Available online at www.sciencedirect.com
SCIENCE DIRECT®
International Journal of Pharmaceutics 299 (2005) 146–154
international journal of pharmaceutics
www.elsevier.com/locate/ijpharm

Antioxidant activity of gamma-oryzanol: Mechanism of action and its effect on oxidative stability of pharmaceutical oils

Claudia Juliano*, Massimo Cossu, Maria Cristina Alamanni, Luisella Piu

Dipartimento di Scienze del Farmaco, Via Muroni 23/A, University of Sassari, Sassari, Italy

Received 2 December 2004; received in revised form 22 April 2005; accepted 18 May 2005

Abstract

Gamma-oryzanol, a phytosteryl ferulate mixture extracted from rice bran oil, has a wide spectrum of biological activities; in particular, it has antioxidant properties and is often used in cosmetic formulations as a sunscreen. The first objective of the present investigation was to elucidate the molecular mechanism(s) of the antioxidant activity of gamma-oryzanol by utilising different in vitro model systems, such as scavenging of stable DPPH$^{\bullet}$ radical, OH$^{\bullet}$ and $O_2^{\bullet -}$ radicals scavenging, and azocompound AMVN-initiated lipid peroxidation. The effect of gamma-oryzanol on the oxidative stability of vegetable oils of pharmaceutical and cosmetic interest was then evaluated in a oxidation accelerate test and compared with the effect of the well-known antioxidants BHA and BHT. Our results demonstrate that gamma-oryzanol is an organic radical scavenger able to prevent AMVN-triggered lipoperoxidation. Moreover, when added to oils at concentrations ranging between 2.5 and 10 mmol/kg, gamma-oryzanol shows a dose-dependent increase of the induction times; in particular, it improved the oxidative stability of oils very prone to lipoperoxidation because of their high content of polyunsaturated fatty acids. On the ground of our results, we can conclude that gamma-oryzanol may have a potential application for the stabilization of lipidic raw materials.

Keywords: Gamma-oryzanol; Antioxidant activity; Oxidative stability; Pharmaceutical and cosmetic oils

1. Introduction

Antioxidant agents are used in pharmaceutical and cosmetic formulations mostly to prevent autooxidative deterioration of lipidic raw materials; antioxidants are also introduced as primary ingredients in cosmetics to scavenge free radicals produced by ultraviolet light and environmental pollutants and involved in skin ageing processes (Lupo, 2001).

Lipid peroxidation occurs through a free radical mediated chain reaction and can cause a change in the organoleptic and technological properties of oils and fats, reducing their shelf life. The initiation phase of this process can be triggered by inorganic oxygen-derived initiators (LOOH-independent initiation) or by the presence in trace amounts of pre-

* Corresponding author. Tel.: +39 079 228735; fax: +39 079 228733.
E-mail address: julianoc@uniss.it (C. Juliano).

0378-5173/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2005.05.018

formed lipid hydroperoxides (LOOH-dependent initiation) (Halliwell and Gutteridge, 1999). Antioxidants delay the beginning of lipid oxidation and are in general defined as "... any substance that, when present in low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate" (Halliwell and Gutteridge, 1999). Traditionally, synthetic phenolic compounds, such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA), are used as antioxidants in fat-containing formulations; however, their harmlessness is at present a controversial point (Ito et al., 1986; Whysner et al., 1994; Williams et al., 1999), therefore the search for natural antioxidants is now becoming an important investigation topic in pharmaceutical and food fields.

Rice bran is a component of raw rice that is obtained when it is removed from the starchy endosperm in the rice milling process (Lakkakula et al., 2004). Unsaponifiable matter of crude rice bran oil contains high levels of components with antioxidant properties: tocopherols/tocotrienols (up to 300 mg/kg of Vitamin E) and gamma-oryzanol (up to 3000 mg/kg) (Shin et al., 1997; Xu and Godber, 1999). Initially, gamma-oryzanol was thought to be a single compound, but now it is known that it is a mixture of at least 10 phytosteryl ferulates; cycloartenyl ferulate, 24-methylenecycloartanyl ferulate and campesteryl ferulate have been identified as the major components, accounting for 80% of gamma-oryzanol in rice bran oil (Xu and Godber, 1999).

Gamma-oryzanol has been reported to possess some health-beneficial properties: improvement of plasma lipid pattern, reduction of total plasma cholesterol and increase of HDL cholesterol levels, inhibition of the platelet aggregation (Cicero and Gaddi, 2001). More interestingly from our point of view, it was reported that gamma-oryzanol exhibits antioxidant properties in in vitro systems, such as pyrogallol autoxidation (Kim et al., 1995), lipid peroxidation induced in porcine retinal homogenate by ferric ion (Hiramitsu and Armstrong, 1991) and cholesterol oxidation accelerated by 2,2′-azobis(2-methylpropionamidine) (Xu et al., 2001); however, experimental data about its antioxidative mechanism(s) of action are so far quite inconclusive.

