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REGULAR ARTICLE

PROOXIDANT EFFECT OF LYCOPENE ON TRIGLYCERIDE OXIDATION

Klaudia Jomova[1], Michael Lawson[2], Lukáš Gaľa[2]*

Address: [1]Constantine the Philosopher University in Nitra, Faculty of Natural Sciences, Department of Chemistry, Tr. A. Hlinku 1, 949 74 Nitra, Slovak Republic

[2] Slovak Technical University, Faculty of Chemical and Food Technology, 812 37 Bratislava, Slovak Republic

* Corresponding author: kjomova@ukf.sk

ABSTRACT

The antioxidant activity of carotenoids arises as a result of the ability of the conjugated double bond structure to delocalise species containing unpaired electrons. However, recent clinical trials explored potential prooxidant behaviour of certain carotenoids. In view of these findings we attempted to perform a study of a potential prooxidant effect of the lycopene. A model system which employed oxidation of triglyceride in the presence of lycopene was studied. The results have shown increased formation of hydroperoxides in the presence of lycopene with respect to control experiment (no lycopene). This prooxidant effect of lycopene was found to be reversed by α-tocopherol.

Keywords: carotenoids, lycopene, triglycerides, free radicals, antioxidant, prooxidant

INTRODUCTION

Free radicals, or more generally Reactive oxygen species (ROS) are well recognised for playing a dual role as both deleterious and beneficial species **(Halliwell, 2007)**. Uncontrolled overproduction of ROS results in oxidative stress, a deleterious process that can be an important mediator of cellular damage **(Jomova, Valko, 2011)**. The damaging effect of

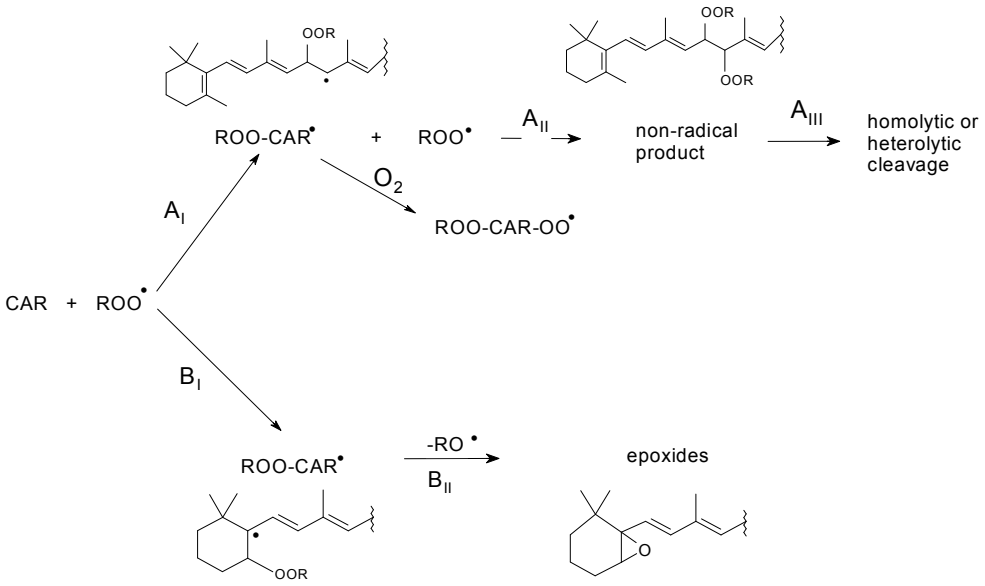
ROS is balanced by the antioxidant action of low-molecular weight antioxidants as well as antioxidant enzymes. Among low-molecular weight antioxidants carotenoids are known to play a key role **(Jomova *et al.*, 2009)**.

It has been widely documented that carotenoids react with a wide range of strongly oxidizing peroxyl radical species via electron transfer producing the radical cation of the carotenoid **(Edge *et al.*, 1997)**. It has also been reported that applying weakly oxidising radicals, such as alkylperoxyl radicals, the reaction proceeds via a hydrogen abstraction process to produce the neutral carotenoid radical **(Jomova *et al.*, 2009)**. In addition, adduct formation with sulfur-centred radicals has also been reported. These three mechanisms can be summarized by the following three reactions and Scheme 1 shown below:

$$CAR + ROO^{\bullet} \rightarrow CAR^{\bullet+} + ROO^{-} \quad \text{(Electron transfer)} \qquad (1)$$

$$CAR + ROO^{\bullet} \rightarrow CAR^{\bullet} + ROOH \quad \text{(Hydrogen abstraction)} \qquad (2)$$

$$CAR + ROO^{\bullet} \rightarrow ROO\text{-}CAR^{\bullet} \quad \text{(Addition)} \qquad (3)$$



