A suitable method for defining the nutritional quality of virgin olive oil

By **S. Ciappellano*, P. Simonetti, G. Bermano, E. Leopardi** and **G. Testolin.**
Department of Food Science and Technology, Division of Human Nutrition,
University of Milan, Via Celoria, 2. 20133 Milan, Italy.

SUMMARY

A suitable method for defining the nutritional quality of virgin olive oil

Modification of the membrane fatty acids affects the structure of the surrounding environment causing a variation in membrane stability, fluidity and permeability.

In this study we developed a red blood cells fragility measurement to evaluate the influence of antioxidant intake on membrane functionality. Rats were fed diets differing in oxidized (peroxide value 400 mEq/kg fat) and antioxidant (vitamin E 7.8mg/kg diet) compounds. The results indicate that the resistance of red blood cells to the oxidative stress test was related to the tocopherols present in diets. The main effect on red blood cells fragility was due to the tocopherols which produced the greatest antioxidant response. The fluidity and resistance of red blood cell membranes were determined by their lipid and antioxidant composition. The higher the antioxidant, the more efficient the red blood cell resistance.

KEY-WORDS: Erythrocyte - Globular fragility - Nutritional quality - Tocopherol - Virgin olive oil.

1. INTRODUCTION

Fats and oils provide the most concentrated source of energy of any foodstuff, supply essential fatty acids, contribute greatly to the feeling of satiety after eating, are carriers of fat-soluble vitamins, and serve to make other foods more palatable (Linscheer et al., 1994; Fidanza, 1984). However, the nutritive value of oxidized fats and oils may be decreased by oxidation of essential fatty acids and tocopherols (Eriksson, 1987; Gardner, 1979; Pearson et al., 1983). Also, because of the presence of many oxidation products, consumption of oxidized fats may cause adverse biological effects (Lunec et al.,1991; Esterbauer et al., 1993). The current model for biomembranes consists of a dynamic, asymmetric lipidic matrix of phospholipids and cholesterol with proteins "floating" on it and "dipping into it" at different levels (Neelands et al., 1983). The function of the enzymes, including the transport systems present on the membrane, depends on the physical characteristics of phospholipids and especially on the fatty acid constituent (Foot et al., 1982). The replacement in the membrane of one fatty acid modifies the structure of the surrounding environment causing a variation in stability and permeability (Vaca et al., 1986).Changes in lipid composition have been observed in mitochondrial and other membranes when animals are fed diets supplemented with either saturated fatty acids or polyunsaturated fatty acids (Innis et al., 1981). Diets lacking essential fatty acids determine changes in the phospholipid structure and a subsequent variation in membrane fluidity (Muriana et al., 1992; Berlin et al., 1992). In red blood cells the changes introduced by dietary lipids may be important because the fluidity of this type of cell affects their flexibility, oxygen transport, and the active receptors depending on this fluidity (Hagve et al.,1991).The aim of this study was to develop a method to evaluate the influence of antioxidant intake on membrane function expressed as red blood cell fragility.

2. EXPERIMENTAL

We studied 80 female Sprague-Dawley rats weighting about 160 g. Four rats were killed immediately as described below. The other were fed a diet AIN-76 (American Institute of Nutrition - 1976) with added 10% strongly oxidized refined olive oil (OX-ROO) (peroxide level 400 mEq O_2/kg of fat), for different time (4, 6, 8, 10, 12 and 19 weeks), in order to produce detectable modifications or damage in blood. At 4 weeks, 4 rats were killed and a group of 16 rats was fed diet extra virgin olive oil (EVOO) until 19th week. At 6 and 8 weeks, 4 rats were killed and a group of 16 rats was treated with the same diet EVOO. The tocopherols content and peroxide level of oils are reported in tables I. The animals were housed in plastic cages with stainless-steel grid floors and fed the diet and water ad libitum. Twice a week the animals were weighed to evaluate their growth performance.