On account of its short term safety (Cicero and Gaddi, 2001), gamma-oryzanol has been proposed as a natural antioxidant to improve the stability of foods (Nanua et al., 2000; Kim and Godber, 2001); moreover, it has been proposed as a UV-A filter in sunscreen cosmetics (Coppini et al., 2001). It seems reasonable to assume that gamma-oryzanol can also be used as antioxidant for pharmaceutical purposes.

Therefore, the aim of the present paper was to elucidate the molecular mechanism(s) of antioxidant activity of gamma-oryzanol by using in vitro previously well-characterized experimental models, and to evaluate the effectiveness of this chemical mixture as antioxidant in raw lipidic materials of pharmaceutical and cosmetic interest.

2. Materials and methods

2.1. Materials

Gamma-oryzanol was purchased from Cruciani Alberto Crual Products (Rome, Italy; batch no. 223/5060328). Gamma-oryzanol concentration was expressed as molarity of cycloarthenyl ferulate, the most represented component of the mixture (p.m. 602,88).

Egg phosphatidilcholine was from Lipid Products (Redhill, UK); 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN) was obtained from Polysciences Inc. (Warrington, PA, USA); 1,10-phenantroline was from Merck (Darmstadt, Germany); H_2O_2 (stabilized, 130 vol) was purchased by ARPI (Rome, Italy), *p*-nitrosodimethylaniline (PNDA) was a kindly gift of Dr. Tadolini. 2,2-Diphenyl-l-picrylhydrazyl (DPPH$^{\bullet}$), ±-α-tocopherol, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), nitroblue tetrazolium (NBT), 2-(*N*-morpholino)ethanesulphonic acid (MES), morpholinepropanesulphonic acid (MOPS), *n*-butanol, $FeCl_2$ and all other chemicals were obtained from Sigma–Aldrich (Milano, Italy). Aqueous solutions were prepared with water purified by MilliQ system (Millipore, Milano, Italy).

As fat raw materials, we chose a number of vegetal oils of pharmaceutical and cosmetic use: avocado oil, castor oil, peanut oil, grape seed oil, wheat germ oil, sesame oil, rosa mosqueta oil, sweet almond oil and *Pistacia lentiscus* berries oil (lentisco oil), in its two varieties "semicotto" and "cotto". Oils were ail purchased from Galeno (Comeana, PO, Italy) with the

exception of "lentisco semicotto" (A) and "lentisco cotto" (B) oils, widely diffused in Sardinia and other Mediterranean areas as popular drugs and foods, which were obtained from *P. lentiscus* ripe fruits in agreement with traditional techniques (Atzei, 2004). In particular, "lentisco semicotto" oil was obtained by treating crushed *Lentiscus* berries with hot water and by collecting the floating oil; when the mixture of "semicotto" oil and water above described is briefly boiled, material collected after cooling is called "lentisco cotto" oil. As stated in the product sheet supplied by the manufacturer, rosa mosqueta oil was stabilized by the addition of alpha-tocopherol (300 ppm). After bottles opening, oils were stored at 4 °C under N_2.

2.2. $OH^{\bullet}$ radical scavenging by gamma-oryzanol

$OH^{\bullet}$ radicals generated in water by the Fenton reaction ($FeCl_2 + H_2O_2$) react with PNDA, causing its decolouration (Rigo et al., 1977). One milliliter of PNDA 45 μM in water, 10 μL of H_2O_2 10 mM and 10 μL of $FeCl_2$ 10 mM were mixed in plastic cuvettes, and 10 μL of ethanolic solutions of gamma-oryzanol at different concentrations were added. Inhibition of PNDA trapping of $OH^{\bullet}$ by gamma-oryzanol and by ethanol was determined by continuously monitoring the decrease in absorbance at 440 nm.

2.3. $O_2^{\bullet -}$ radical scavenging by gamma-oryzanol

Superoxide radicals ($O_2^{\bullet -}$), generated during the autoxidation of $FeCl_2$ 100 μM in MOPS buffer (5 mM, pH 7.5) (Tadolini, 1987), reduce nitroblue tetrazolium salts (2.5 mM) to formazan, a blue product with an absorbance peak at 560 nm. The variation of absorbance at 560 nm was monitored in the absence and presence of gamma-oryzanol (10 μM) in order to determine superoxide scavenging effect of our compound.