Scheme 1 Pathways of the reactions of carotenoids with peroxyl radicals ($ROO^{\bullet}$)

The efficiency of carotenoids to act as antioxidants is dependent on their co-existence and interaction with other antioxidants present in the vicinity of carotenoids. This applies mainly to vitamin E and vitamin C. Carotenoids have been documented to lose their antioxidant activity at high concentrations and/or at high partial pressures of oxygen **(El-Agamey *et al.*, 2004)**. However, this does not necessarily mean, that unlike in vitro systems,

carotenoids act as prooxidants under in vivo conditions. The aim of this work was to elucidate the effect of carotenoids on the oxidation of oils. In this study the effect of lycopene on oxidation of lipids substantiated by the formation of hydroperoxides is presented.

MATERIALS AND METHODS

Chemicals

All chemicals were obtained from commercial sources and were used as received.

Instrumental

The SFC apparatus used in this work consists of a Model 100D syringe pump with control unit, SFX 2-10 supercritical Fluid Extractor (ISCO, USA) and Forward Optical Scanning Detector with a data processing unit (Spectra-Physics, USA). The scheme of the apparatus is shown in Figure 1 **(Hui *et al.*, 1994).**

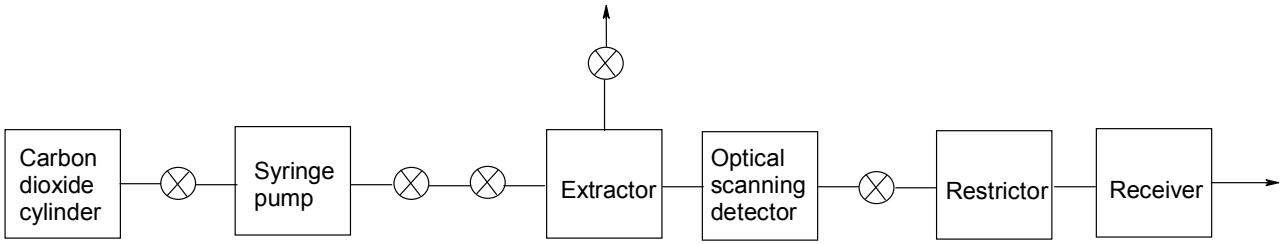


Figure 1 Schematic drawing of SFE apparatus

Supercritical extraction of triglycerides

Triglycerides can be efficiently extracted using supercritical fluid extraction (SFE). It is known that the solubility of triacyglycerols in liquid as well as in supercritical carbon dioxide increases with pressure **(Brannolte *et al.*, 1983)**. At pressures lower than 250 bar the solubility of triacyglycerols is higher in liquid than in supercritical CO_2, while conversely, at pressures higher than 250 bar it is higher in supercritical CO_2. The extraction of triglycerides is routinely carried out at temperatures around 35^{o}C and pressures in the range of 300 – 400 bar. However, better extraction efficiency can be achieved at pressures higher than 400 bar

and elevated temperatures up to 60°C. Such extraction conditions were adopted in our study of SFE from coconut copra which is a naturally rich source of triglycerides. A 57% yield was achieved at pressure 620 bar and temperature 65°C with total amount of used CO_2 approximately 14 kg per kg extract.

Oxidation studies

After adding lycopene (30 μg/g) in hexane to triglycerides, the hexane was evaporated under a mild stream of argon. The samples were oxidised under air in sealed glass bottles using fluorescent lamps in a light cabinet (the light intensity was 13000 lux) at ambient temperature.

The control experiment was represented by a sample containing triglyceride without carotenoid. Oxidation of triglycerides was assessed by measuring formation of hydroperoxides and quantified using a peroxide value (PV). The PV is determined by measuring the amount of I_2 formed by the reaction of peroxides with I^-. The PV is defined as the amount of peroxide oxygen per 1 kilogram of fat or oil and is expressed in milliequivalents. Further details can be found elsewhere. The loss of lycopene following oxidation was monitored spectrophotometrically (spectrophotometer Perkin Elmer, UK) at 455 nm.

