Free Radicals in Virgin Olive Oil: A Spin Trapping EPR Study

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ABSTRACT

The spin trap 5 5-dimethyl-1-pyrroline-*N*-oxide (DMPO) has been used as a probe for monitoring the oxidation properties of virgin olive oil and the detection of free radicals produced in the oil during storage When DMPO is added in an olive oil sample (or in oxidised triolein) a 12-line Electron Paramagnetic Resonance (EPR) signal is recorded suggesting the trapping of alkoxyl radicals In presence of ethanol the formation of hydroxyl radicals is also detected The 12-line spectrum changes gradually with time and after several days is transformed to a 3-line spectrum Filtration of the olive oil increases the formation of DMPO spin adducts A putative mechanism for the generation of the recorded spin adducts is discussed

INTRODUCTION

It has long been known that oxidative rancidity is the main deteriorative change of olive oil during storage and it is due to the oxidation of unsaturated fatty acids and the subsequent formation of compounds possessing unpleasant taste and odour (1) The oxidation process affecting the stability of vegetable oils is often called autoxidation and involves a free radical mechanism It is assumed that hydroperoxide groups attach to the carbon atom of unsaturated fatty compounds and

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subsequently the breakdown of hydroperoxides gives a chain reaction of autoxidation (2)

Of the available methods for the detection of free radicals, only spin trapping offers the opportunity to simultaneously measure and distinguish among a variety of important chemically or biologically generated free radicals Most of the spin trapping agents used have a nitrone-type group which is able to form a nitroxide (spin-adduct) during the trapping of the free radical, making it detectable in EPR spectroscopy (3) (Fig 4) Among several nitrones used as spin traps DMPO has received the most attention since useful information about the nature of the added radical can be obtained from the size of the nitrogen as well as of beta- and gamma-hydrogen splitting because of the cyclic nature of this molecule (4-5) In this study we have used DMPO as a probe for monitoring free radical formation in virgin olive oil

MATERIALS AND METHODS

Virgin olive oil samples were generously gifted by ELAIS SA, Greece and by the National Agricultural Research Foundation, Subtropics and Olive Institute of Chania Greece Olive oil samples were filtered through a large coarse fluted paper EPR spectra were recorded on a Bruker ER 200D spectrometer system operating at the X-band The samples were contained in an E-248 cell at room temperature Typical instrument settings were centre field 3471 G scan range, 100G, time constant 500ms microwave power 7 96mW microwave frequency, 9 77GHz and modulation amplitude 1G Reaction mixtures were incubated at 25 °C, under ambient light or in the dark for various time periods before EPR measurement When necessary, the reaction mixtures were deoxygenated by bubbling with nitrogen and then incubated under nitrogen atmosphere in sealed tubes

RESULTS AND DISCUSSION

When DMPO was added in an olive oil sample in presence of ethanol, a six -line EPR signal was recorded due to the formation of DMPO-hydroxyethyl radical adducts after the reaction of hydroxyl radicals with ethanol (Fig 1a) In absence of ethanol a more complicated EPR signal was recorded 10 min after the addition of DMPO (Fig 1b) This spectrum gradually changed with time and after several days it was transformed to a three line EPR spectrum (Fig 1c) Similar spectra were recorded when DMPO was added to an oxidised triolein solution, both immediately

after the addition of the spin trap and after several days In contrast, non-oxidised triolein was EPR silent (data not shown) Analysis of the first 12 line spectrum and calculation of the hyperfine splittings suggests the trapping of alkoxyl radicals The outermost lines are assigned to a carbon centred radical adduct (6) Nevertheless, we cannot exclude the involvement of peroxyl radical adducts In this case their 6-line pattern should be masked under the alkoxyl radical signal The three line spectrum may has been produced by replacement of beta hydrogen with an oxidizing agent and thus the hyperfine splitting is provided by the nitrogen atom only The formation of the 12 line EPR spectrum was inhibited by the antioxidant methyl catechol and by compounds present in unfiltered olive oil In several cases, the intensity of the 3-line spectrum of the unfiltered oil was also drastically reduced (not shown)