2.4. DPPH$^{\bullet}$ radical reduction by gamma-oryzanol

Free radical scavenging activity of gamma-oryzanol was tested against a methanolic DPPH$^{\bullet}$ solution (Joyeux et al., 1995). The degree of decolouration of this solution indicates the scavenging efficiency of the added compound. Different amounts of gamma-oryzanol (in 0.75 mL of methanol) were added to 1.5 mL of DPPH$^{\bullet}$ solution (20 mg/mL, stored at −20° until use); the blank contained 0.75 mL of methanol. The absorbance decrease of DPPH was determined after 5 min of incubation at room temperature at 517 nm, with a Perkin-Elmer Lambda 3 UV/vis spectrophotometer. The percentage of DPPH$^{\bullet}$ decolouration was calculated as follows:

$$\text{decolouration percentage} = \left[1 - \left(\frac{\text{absorbance with compound}}{\text{absorbance of the blank}}\right)\right] \times 100.$$

As comparison, alpha-tocopherol activity was evaluated in the same experimental conditions.

2.5. Effect of gamma-oryzanol on AMVN initiated lipid peroxidation

AMVN is a hydrophobic azoinitiator of lipoperoxidation, which decomposes at 41 °C generating radicals which react with oxygen to give peroxyl radicals (Halliwell and Gutteridge, 1999):

$$X\text{–}N = N\text{–}X \rightarrow N_2 + 2X^{\bullet}$$

$$2X^{\bullet} + O_2 \rightarrow XOO^{\bullet}$$

Multilamellar liposomes containing AMVN were prepared in a round bottom tube in agreement with Tadolini et al. (2000) by adding in the following order: PC, AMVN and, when present, gamma-oryzanol or alpha-tocopherol, and by removing the solvent with a nitrogen stream in ice after each addition. The thin film obtained after evaporation was vortexed for 10 min at 4 °C with 5 mL of 5 mM MES buffer (0.1 mM EDTA, pH 6.5) in order to obtain 8 mM PC, 2 mM AMVN and the desired concentration of antioxidant. In some experiments, liposomes were prepared in the same way but without gamma-oryzanol (or alpha-tocopherol), which were added afterwards to the liposome suspension in order to verify its capability to penetrate into pre-formed multilamellar vescicles. Lipid peroxidation was triggered by incubating the mixture at 41 °C under air in the dark. At fixed times, lipid peroxidation was evaluated by measuring the oxidation index of the liposome suspension: 100 μL samples of the mixtures were extracted with 1 mL of *n*-butanol; phases were separated by centrifugation at 2500 rpm for 10 min, UV

spectra of the upper organic phases were recorded, and the oxidation indexes were determined by the Abs_{234nm}/Abs_{215nm} ratio.

2.6. Evaluation of antioxidant activity of gamma-oryzanol in oils

The antioxidant activity of gamma-oryzanol in oil samples was evaluated by the conductometric method developed by Hadorn and Zuercher (1974), which uses accelerated oxidation conditions. The principle of this test is based on the conductometric determination of volatile degradation products. A flow of air (20 L/h) was bubbled successively through the oil heated at 110 °C and cold water. In this process, the volatile oxidation products were stripped from the oil and dissolved in the water, increasing the water conductivity (Fig. 1). The variations in electric conductivity were registered during the process with the aid of an YSI mod.33 conductimeter (Yellow Spring Instruments Co. Inc., Yellow Spring, OH).

The time taken until there is a sharp increase of conductivity is termed the induction time (IT), and it is expressed in hours. IT is determined by the intersection of the baseline with the tangent to the conductivity curve (Fig. 2).

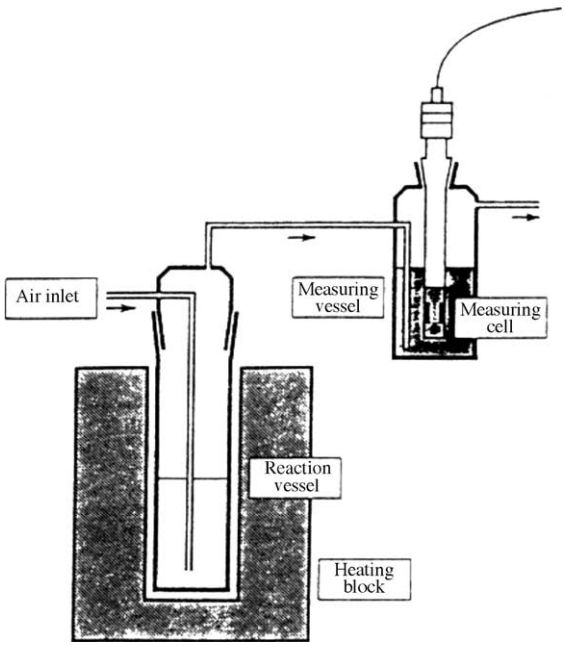

Fig. 1. Scheme of the device used for the measurements of the oxidation time of oils by conductometric method.