Table I
Tocopherols content and peroxide level in the different olive oils

	Peroxide level mEq 0_2/kg	Tocopherols mg/100g
Oxidized refined	311.3	0.0
Extra virgin	20.6	7.8

Four animals per group were killed at various time points, as indicated in figure 1, by bleeding from the abdominal aorta under ether anesthesia, and erythrocyte fragility was measured as reported in figure 2. The heparinized blood was centrifuged at 1,000xg for 10 minutes; erythrocytes were washed 3 times with an equal volume of isotonic saline and the hemoglobin assayed according to Crosby et al. (1954). Red blood cells were then diluted to 1.8 g Hb/dl (5% hematocrit) on the basis of hemoglobin estimation. Susceptibility to lipid peroxidation and consequent globular fragility were determined by a modification of the Clemens and Bursa-Zanetti method (1989). Equal volumes of the final cell suspension and to 2.5% (735 mM) hydrogen peroxide solution were mixed (final hematocrit 2.5%) in tubes that were incubated at 37°C in a mixing bath for 60 min. The surnatant was separated by centrifugation, and the rate of hemolysis was determined photometrically by measuring the hemoglobin concentration after conversion to cyanmethemoglobin at 540 nm (Crosby et al., 1954) utilizing a Varian UV-Vis spectrophotometer model CARY 3E.

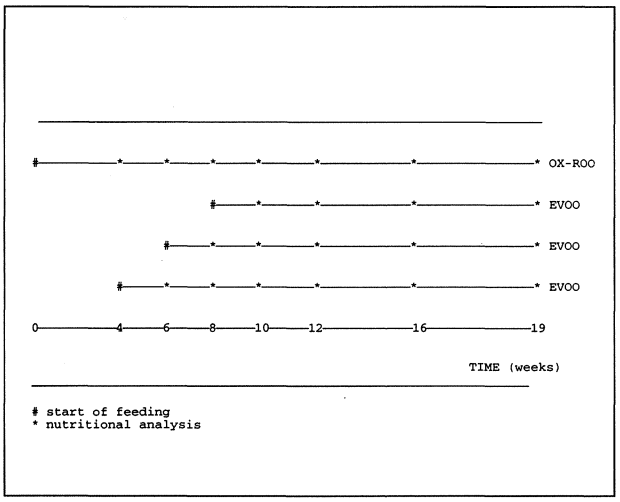

Figure 1
Experimental design of the animals dietary treatments (OX-ROO: oxidized refined olive oil; EVOO: extra virgin olive oil)

3. RESULTS

The tocopherol content and peroxide level in the two oils employed in our experiment were appreciably different (table I). The growth performance of the different groups was not signicantly influenced by the presence of antioxidant or peroxides (figure 3). Red blood cell resistance to the oxidative stress test was related to the tocopherols present in the diets. When the animals were fed on oxidized olive oil, resistance to lysis dropped dramatically; only about 20% of red blood cells remained unlysed. The resistance of red blood cells to the oxidative stress test was fully restored by feeding EVOO which is rich in tocopherols (figures 4-6).

