DETECTION OF FREE RADICALS PRODUCED IN VIRGIN OLIVE OIL. A SPIN TRAPPING AND EPR STUDY

D.Charalabopoulos, S.Avramiotis, T.G.Sotiroudis & A.Xenakis

Industrial Enzymology Unit, Institute of Biological Research and Biotechnology, The National Hellenic Research Foundation, 48 Vassileos Constantinou Ave., Athens 116 35, Greece

It is well known that oxidative rancidity is the main deteriorative change of olive oil during storage. This 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 get attached to the carbon atom of unsaturated fatty compounds and 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). 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 splittings 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.

When DMPO was added in a fresh olive oil sample a complicated multiline EPR signal was recorded immediately after the addition of spin trap. This spectrum changes gradually with time and after several days was transformed to a three line EPR spectrum. Analysis of the multiline spectrum and computer simulation indicated that three different DMPO radical adducts were present. Based on the hyperfine coupling constants, peroxyl, alkoxyl and carbon-centred radical adducts were identified, while peroxyl radicals represented the majority (~70%) of the adducts formed. Similar spectra were recorded when DMPO was added to oxidised (in air) triolein, both immediately after the addition of the spin trap and after several days. However oxidation of triolein by Cu2+ or Fe2+ resulted in the formation of alkoxyl radical adducts almost exclusively. In contrast, non-oxidised triolein was EPR silent. The three line spectrum may have been produced by replacement of beta hydrogen with an oxidising agent and thus the hyperfine splitting is provided by the nitrogen atom only. The formation of the multiline EPR spectrum was blocked by heating or storage of the olive oil for

several months. Nevertheless, heating or storage of oxidised triolein do not affect significantly the formation of the multiline spectrum.

Interestingly, addition of DMPO in olive oil or oxidised triolein dissolved in toluene resulted in the formation of a six-line EPR spectrum, which upon simulation revealed the presence of alkoxyl radical adducts only. The formation of these radical adducts persisted in thermal treatment or storage of the olive oil.

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 three line DMPO adduct. 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. 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.

This work was supported by a grant from the Programme EKBAN-691 of the Greek General Secretariat for Research and Technology with the collaboration of ELAIS S A

REFERENCES

1 Kiritsakis, A & Markakis, P (1987) *Adv Food Res* 31, 453-482
2 Logani, M K and Davies, R E (1979) *Lipids* 15, 485-495
3 Finkelstein, E , Rosen, G M and Rauckman, E J (1980) *Arch Biochem Biophys* 200, 1-16
4 Pou, S , Hassett, D J , Britigan, B E , Cohen, M S and Rosen, G M. (1989) Anal Biochem 177, 1-6
5 Janzen, E G and Liu, J I-P (1973) J Magn Res 9, 510-512