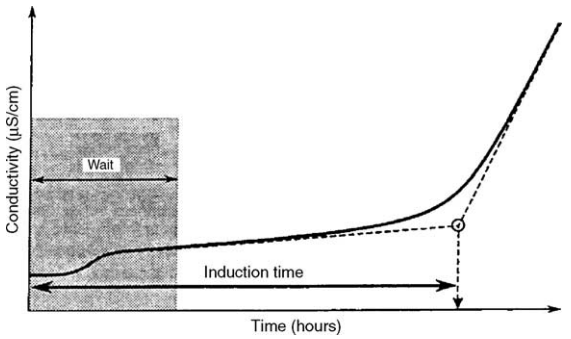

Fig. 2. Oxidation curve and induction time determination.

The IT was evaluated on oils in the presence and absence (test) of gamma-oryzanol, BHA and BHT at different concentration (5.0, 10.0 and 20.0 mmol/kg of oil). The antioxidative index was calculated as: $AI = It_s/It_o$, where It_s is the induction period of oil with antioxidant addition and It_o is the induction period of oil alone.

3. Results

3.1. $OH^{\bullet}$ radical scavenging

Fig. 3 shows the effect of different concentrations of gamma-oryzanol on scavenging of $OH^{\bullet}$ radicals generated by Fenton reaction. In these experimental conditions, the slight activity of $OH^{\bullet}$ scavenging observed is only ascribable to ethanol vehicle, while gamma-oryzanol does not react with $OH^{\bullet}$ radicals and therefore is not able to interfere with its reaction with PNDA.

3.2. $O_2^{\bullet -}$ radical scavenging

Fig. 4 shows the $O_2^{\bullet -}$ generation during spontaneous autoxidation of $FeCl_2$ in MOPS buffer at pH 7.5, in the presence and absence of gamma-oryzanol. The superoxide production is revealed by a gradual increase of absorbance at 560 nm, due to the reduction of NBT to formazan. The reduction rate of NBT is not affected by gamma-oryzanol addition (10 μM), showing that, at least in our experimental conditions, this compound is not able to scavenge superoxide radicals.

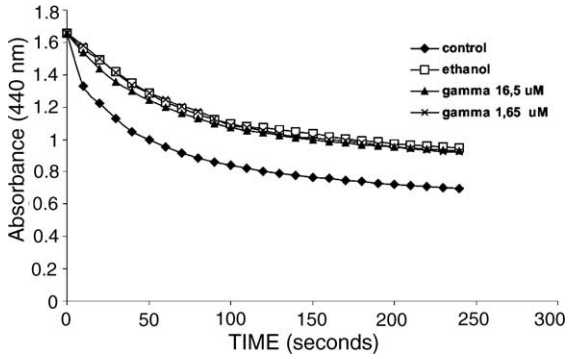

Fig. 3. OH• radicals produced by Fenton reaction react with PNDA (a yellow substance) decreasing its absorbance at 440 nm (control). Ethanol (1%, v/v, in the mixture reaction) reduces PNDA decolouration because of its well-known OH• -scavenging activity. The effect of different gamma-oryzanol amounts (added as alcoholic solutions) does not significantly differ from that one of the ethanol, being only ascribable to the solvent activity. Values plotted are means of triplicate assay; the results are representative of five experiments.

3.3. DPPH• radical reduction by gamma-oryzanol

Fig. 5 shows the results obtained in the DPPH• decolouration test with increasing concentrations of gamma-oryzanol and alpha-tocopherol (up to 238 μM). Gamma-oryzanol possesses a clear, dose-dependent DPPH scavenging activity, although it is weaker than alpha-tocopherol, used as antioxidant of reference.

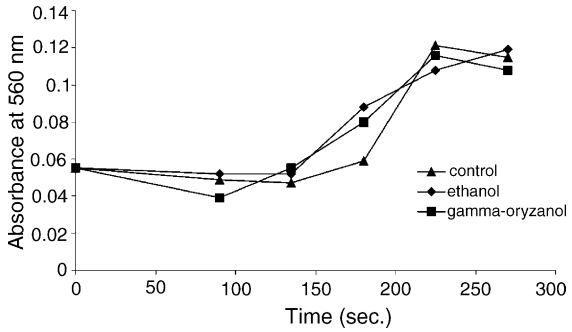

Fig. 4. Effect of gamma-oryzanol on the reaction of NBT with superoxide radicals generated by spontaneous autoxidation of Fe^{2+} in MOPS buffer at pH 7.5. NBT is reduced at blue formazan absorbing at 560 nm. The reduction rate of NBT is not affected by gamma-oryzanol addition (10 μM). The results are representative of four experiments.

