

SPIN TRAPPING AND RADICAL SCAVENGING METHODS IN STUDIES OF BIOGLYCEROL

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Bioglycerol - 1,2,3-propanetriol consisting fraction from biodiesel production- was examined towards its antioxidant activity. All investigated glycerol fractions (GF) exhibited high, in comparison with biodiesel and oil (origin material), free radical scavenging properties. Free radical scavenging activity measured by EPR spectroscopy paralleled standard antioxidant/anticorrosive investigations (Herbert Test, Folin-Ciocalteu method).

Spin trapping electron paramagnetic resonance (ST EPR) spectroscopy and density functional theory (DFT) were used to study the structure of antioxidant species present in GF. The reaction with H_2O_2 was examined via EPR measurements of formed PBN spin adducts. Additionally, pure glycerol, α - and δ -tocopherol along with oil were used as reference chemical systems, since they are potential constituents of GF. The hyperfine parameters calculated from simulated glycerol fraction-PBN adduct EPR spectra indicate the α -tocopherol origin of PBN trapped radicals. Results obtained from DFT calculations of EPR parameters stay in good accordance with experimental ones especially when the solvent effects are included in the calculations. DFT results exclude trapping of oxygen-centered radical and suggest carbon-centered type.

INTRODUCTION

Glycerol fraction (GF) called also bioglycerol is a product of transesterification reaction of edible oils or/and animal fats. During last decades the main interest of transesterification products was pointed only at biodiesel. Glycerol fraction was treated as an unwanted waste material. The problem of bioglycerol overproduction indicated exploration of its new properties (Pagliaro & Rossi, 2008) which will be helpful in finding of the new utilization for the raw fraction. One of such feature is antioxidants content in the fraction. Phenolic antioxidants in vegetable oils were already studied (Koski, Psomiadou, Tsimidou, Hopia, Kefalas, Wähälä & Heinonen, 2002; Isbell, Abbott & Carlson, 1999; Okogeri & Tasioula-Margari, 2002), but their possibly changed structure and concentration when transferred to glycerol fraction during biodiesel production was not investigated yet. Crude glycerol fractions from various biodiesel technologies were found to be a very interesting subject of antioxidant studies by electron paramagnetic resonance (EPR) spectroscopy. The issue of finding the antioxidant routes in the transesterification process from oil to the products is of great importance.

EXPERIMENTAL DETAILS

Materials.

Raw glycerol fractions, oils and biodiesel samples from 7 different biodiesel producers (applied with different, patented technologies) were investigated. Fractions originated in various technological scale, from industrial (100 000 tonnes of biodiesel a year) to a laboratory scale. Also different oils, catalysts and alcohols were used (Jerzykiewicz, Cwiela & Jerzykiewicz, 2009). Raw samples are meant as crude glycerol fractions before removal of soap and neutralization. All glycerol fractions were pre-treated by distilling off water and alcohol.

α -Tocopherol, δ -tocopherol, galvinoxyl radical, *N*-t-butyl- α -phenylnitrone (PBN) and caffeic acid were purchased from Sigma-Aldrich; 1,2,3-propanetriol, ethyl alcohol, hexane and hydrogen peroxide were purchased from POCH S.A. (Polish Chemical Reagents).

Electron Paramagnetic Resonance investigations.

Primary EPR investigations were performed for the raw samples without addition of any solvent. Scans were accumulated 5 times due to the low concentration of radicals.

Advanced EPR analysis was conducted using free radical scavenging and spin trapping method. The measurements of the oils and esters samples were performed for hexane whilst glycerol samples for ethyl alcohol (Jerzykiewicz *et al.*, 2009). Galvinoxyl free

radical (Sigma-Aldrich) solution (1.1mmol/dm³) in ethyl alcohol/hexane accordingly was used for scavenging investigations. The blank sample was located in one cavity, and the analyzed sample in the second one. For quantitative calculation of antioxidants gallic and caffeic acid were used. The same scavenging procedure was used in UV-Vis measurements to compare and confirm the EPR results.