Storage of olive oil with DMPO for several days in the dark and in absence of air produces adducts showing again a major three line spectrum Nevertheless, in this case the signal comprises additional splittings suggesting that the transformation of the 12 line initial adduct to the final oxidised product was slowed down under these specific conditions (data not shown)

The three line EPR spectrum of the DMPO adduct in olive oil reminds that of an immobilised nitroxide free radical with anisotropic motion, as indicated by differences of individual linewidths Thus if we compare the EPR spectra of the amphiphilic spin label 5-doxyl stearic acid dissolved in olive oil and isooctane, that is, two solvents with different viscosities but with similar low dielectric constants, we can observe that the spectrum of the spin label in olive oil is similar to that of the DMPO adduct (not shown) However the profile of the EPR spectrum of a hydrophilic TEMPO-nitroxide in olive oil is similar to that of a nitroxide radical tumbling rapidly in solution (not shown) This implies that the immobilisation of the DMPO adduct in olive oil is not due to the higher viscosity of the solvent but to the strong hydrophobic interactions between the oil matrix and the lipid chains of the trapped radicals

In conclusion our results have shown that the formation of stable DMPO spin adducts following oxidation reactions in virgin olive oil could help to investigate the mechanisms of the oxidation processes affecting the stability of the oil Moreover, EPR spin-trapping methodology could be used in parallel with other established

laboratory tests as an alternative in assessing the quality of virgin olive oil

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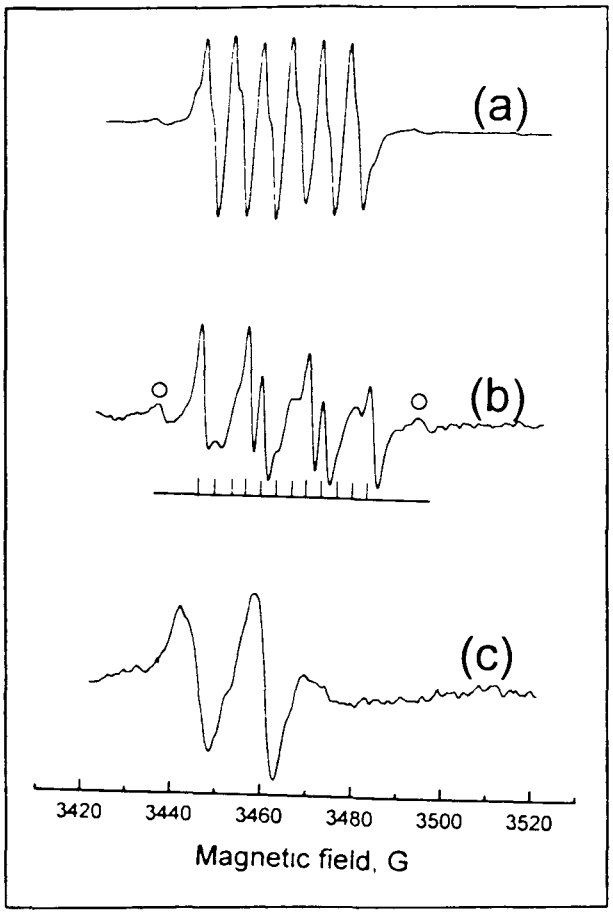

Figure 1 EPR spectra of free radicals formed in virgin olive oil in presence of 18 mM DMPO
a) spin trapping of hydroxyl radicals in presence of 0 17 M ethanol
b) 10 min after addition of DMPO in the absence of ethanol Analysis of the spectrum in terms of lines from the alkoxyl radical species is as shown by the stick diagram, lines marked O are assigned to a carbon-centred radical adduct
c) 7 days after addition of DMPO in the absence of ethanol

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