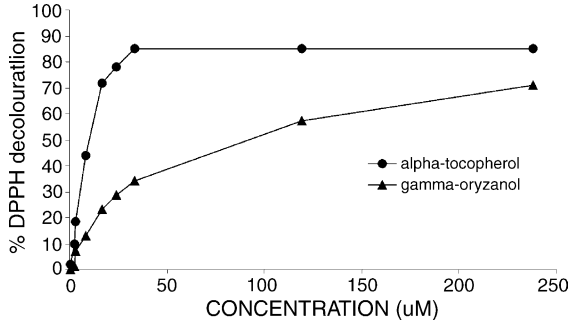

Fig. 5. Effect of gamma-oryzanol concentration on DPPH reduction. DPPH• was incubated with increasing concentrations of either gamma-oryzanol or alpha-tocopherol for 5 min and its absorbance decrease was evaluated at 517 nm. Values plotted are means of triplicate assay; the results are representative of five experiments.

3.4. AMVN initiated lipid peroxidation

The peroxidation of PC liposomes was triggered by the thermic decomposition of the lipophilic free radical generator AMVN, incorporated into lipid vescicles. In these experimental conditions, control liposomes were oxidized at a constant rate as shown by the regular increase of the oxidation index (Fig. 6a). In the presence of gamma-oryzanol, peroxidation rate is reduced in a dose-dependent way; 50 μM gamma-oryzanol was more efficient than 10 μM alpha-tocopherol (Fig. 6a). Interestingly, when 100 μM gamma-oryzanol was exogenously added to preformed PC liposomes as ethanolic solution, it was no more effective in lipoperoxidation inhibition (Fig. 6b); this fact demonstrates that gamma-oryzanol is not able to compartimentalize into liposomes, probably because of its solubility or its physico-chemical features.

3.5. Evaluation of antioxidant activity of gamma-oryzanol in oils

The oxidation behaviour of the oil samples was previously studied. Fig. 7 represents the conductivity curves of the examined oils versus time; the corresponding induction times in hours are reported in Fig. 8. Some oils (castor oil, avocado oil) resulted to be very resistant to heat-induced lipoperoxidation; in particular, it was not possible to evaluate the induction time of castor oil. On the contrary, rosa mosqueta oil, *P.*

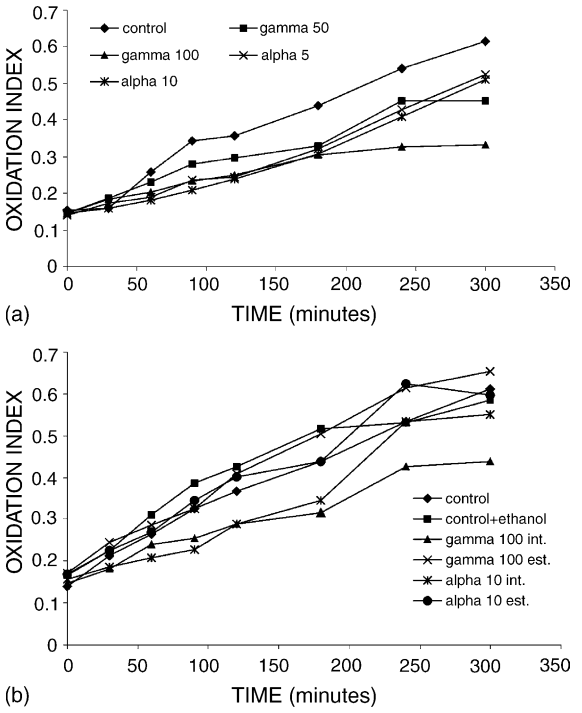

Fig. 6. Effect of gamma-oryzanol on PC liposomes peroxidation triggered by AMVN. Peroxidation was evaluated by oxidation index, which depends on conjugated dienes formation. (a) Gamma-oryzanol is incorporated at two different concentrations (50 and 100 μM) into liposomes. (b) Effect of gamma-oryzanol (50 and 100 μM) both incorporated and exogenously added to PC liposomes.

lentiscus oil B and grape seed oil proved to be very sensitive to the oxidation, because of their high content in polyunsaturated fatty acids. The other oil samples were characterized by a intermediate sensitivity to lipoperoxidation.

The addition to oils of increasing gamma-oryzanol concentrations (2.5–10 mmol/kg oil) produced in general a progressive increase of the AI values of all the samples, reaching a maximum effect at 10 mmol/kg (Fig. 9). At this concentration, the antioxidant activity of gamma-oryzanol was comparable, in most cases, to that one of the same concentration of BHT, although BHA resulted in general more effective (Fig. 10).