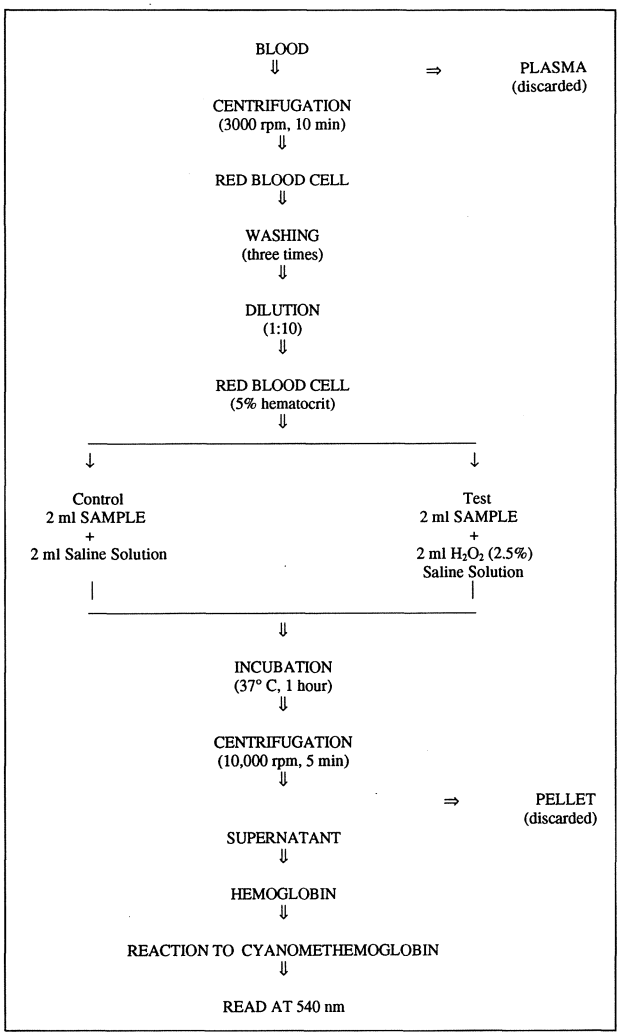

Figure 2
Procedure for the red blood cell preparation and the globular fragility determination.

4. DISCUSSION

At present, the utilized methods to study the oxidative status, TBA-test, Cyt.P450, etc., are laborious and, some times, sensitive to different organic or inorganic substances (Ohkawa et al., 1979; Omura et al., 1964; Eriksson et al., 1982).

This developed method on the globular resistence is able to evidentiate the global oxidative status both in animals and humans. In fact the red blood cells lysis in OX-ROO treated animals is significantly different from animals fed oils containing tocopherol (EVOO). Furthermore our results indicate that tocopherols content significantly ($p<0.01$) influences the resistance of red blood cell membranes to oxidative stress. The evaluation of red blood cells fragility results a useful index to assess the intake of oxidant or antioxidant compounds in oils and easy to use.

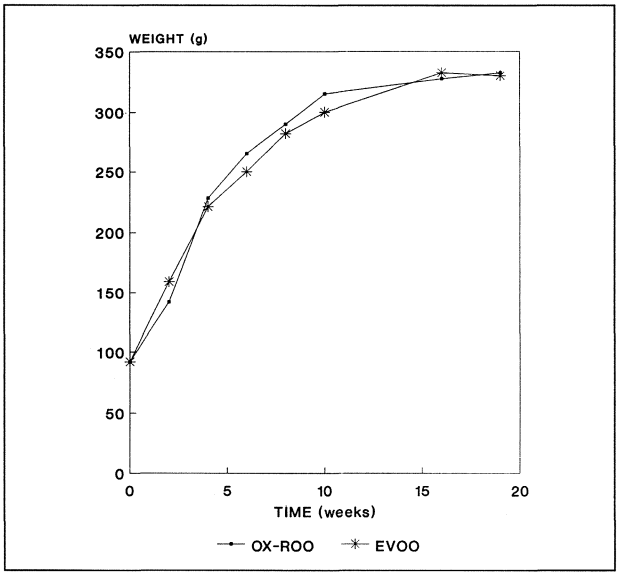

Figure 3
Growth performance of rats fed OX-ROO and EVOO oils.

