pm 0.050$	x	x	x	x
+ Vit.E	0.030 $\pm 0.010$	0.240 $\pm 0.010$	1.540 $\pm 0.040$	x	x	x	x	x
+ Vit. A + E	0.060 $\pm 0.210$	0.140 $\pm 0.010$	1.500 $\pm 0.060$	x	x	x	x	x

\*h= hours after placing sample glass jars in the incubator that maintains a constant 60°C.

## Results

From many scientific reports it is known that one of the final products of lipid peroxidation is MDA [2, 12]. Quantitation of its content allows one to estimate the peroxidation process of PUFAs. The influence of the aging processes, resulting from peroxidation occurring in selected oils to which practical antioxidant was added, was observed using Schaal Oven test. This test assures that the process of aging runs in controlled conditions and increased temperature deviates peroxidation. The following oils were taken to examination: rapeseed oil, olive oil (refined) and grape seed oil. Vitamin A and/or E in recommended quantities as antioxidants were added. The content of MDA in tested oil samples was measured by the spectrophotometrical method with a coefficient variability calculated to be 3.48% and standard deviation to be 0.00388. It was ascertained that the addition of examined vitamins caused essential changes in the contents of MDA in each test stage. For the control samples in zero time we detected the following concentration of MDA:

- in rapeseed oil  $0.04\mu\text{M}/\text{dm}^3$ ,
- in olive oil  $0.04\mu\text{M}/\text{dm}^3$ ,
- in grape seed oil  $0.03\mu\text{M}/\text{dm}^3$ .

When vitamin A was added to the samples the concentration of MDA in the examined rapeseed oil after 120 hours of experiment reached the highest values at about  $1.48\mu\text{M}/\text{dm}^3$  (Fig. 1). When the added antioxidant was vitamin E, after 168 hours the concentration of MDA reached the highest value. The same concentration was also detected in the samples with mixed vitamin A and E.

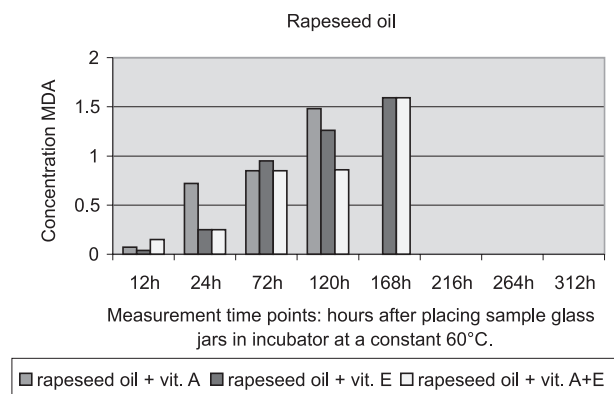


Fig. 1. Concentration of MDA in rapeseed oil samples containing antioxidants subjected to Schaal Oven test.

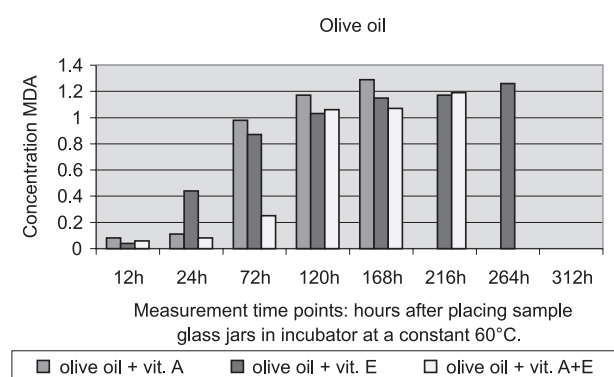


Fig. 2. Concentration of MDA in olive oil samples containing antioxidants subjected to Schaal Oven test.

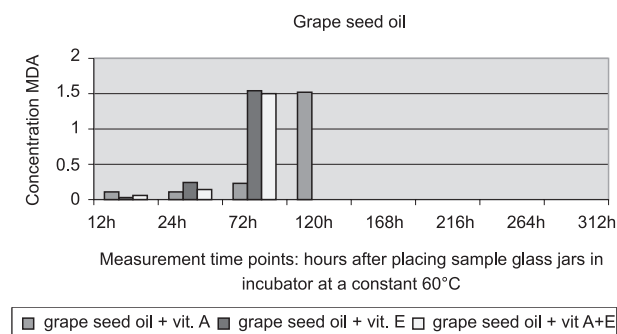


Fig. 3. Concentration of MDA in grape seed oil samples containing antioxidants subjected to Schaal Oven test.

It was ascertained that tests with the addition of vitamin E and A and E mixed were suitable to five measurements but oil with the addition of vitamin A was suitable only for four. After added vitamin A concentration of MDA in olive oil (refined) reached after 168 hours of experiment the highest value about  $1.29\mu\text{M}/\text{dm}^3$  (Fig. 2). In the samples with vitamin E we observed that the highest concentration of MDA was obtained after 264 hours and reached the value  $1.26\mu\text{M}/\text{dm}^3$ . The highest concentration of MDA was proved after adding vitamins A, and lowest after add-

ing a mixture of vitamins A and E at about  $1.1\mu\text{M}/\text{dm}^3$ . The samples with vitamin E were the longest suitable for examination (264 hours).