Spin trapping investigations were performed using 0.5ml of (0.067M) PBN solution for every 0.2500g of bioglycerol or oil (Jerzykiewicz, Ćwieląg-Piasecka, Witwicki & Jezierski, 2010). As a reference material pure glycerol, α - and δ -tocopherol and oil were used.

All Electron Paramagnetic Resonance (EPR) spectra were recorded using Bruker ESP300E spectrometer with a 100 kHz magnetic field modulation equipped with a Bruker NMR gaussmeter ER 035M and a Hewlett-Packard microwave frequency counter HP 5350B operating at X-band frequencies at room temperature. Microwave power, 20 mW, modulation amplitude of 1 G and double resonance cavities were used. Measurements were performed for both cavities over the same period of time with at least five replications.

For calculations of the EPR spectra the WINEPR and SimFonia of Bruker program was used.

All computations were performed with GAUSSIAN 09 suite of programs. Geometries were optimized at the UB3LYP/6-31G(d,p) theory level. The UB3LYP/EPR-II and UPBE0/EPR-II methods were employed to calculate the EPR properties. To caption the major effects of solvation Tomasi's polarized continuum method (PCM) was employed using its integral equation formalism variant (IEFPCM) to mimic ethanol ($\epsilon = 24.852$) as a solvent. Furthermore, to take into account the specific interactions between the solvent molecules and solute, the more elaborated computations including the PCM and two ethanol molecules interacting with the nitric oxide radical center were performed.

RESULTS AND DISCUSSION

Galvinoxyl radical scavenging

Methyl esters, oils, and bioglycerols show different effectiveness of galvinoxyl radical scavenging (Fig. 1). GF were found to have the strongest free radical scavenging activity and most of investigated glycerol samples quenched the galvinoxyl radical in the first few minutes of measurements. Oil samples have medium scavenging properties and esters shows the smallest effect.

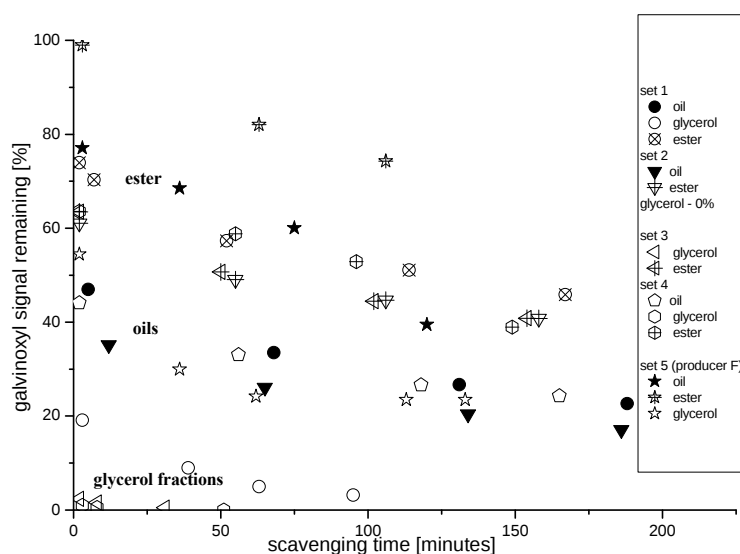


Fig 1. Dependence of galvinoxyl radical scavenging in oils and its product vs. time.

Important is also the fact of different antioxidant concentration for different glycerol fractions (two examples given in table 1). Galvinoxyl radical scavenging activity vary for different producers and is independent from the scale of technology, type of used catalyst or alcohol. Diversity of radical quenching ability divide glycerol fractions among three groups of

low, medium and high antioxidant properties. Bioglycerol with the highest galvinoxyl radical scavenging properties are the same samples which for crude fractions exhibit EPR signal ($g=2.0046$) attributed to oxygen centered radical from polyphenolic antioxidants.