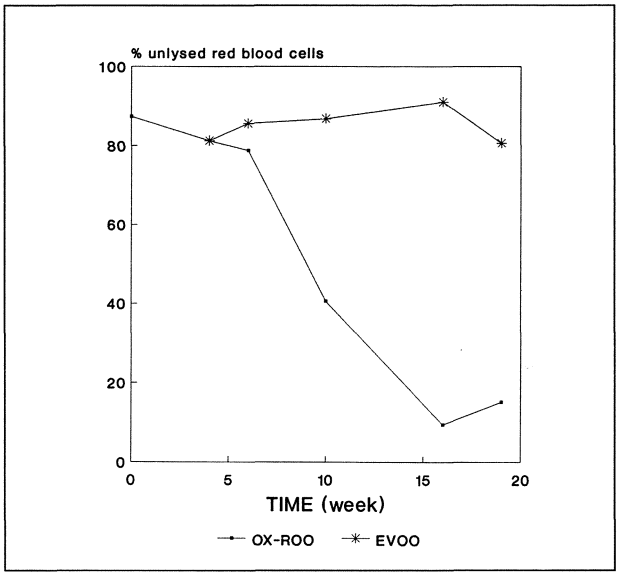

Figure 4
Globular fragility, expressed as % of unlysed red blood cells after 4 weeks, in rats fed oxidized oil (OX-ROO) and extra virgin olive oil (EVOO).

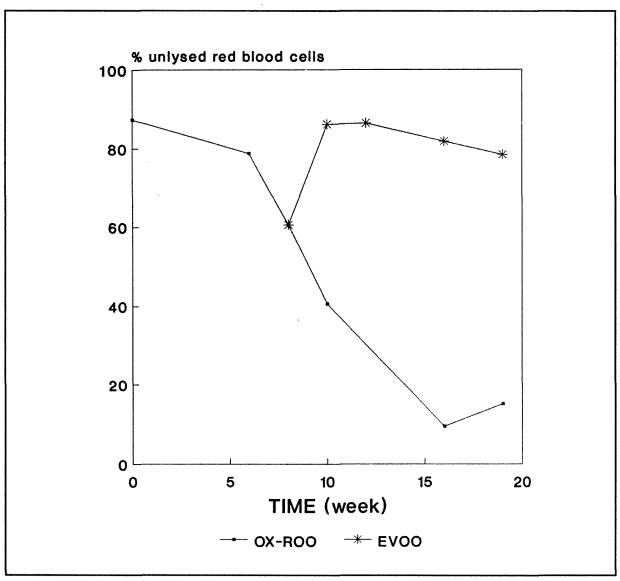

Figure 5
Globular fragility, expressed as % of unlysed red blood cells after 8 weeks, in rats fed oxidized oil (OX-ROO) and extra virgin olive oil (EVOO).

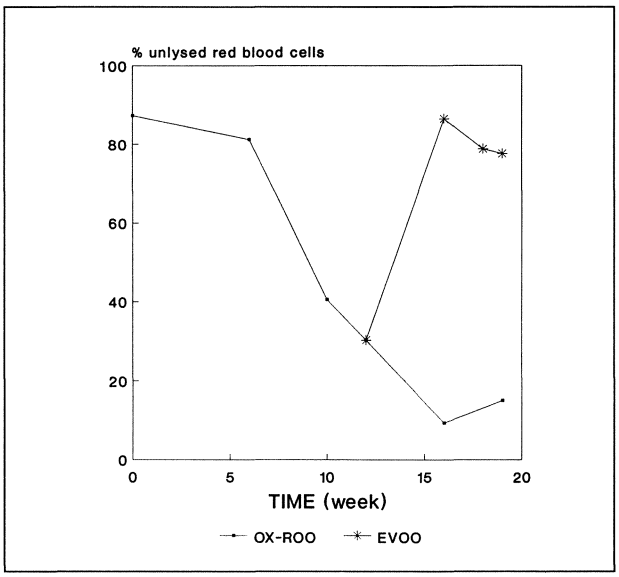

Figure 6
Globular fragility, expressed as % of unlysed red blood cells after 12 weeks, in rats fed oxidized oil (OX-ROO) and extra virgin olive oil (EVOO).