4. Discussion

Since there are multiple ways in which a substance can exert its antioxidant activity, we decided to assess the antioxidant mechanism of gamma-oryzanol with different in vitro models. First of all, we have applied experimental models where inorganic oxygen-derived radicals, such as $OH^{\bullet}$ and $O_2^{\bullet -}$, are generated. It is well known that they can be produced by a metal-driven reduction of oxygen and can trigger the so-called "LOOH-independent" initiation of lipid peroxidation (Halliwell and Gutteridge, 1999). Actually, our results seem to exclude that gamma-oryzanol can interfere with this lipoperoxidation mechanism, because, at least

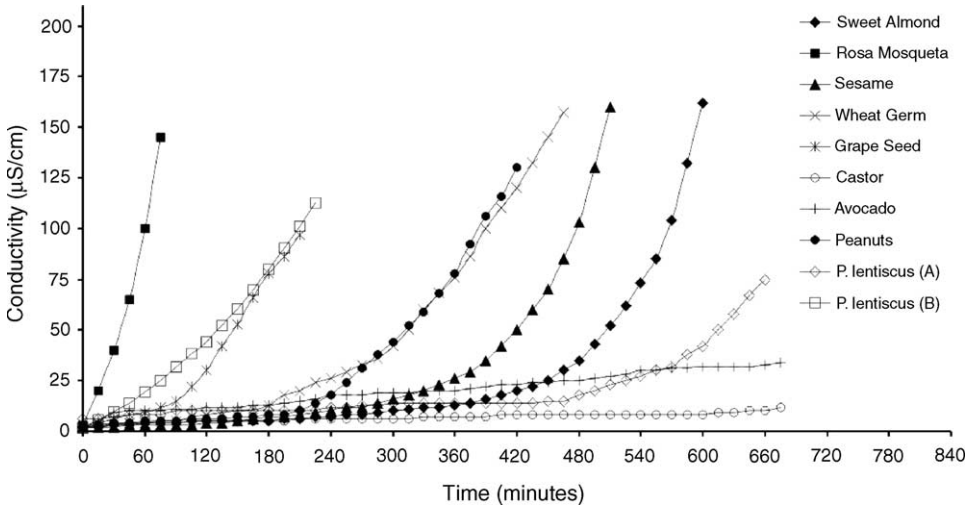

Fig. 7. Induction curves of the examined oils vs. time (mean of three determination).

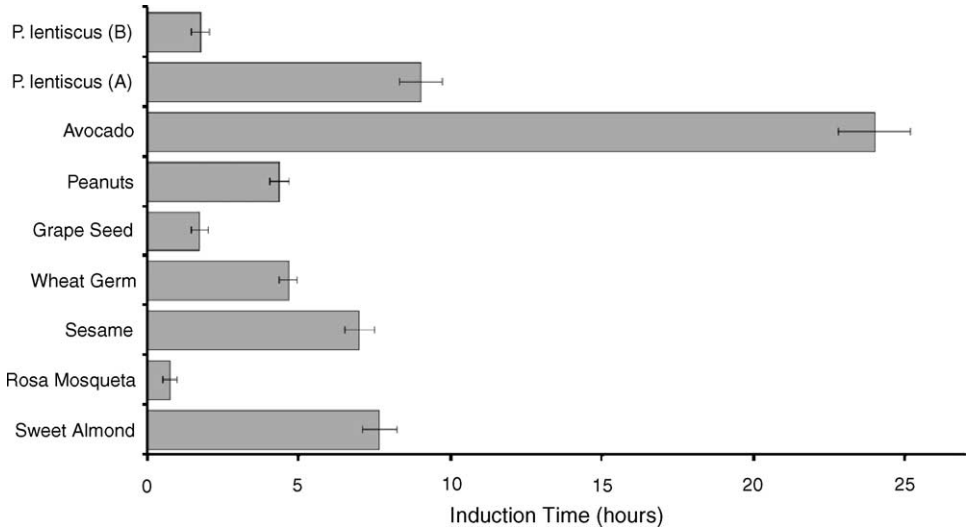

Fig. 8. Induction times of different oils samples (mean ± S.D. of three determination). Error bars indicate standard deviation.

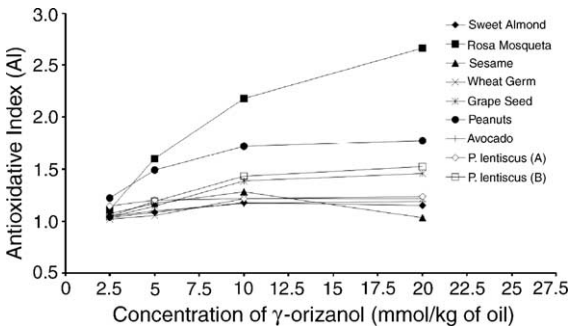

Fig. 9. Dose-dependence of the antioxidant activity of gamma-oryzanol dissolved in oil in concentration range 2.5–20.0 mmol/kg of oil (mean of three determination).

in our experimental conditions, it did not prove to be a $OH^{\bullet}$ nor $O_2^{\bullet -}$ scavenger.