Table 1. Antioxidant concentration (gallic acid as a standard) and hyperfine parameters of PBN-bioglycerol and α -tocopherol adducts

	Antioxidant concentration	First day		1 week		3 weeks	
	[mmol/l]	$a_{\text{iso}}(^{14}\text{N})$	$a_{\text{iso}}(^1\text{H})$	$a_{\text{iso}}(^{14}\text{N})$	$a_{\text{iso}}(^1\text{H})$	$a_{\text{iso}}(^{14}\text{N})$	$a_{\text{iso}}(^1\text{H})$
Bioglycerol 1	5.75	15.3	3.8	15.3	3.8	15.3	3.8
Bioglycerol 2	0.20	15.3	3.8	15.3	3.2	15.5	1.8
α -tocopherol	n/a	-	-	15.3	3.9	15.3	3.8

Spin trapping

Parameters obtained from simulation of PBN-adduct EPR spectra (fig. 2, tab. 1) define unequivocally that antioxidants present in glycerol fraction from biodiesel production have structure of α – tocopherol. Hyperfine splitting constants of bioglycerol samples having the highest antioxidant content remained unchanged during the whole experiment and were the same as for α – tocopherol ($a_{\text{iso}}(^1\text{H}) = 3.8\text{G}$, $a_{\text{iso}}(^{14}\text{N}) = 15.3\text{G}$). These parameters for samples with medium and low antioxidant content differ in time. At the beginning of the experiment parameters are the same as for α – tocopherol, but in time when antioxidant is consumed and the parameters change to those similar to oil (lipid-PBN adduct; $a_{\text{iso}}(^1\text{H}) = 1.8\text{G}$, $a_{\text{iso}}(^{14}\text{N}) = 15.1\text{G}$).

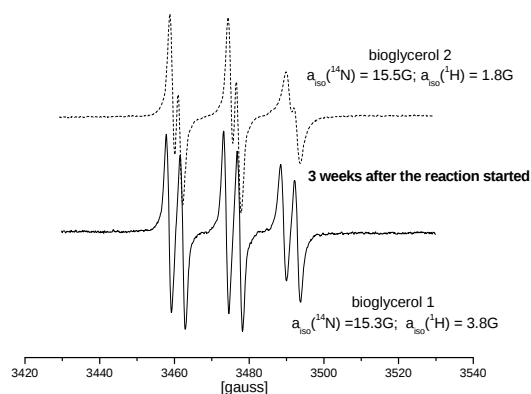


Figure 2. EPR spectra of PBN bioglycerol adducts.

The comparison of experimental and DFT predicted $a_{\text{iso}}(^1\text{H})$ values reveals that the carbon centered α – tocopherol radical is trapped by PBN. However, this fact cannot be unambiguously associated with the antioxidant activity. Though it was shown that antioxidant activity of α -tocopherol against hydroxyl radicals is not selective and can take place at several positions. Moreover one should keep in mind that in experiments reported herein α -tocopherol was exposed

to hydroxyl radicals constantly, even after exhaustion of its antioxidant capability, therefore the probability of formation and trapping of carbon centered radical was additionally increased.

Computational approach

The DFT calculations showed that accurate inclusion of solvent effects (combination of PCM method and explicit ethanol molecules) is mandatory for correct prediction of $a_{\text{iso}}(^1\text{H})$, $a_{\text{iso}}(^{14}\text{N})$ and g-tensor in radical systems under studies.

CONCLUSIONS

The presented results show that most well-known phenolic antioxidants occurring naturally in oils during esterification reaction are transferred to the glycerol fraction. Additionally, hyperfine parameters obtained from simulated spectra and calculated by DFT method suggest that the carbon-centered α – tocopherol radical is trapped by PBN

The proven high content of phenolic antioxidants in the glycerol fraction indicates new possibilities of using the fraction without very expensive purification processes.

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