RESULTS AND DISCUSSION

The results suggested that lycopene at the concentration of 30 μg/g acts as a prooxidant. This is documented in Figure 2 which shows the effect of lycopene on the formation of hydroperoxides (peroxide value expressed as miliequivalents per kg) in the system consisting of triglycerides oxidised under light at ambient temperature.

Of great surprise is the fact that there is an increase in the amount of formed hydroperoxides in the sample of triglycerides containing lycopene with respect to control (no lycopene added). Thus these experiments clearly confirmed a prooxidant behaviour of lycopene added into the triglyceride system.

We also studied if the prooxidant behaviour of lycopene can be reversed by α-tocopherol (Figure 2). The results have shown the reduction in the amount of formed

hydroperoxides which clearly documents a significant antioxidant effect of α-tocopherol in the studied system.

Enhanced formation of organic hydroperoxides is especially dangerous when metal ions are present in the vicinity of the site of formation. This has been substantiated by the many experimental studies describing the extensive free radical formation from lipid hydroperoxides catalyzed by the transition metals **(Valko, 2005)**. Lipid hydroperoxides can react fast with Fe^{2+} to form lipid alkoxyl radicals (RO•), or much slower with Fe^{3+} to form lipid peroxyl radicals (ROO•).

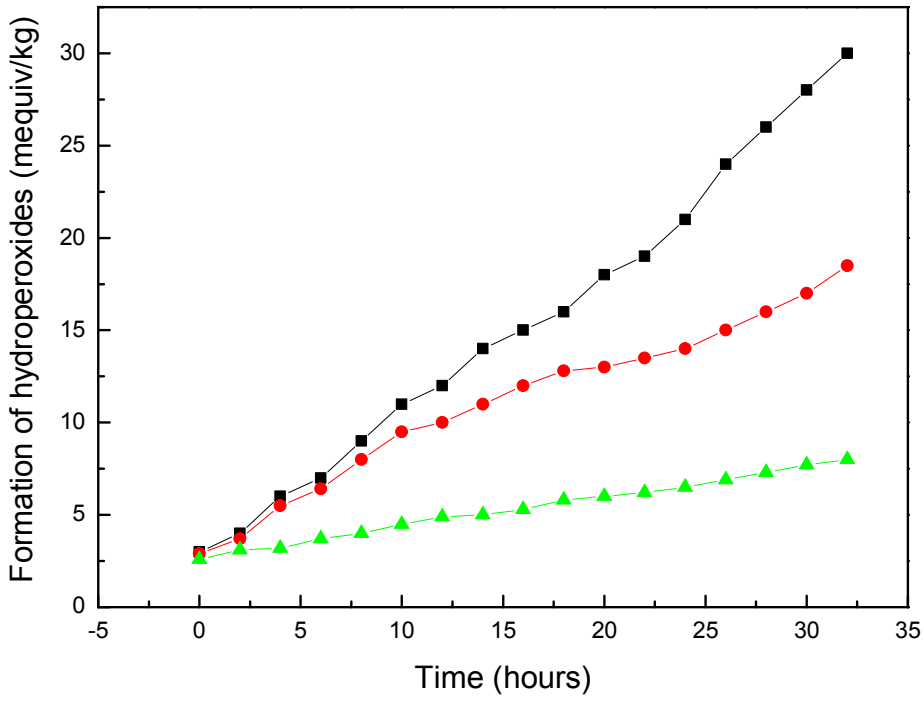


Figure 2 Effect of lycopene alone or in combination with α-tocopherol on the formation of hydroperoxides in the system of oxidized triglycerides under light at 25 °C. (● control experiment - no lycopene; ■ 30 μg/g lycopene; ▲ 30 μg/g lycopene plus 10 μg/g α-tocopherol).

Peroxyl radicals can be rearranged via a series of reactions including a cyclisation reaction to endoperoxides with the final product of the peroxidation process being malondialdehyde (MDA) **(Marnett, 2000)**. The major aldehyde product of lipid peroxidation other than malondialdehyde is 4-hydroxy-2-nonenal (HNE). MDA is mutagenic in bacterial and mammalian cells and carcinogenic in rats. Hydroxynonenal is weakly mutagenic but appears to be the major toxic product of lipid peroxidation.

CONCLUSION

This work points to the importance of mapping of experimental conditions (carotenoid concentration, partial pressure of oxygen, presence of redox active transition metals) under which carotenoids may behave as prooxidants.

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