BIBLIOGRAPHY

Berlin, E., Bhathena, S.J., Judd, J.T., Nair, P.P., Peter, R.C., Bhagavan, H.B., Ballard-Barbash, R., Taylor, P (1992).-"Effects of omega-3 fatty acid and vitamin E supplementation on erythrocyte membrane fluidity, tocopherols, insulin binding, and lipid composition in adult men".– J. Nutr. Biochem. 3, 392-400.

Crosby, W.H., Munn, J.I., Furth, F. W. (1954).-"Standardizing a method for clinical hemoglobinometry".- U.S. Armed Forces Medicine J. 5, 693-703.

Eriksson, L.C., De Pierre, J.W. (1982) "Preparation and properties of microsomal fractions" in "Hepatic cytochrome P450 monooxygenase system" p. 9.- J.B. Schenkman and D.Kupfer, (eds). - Pergamon Press, Oxford.

Eriksson, C.E (1987).- "Oxidation of lipids in food system" in "Autoxidation of unsaturated lipids"- p.207. H.W.-S. Chan (Ed.). - Academic Press Inc., London.

Esterbauer, H., Wag, G., Puhl, H. (1993).-"Lipid peroxidation and its role in atherosclerosis".-Br. Med. Bull. 49, 566-576.

Fidanza, F. (1984).-"I lipidi" in "Nutrizione umana" - 1 Ed. p. 64. - F. Fidanza and G. Liguori (Ed.). - Idelson Inc., Naples.

Foot, M., Cruz, T.F., Clandinin, M.T. (1982). -"Influence of dietary fat on the lipid composition of rat brain synaptoso-mal and microsomal membranes".-Biochem. J. 208, 631-640.

Gardner, H.W. (1979).-"Lipid hydroperoxide reactivity with proteins and aminoacids: a review".-J. Agric. Food Biochem. 27, 220-229.

Hagve, T.A., Johansen, Y., Christopherson, B. (1991).-"The effect of n-3 fatty acids on osmotic fragility of rat erythrocytes".-Biochim. Biophys. Acta 1084, 251-254.

Innis, S.M., Clandinin, M. (1981).-"Dynamic modulation of mitochondrial inner-membrane lipids in rat heart by dietary fat".-Biochem. J. 193, 155-167.

Linscheer, W.G., Vergroesen, A.J. (1994).-"Lipids" in "Modern nutrition in health and disease" - 8nd Ed. p. 47. - M.E. Shils and V.R. Young (Ed.). - Lea & Febiger Inc., Philadelphia.

Lunec, J., Griffiths, H. (1991).-"Oxygen radicals: their role in disease processes" in "Free radicals in biotechnology and medicine".-Analytical Proceedings 27, 212-213.

Muriana, F.J.G., Ruiz-Gutierrez, V. (1992).-"Effect of n-6 and n-3 polyunsaturated fatty acids ingestion on rat liver membrane-associated enzymes and fluidity".-J. Nutr. Biochem. 3, 659-663.

Neelands, P.J., Clandinin, M.T. (1983).-"Diet fat influences liver plasma membrane lipid composition and glucagon-stimu-lated adenylate cyclase activity".- Biochem. J. 212, 573-583.

Ohkawa, H., Ohishi, N., Yagi, K. (1979). - "Assay for lipidic peroxides in animal tissues by thiobarbituric acid reaction". - Anal. Biochem., 95, 351-358.

Omura, T., Sato, R. (1964). - "The carbon monoxide-binding pigment of liver microsomes".- J. Biol. Chem., 239, 2370-2378.

Pearson, A.M., Gray, J.I., Wolzak, A.M., Horenstein, N.A. (1983).-"Safety implications of oxidized lipids in muscle foods". -Food Technology, 121-128.

Vaca, C.E., Harms-Ringdhal, M. (1986).-"Lipid peroxidation in the rat liver S9 fraction: influence of membrane lipid composition".-Mutation Res. 162, 21-32.