We verified then the possibility that gamma-oryzanol may affect the lipoperoxidation interfering with the organic radicals. To this purpose we utilized as experimental model the phosphatidylcholine liposomes peroxidation initiated by azoinitiator AMVN. Our results demonstrate that gamma-oryzanol is able to inhibit lipid soluble organic radicals at concentration of 50–100 μM. However, this effect can be observed only when gamma-oryzanol is incorporated within liposomes, while, when exogenously added to the liposome suspension, it is no more able to inhibit lipoperoxidation, probably because it cannot spontaneously localize within the lipid phase. In our experimental conditions, gamma-oryzanol appeared to be a chain-breaking antioxidant less efficient than alpha-tocopherol, which scavenges organic radicals at lower concentrations. The effect of gamma-oryzanol on organic radicals is confirmed by its ability in scavenging the lipid-soluble $DPPH^{\bullet}$ radical.

The free radical scavenging action of gamma-oryzanol and their protective effect against lipoperoxidation make it a good candidate for use as natural antioxidant of technological interest. To verify its applicability in protection of lipidic raw materials, different oil samples were added with increasing concentrations of gamma-oryzanol and then subjected to an accelerated oxidation test. Gamma-oryzanol produced a dose-dependent increase of the induction time, with a maximum effect at 10 mmol/kg and an efficiency comparable to that one of BHT. Its protective effect from lipoperoxidation induced by heating and O_2 exposition was especially interesting in oils rich in polyunsaturated fatty acids, such as rosa mosqueta oil (a concentrated solution of linoleic (41%) and linolenic (39%) acid; Moreno Gimenez et al., 1990), and grape seed oil (which contains 72–76% of linoleic acid; Cao and Ito, 2003).

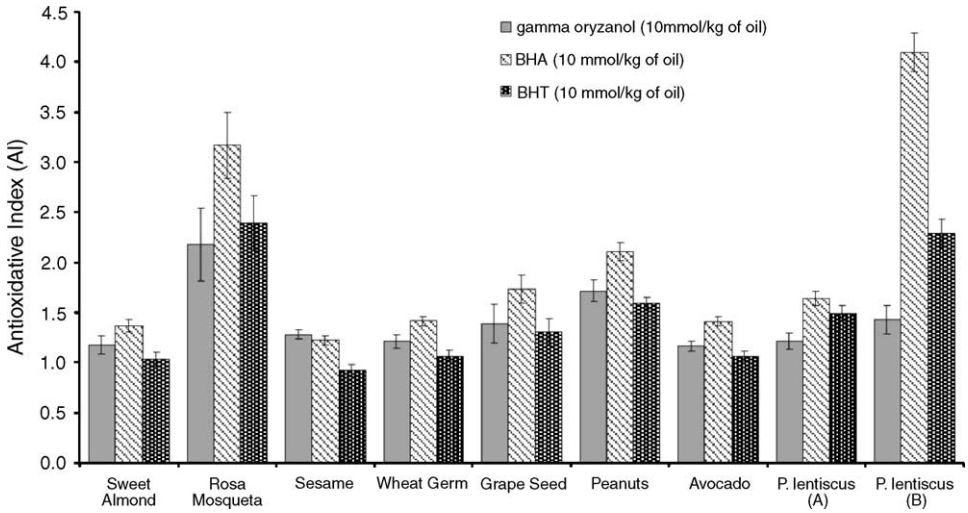

Fig. 10. Antioxidant activity of gamma-oryzanol (10 mmol/kg of oil) in comparison with the synthetic antioxidants BHA and BHT at the same concentration dissolved in the different oils (mean ± S.D. of three determination).

To conclude, the results from our investigation indicate that gamma-oryzanol may have potential application as a natural antioxidant for the stabilization of oils; its antioxidant properties are not exceptional but they could be enhanced by the association with other natural antioxidants, obtaining mixtures able to replace the more common synthetic stabilizers. It is very interesting that gamma-oryzanol possesses many other biological activities (e.g., sunscreen, anticholesterol), which make it an useful multifunctional ingredient for pharmaceutical and cosmetic formulations and for foods. Moreover, rice bran oil, naturally rich in gamma-oryzanol and in tocopherols, could be suggested as a novel excipient for topic application. Future development of our work will point to the assessment of gamma-oryzanol as antioxidant in complex lipophilic formulations, such as ointments and emulsions.

Acknowledgements

The authors are grateful to Professor Bruna Tadolini (Dipartimento di Scienze Biomediche di Sassari, Italy) for the fruitful discussions concerning the mechanism of the antioxidant action of gamma-oryzanol. This work has been supported by a grant from ex 60% MIUR.