The next examined oil was grape seed oil (Fig. 3). When vitamin A was the antioxidant the highest concentration of MDA was reached after 120 hours of experiment. With vitamin E like vitamins A and E mixed, the highest concentration of MDA was obtained after 72 hours and reached about  $1.54\mu\text{M}/\text{dm}^3$ .

## Discussion

MDA and other aldehydes are generally considered the end products of lipid peroxidation and are widely measured as the indexes of lipid peroxidation [2, 4, 2]. It is also the oxidation product of amino acids and DNA. Long et al. [13] concluded that MDA is an inhibitor of mitochondrial respiration, mitochondrial complexes I and II [13]. They suggested that a different strategy should be taken to prevent lipid peroxidation in an organism. For quantitative measured MDA in oils samples Shibamoto compared few methods [14]. In our studies we used the spectrophotometrical method for determination concentration of MDA in examined oil samples [10, 11].

We observed that obtained different results in amounts of MDA depend on used tested oil [3, 11]. Rapeseed oil possesses about 20% of polyunsaturated fatty acids. Grape seed oil in its composition possesses 70% of polyunsaturated fatty acids. The result of the present study showed that this oil was characterized with the smallest persistence and after 3 days was not suitable for investigation. Also in analysis of results it was ascertained that in this oil the highest concentration of MDA  $1.54\mu\text{M}/\text{dm}^3$  was observed in the shorter time. Olive oil characterizes only 10.5% participation of polyunsaturated fatty acids in its composition and about 70% monounsaturated fatty acids as indicated by many authors. Gallina-Toshi et al. [15] concluded that the characteristic resistance to oxidation of virgin olive oil is related to its unique fatty acid composition in addition to several minor components that have antioxidant properties. Similar results were obtained by Hrnčirik and Fritsche [8]. Mateos et al. [16] confirmed that the stability of olive oils mainly depends on triacylglycerol composition and concentrations of *o*-diphenols and alpha-tocopherol. The obtained results confirm the fact that composition of fatty acids has a significant influence on the amount of MDA in examined oils. The aging process is the process of peroxidation. Artajo et al. [17] indicated that olives and olive oil, especially extra virgin olive oil, contain a variety of bioactive compounds (phytochemicals) widely considered to be potentially natural antioxidants. Bendini et al. [18] examined the protective effects of extra virgin olive oil phenolics on oxidative stability [18]. The important role of phenolic compounds in protection from autoxidation was confirmed. Schroeder et al. suggested that carotenes rather than phenols are primary substrate for lipid-derived radicals when the red palm oil were taken into consider-

ation [19]. Pellegrini et al. applied "heating experiments" and reported that total antioxidant activity of extra virgin olive oil is mainly due to their phenol and aliphatic phenol content [20]. It has been reported that olive oil high in phenolic compounds could modulate the oxidative/antioxidative status of healthy men [21]. It is well known that different steps used for preparation of olive oil (refined) determine differences in the quantities of phenol. The phenolic compounds are among the substances eliminated during the refining process [22] Faine et al. [23] suggests that the beneficial effects of olive oil are not only related to its high content of oleic acid, but also to the antioxidant potential of its polyphenols. They concluded from experiments that olive oil was more effective than its isolated components in improving lipid profile. Refined olive oil is a product that meets the characteristics of food regulations, but which, due to the decrease in the aliphatic phenol content and in polyunsaturated fatty acids, has decreased some of its excellent nutritional properties. The process with the greatest influence is saponification [24]. Quilles et al. have reported that the supplementation of refined olive oil with 200 mg/kg of vitamin E increases the stability of this oil under pro-oxidant conditions, and its intake decreases the oxidative damage generated by adriamycin in rats [25].

The current study results demonstrated and released during the aging process of the tested edible vegetable oils toxic substance such as MDA was formed. The amount of MDA depends on the antioxidant used and the type of tested oil.