References

Atzei, A.D., 2004. Le piante nella tradizione popolare della Sardegna, first ed. Delfino, Sassari.

Cao, X., Ito, Y., 2003. Supercritical fluid extraction of grape seed oil and subsequent separation of free fatty acids by high-speed counter-current chromatography. J. Chromatogr. A 1021, 117–124.

Cicero, A.F.G., Gaddi, A., 2001. Rice bran oil and γ-oryzanol in the treatment of hyperlipoproteinemias and other conditions. Phytother. Res. 15, 277–289.

Coppini, D., Paganizzi, P., Santi, P., Ghirardini, A., 2001. Capacità protettiva nei confronti delle radiazioni solari di derivati di origine vegetale. Cosmetic News 136, 15–20.

Hadorn, H., Zuercher, K., 1974. Determination of the antioxidant stability of oils. Deutsch. Lebens. Rund. 70, 57–65.

Halliwell, B., Gutteridge, J.M.C., 1999. Free Radicals in Biology and Medicine, third ed. Oxford University Press, New York, p. 106.

Hiramitsu, T., Armstrong, D., 1991. Preventive effect of antioxidants on lipid peroxidation in the retina. Ophtalmic Res. 23, 196–203.

Ito, N., Hirose, M., Fukushima, S., Tsuda, R., Shirai, T., Tatematsu, M., 1986. Studies on antioxidants: their carcinogenic and modifying effects on chemical carcinogenesis. Food Chem. Toxicol. 24, 1071–1082.

Joyeux, M., Lobstein, A., Anton, R., Mortier, F., 1995. Comparative antilipoperoxidant, antinecrotic and scavenging properties of terpenes and biflavones from Ginkgo and some flavonoids. Planta Med. 61, 126–129.

Kim, J.-S., Godber, J.S., 2001. Oxidative stability and vitamin E levels increased in restructured beef roast with added rice bran oil. J. Food Qual. 24, 17–26.

Kim, J.-S, Han, D., Moon, K.D., Rhee, J.S., 1995. Measurement of superoxide dismutase-like activity of natural antioxidants. Biosci. Biotechnol. Biochem. 59, 822–826.

Lakkakula, N.R., Lima, M., Walker, T., 2004. Rice bran stabilization and rice bran oil extraction using ohmic heating. Bioresour. Technol. 92, 157–161.

Lupo, M.P., 2001. Antioxidants and vitamins in cosmetics. Clin. Dermatol. 19, 467–473.

Moreno Gimenez, J.C., Bueno, J., Navas, J., Camacho, F., 1990. Treatment of skin ulcer using oil of mosqueta rose. Med. Cutan. Ibero Lat. Am. 18, 63–66.

Nanua, J.N., McGregor, J.U., Godber, J.S., 2000. Influence of high-oryzanol rice bran oil on the oxidative stability of whole milk powder. J. Dairy Sci. 83, 2426–2431.

Rigo, A., Stevanato, R., Finazzi-Agrò, A., Rotilio, G., 1977. An attempt to evaluate the rate of the Haber–Weiss reaction by using $OH^{\bullet}$ radical scavengers. FEBS Lett. 80, 130–132.

Shin, T., Godber, J.S., Martin, D.E., Wells, J.H., 1997. Hydrolitic stability and changes in E vitamers and oryzanol of extruded rice bran during storage. J. Food Sci. 62, 704–708.

Tadolini, B., 1987. Iron autoxidation in Mops and Hepes buffers. Free Radic. Res. Commun. 4, 149–160.

Tadolini, B., Juliano, C., Piu, L., Franconi, F., Cabrini, L., 2000. Resveratrol inhibition of lipid peroxidation. Free Radic. Res. 33, 105–114.

Whysner, L., Wang, C.X., Zang, E., Iatropoulos, M.J., Williams, G.M., 1994. Dose response of promotion by butylated hydroxyanisole in chemically initiated tumours of the rat forestomach. Food Chem. Toxicol. 32, 215–222.

Williams, G.M., Iatropoulos, M.J., Whysner, J., 1999. Safety assessment of butylated hydroxyanisole and butylated hydroxytoluene as antioxidant food additives. Food Chem. Toxicol. 37, 1027–1038.

Xu, Z., Godber, J.S., 1999. Purification and identification of components of γ-oryzanol in rice bran oil. J. Agric. Food Chem. 47, 2724–2728.

Xu, Z., Hua, N., Godber, J.S., 2001. Antioxidant activity of tocopherols, tocotrienols, and gamma-oryzanol components from rice bran against cholesterol oxidation accelerated by 2,2′-azobis(2-methylpropionamidine) dihydrochloride. J. Agric. Food Chem. 49, 2077–2081.