## References

1. DRAPER H.H., HADLEY M. A review of recent studies on the metabolism of exogenous and endogenous malondialdehyde. *Xenobiotica* **20**, (9), 901, **1990**.
2. GUILLEN-SANS R., GUZMAN-CHAZOS M. The thiobarbituric acid reaction in foods, *Critical Reviews in Food Science and Nutrition* **38**, 316, **1998**.
3. NIEDWOROK E., NOGA B., DUL L., CAŁYNIUK B., SZCZEPAŃSKA E. Determination of the concentration of malonaldehyde (MDA) as a toxic product of the lipid peroxidation process in consuming vegetable oils. *Proceedings of 11<sup>th</sup> International Rapeseed Congress*. Ed.H.Sorensen, Biochemistry Natural Product Research Group Chemistry Dpt VL, Copenhagen, Denmark Part **2**, 559, **2003**.
4. JANERO D.R. Malondialdehyde and thiobarbituric acid reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Rad. Biol. Med.* **9**, 515, **1990**.
5. PSOMIADOU E., TSIMIDOU M. Stability of virgin olive oil. 2. Photo-oxidation studies. *J Agric Food Chem.* **50**,(4), 722, **2002**.
6. PSOMIADOU E., TSIMIDOU M. Stability of virgin olive oil. 1. Autoxidation studies *J Agric Food Chem.* **50**,(4), 716, **2002**.
7. YAU T.M. Mutagenicity and cytotoxicity of malonaldehyde in mammalian cells. *Mechanism of Ageing and Development* **11**, 137, **1979**.
8. HRNCIRIK K., FRITSCHKE S. Relation between the endogenous antioxidant system and the quality of extra virgin olive oil under accelerated storage conditions. *J Agric Food Chem.* **53**,(6), 2103, **2005**.
9. ANTOLOVICH M., PRENZLER P.D., PATSALIDES E., Mc DONALD S., ROBARDS K. Methods for testing antioxidant activity. *Analyst.* **127**, **2002**.
10. Method-instruction Bioxytech LPO-586 (Oxis, USA)
11. NIEDWOROK E., CAŁYNIUK B., KOKOT T., NOWAKOWSKA-ZAJDEL E. Antioxidant effect of vitamins A and E on consequences aging process different edible oils. *Polish J. Environ. Stud.* **15**,(2b), 489, **2006**.
12. MOORE K., ROBERTS J. Measurement of lipid peroxidation, *Free Radical. Res.* **28**, (26), 659, **1998**.
13. LONG J., WANG X., GAO H., LIU Z., LIU CH., MIAO M., LIU J. Malonaldehyde acts as a mitochondrial toxin: Inhibitory effects on respiratory function and enzyme activities in isolated rat liver mitochondria. *Life Science* **79**.1466, **2006**.
14. SHIBAMOTO T. Analytical methods for trace levels of reactive carbonyl compounds formed in lipid peroxidation systems. *J.Pharm Biomed. Analysis* **1**,(1), **12**, **2006**.
15. GALLINA-TOSCHI T., CERRETANI L., BENDINI A., BONOLI-CARBOGNIN M., LERCKER G. Oxidative stability and phenolic content of virgin olive oil: an analytical approach by traditional and high resolution techniques. *J Sep Sci.* **28**, (9-10), 859, **2005**.
16. MATEOS R., TRUJILLO M., PEREZ-CAMINO M.C., MOREDA W., CERT A. Relationships between oxidative stability, triacylglycerol composition, and antioxidant content in olive oil matrices. *J Agric Food Chem.* **53**, (14).5766, **2005**.
17. ARTAJO L., ROMERO M., MORELLÓ J., MOTILVA M. Enrichment of refined olive oil with phenolic compounds: evaluation of their antioxidant activity and their effect on the bitter index. *J Agric Food Chem.* **54**, 6079, **2006**.
18. BENDINI A., CERRETANI L., VECCHI S., CARRASCO-PANCORBO A., LERCKER G. Protective effects of extra virgin olive oil phenolics on oxidative in the presence or absence of copper ions. *J Agric Food Chem.* **54**, 4880, **2006**.
19. SCHROEDER M.T., BECKER E.M., SKIBSTED L.H. Molecular mechanism of antioxidant synergism of tocotrienols and carotenoids in palm oil. *J Agric Food Chem.* **54**, (9), 3445, **2006**.
20. PELLEGRINI N., VISIOLI F., BURATTI S., BRIGHENTI F. Direct analysis of total antioxidant activity of olive oil and studies on the influence of heating. *J Agric Food Chem.* **49**, (5), 2532, **2001**.
21. WEINBRENNER T., FITÓ M., TORRE R., GUILLERMO T., RIJKEN SP, TORMOS C., COOLEN S., ALBALADEJO M., ABANADES S, SCHRODER H., MARRUGAT J., COVAS M.I. Olive Oils High in Phenolic Compounds Modulate Oxidative/Antioxidative Status in Men *J. Nutr.* **13**, 2314, **2004**.
22. GARCÍA A., RUIZ-MÉNDEZ M.V., ROMERO C., BRENES M. Effect of refining on the phenolic composition of crude olive oils. *J. Am. Oil Chem. Soc.* **83**, (2), 159, **2006**.
23. FAINE L., RODRIGUES H., GALHARDI C., EBAID G., DINIZ Y., PADOVANI C., NOVELLI E. Effects of olive oil and its minor constituents on serum lipids, oxidative stress, and energy metabolism in cardiac muscle. *Can J Physiol Pharmacol.* **84**, (2), 239, **2006**.

24. BONILLA POLO A., MURILLO RAMOS J.J., GONZALEZ BONILLO J., SANZ PEREZ B. Variations in fatty acids, tocopherol and other quality parameters of virgin olive subjected to refining process. *Nutr.Hosp.* **12**, (6), 309, **1997**.
25. QUILES J., RAMÍREZ-TORTOSA M., IBÁÑEZ S., GONZÁLEZ, DUTHIE G., HUERTAS J., MATAIX J. Vitamin E supplementation increases the stability and in vivo anti-oxidant capacity of refined olive oil. *Free Radic. Res.* **31**, (1),129, **1999**.