Meat Science 83 (2009) 201–208

Contents lists available at ScienceDirect

Meat Science

journal homepage: www.elsevier.com/locate/meatsci

# The effect of lutein, sesamol, ellagic acid and olive leaf extract on lipid oxidation and oxymyoglobin oxidation in bovine and porcine muscle model systems

J.E. Hayes [a], V. Stepanyan [a], P. Allen [a], M.N. O'Grady [b], N.M. O'Brien [b], J.P. Kerry [b,*]

[a] Ashtown Food Research Centre, Teagasc, Ashtown, Dublin 15, Ireland
[b] Department of Food and Nutritional Sciences, University College Cork, National University of Ireland, Western Road, Cork, Ireland

ARTICLE INFO

*Article history:*
Received 13 February 2009
Received in revised form 24 April 2009
Accepted 28 April 2009

*Keywords:*
Lutein
Sesamol
Ellagic acid
Olive leaf extract
Lipid oxidation
Oxymyoglobin oxidation
Antioxidant

ABSTRACT

The effect of lutein (100, 200, 300 μg/ml), sesamol (500, 1000, 2000 μg/ml), ellagic acid (300, 600, 900 μg/ml) and olive leaf extract (100, 200, 300 μg/ml) on oxymyoglobin oxidation and lipid oxidation in bovine and porcine muscle model systems (25% *M. longissimus thoracis et lumborum* homogenates) was examined. Radical scavenging activity, using the DPPH assay, and iron-chelating activities of lutein, sesamol, ellagic acid and olive leaf extract were assessed at concentrations ranging from 200 to 1000 ppm. The radical scavenging activity was of the order: ellagic acid > sesamol > olive leaf extract > lutein. None of the natural antioxidants examined exhibited iron chelating activity. Following induced lipid oxidation ($FeCl_3$/sodium ascorbate addition), lipid oxidation and oxymyoglobin oxidation were measured after 24 h at 4 °C. In bovine and porcine muscle model systems, lipid oxidation decreased ($P < 0.001$) following addition of each of the natural antioxidants relative to the control and antioxidant potency followed the order: sesamol > ellagic acid > olive leaf extract > lutein. Ellagic acid and olive leaf extract decreased oxymyoglobin oxidation ($P < 0.001$) while sesamol increased oxymyoglobin oxidation in both systems. The natural antioxidants examined may have applications in the development of nutritional enhanced meat products with enhanced shelf-life characteristics.



## 1. Introduction

Meat colour is influenced by the amount and chemical state of the myoglobin pigment. Oxidation of the cherry red oxymyoglobin to brown metmyoglobin results in meat discoloration. Oxymyoglobin is responsible for the red colouration in highly pigmented muscles such as beef and pink colouration in the less pigmented porcine muscles (Miller, 2002). Antioxidants delay auto oxidation by inhibiting the formation of free radicals during the initiation step of the oxidation reaction or by interrupting propagation of the free radical chain, protecting lipids from oxidation and stabilising oxymyoglobin (O'Grady et al., 1996). Oxidative processes such as lipid oxidation and oxymyoglobin oxidation in meat is a challenging problem to the meat industry. Synthetic antioxidants have been commonly used by the meat industry to reduce lipid oxidation but in recent years consumers and food manufacturers have been opting for meat products with all natural or clean labels. This prompted a surge in the use of natural antioxidants due to the increased limitations on the use of synthetic antioxidants.

Lutein is an oxygenated carotenoid (xanthophylls) abundantly present in dark green leafy vegetables and is one of the most important dietary antioxidants for eye health. Lutein significantly reduces the risk of age-related macular degeneration, atherosclerosis and UV damage (Chen et al., 2002; Mares-Perlman, Millen, Ficek, & Hankinson, 2002; O'Connell et al., 2008). Antioxidant activity of lutein is based on its singlet oxygen quenching ability. Lutein is not synthesised in the body and for this reason it must be ingested via foods that contain it. Ellagic acid is a polyphenol antioxidant found in numerous fruits and vegetables including raspberries, strawberries and other plant foods. Ellagic acid has four phenolic OH groups with a fused benzofuran structure and has a variety of biological activities, including potent antioxidant, anticancer, antimutagen, anti-inflammatory and cardioprotective activity (Ezdihar, Vodhanel, Holden, & Abushaban, 2006; Lei et al., 2003; Priyadarsini, Khopde, Kumar, & Mohan, 2002). Sesamol is a natural organic compound which is a component of sesame oil. Sesamol is a unique dietary phenolic compound as it is stable in physiological pH range, soluble in both aqueous and lipid phases and stable at high cooking temperatures (Joshi, Kumar, Satyamoorthy, Unnikrisnan, & Mukherjee, 2005). Sesamol exhibits anticarcinogenic activity and inhibits atherosclerosis (Decker, 1995). Olive leaf extract is a phenolic compound derived from olive leaves and is known to have anti-oxidative, antimicrobial, antiviral and anti-inflammatory properties and to protect low density lipoprotein from oxidation, the capacity to lower blood pressure in animals and to inhibit lipid oxidation (Bouaziz, Fki, Jemai, Ayadi, & Sayadi, 2008; Khayyal et al., 2002; Micol et al., 2005; Visioli & Galli, 2002).

* Corresponding author. Tel.: +353 21 4903798; fax: +353 21 4270001.
*E-mail address:* joe.kerry@ucc.ie (J.P. Kerry).

0309-1740/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2009.04.019

Meat is biologically complex and consequently, different model systems have been developed in vitro, to gain a better understanding of lipid and oxymyoglobin oxidation processes. Such models involve chemical oxidation induced by transition metals, such as iron or copper, which can generate free radicals. These metal-catalysed oxidation systems have been used to enhance both lipid and oxymyoglobin oxidation. The objective of this study assess antioxidant efficacy in terms of free radical scavenging and iron chelating activity of lutein, sesamol, ellagic acid and olive leaf extract and to determine the influence of these compounds on lipid and oxymyoglobin oxidation following ferric chloride/sodium ascorbate induced oxidation in beef and pork muscle model systems.

## 2. Materials and methods

### 2.1. Reagents

All general laboratory chemicals were obtained from Lennox Laboratory Supplies (Dublin, Ireland). All chemicals used were 'AnalaR' grade. 1,1-Diphenyl-2-picrylhydrazyl free radical (DPPH), ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), L-ascorbic acid ($C_6H_7O_6Na$), L-histidine ($C_6H_9N_3O_2$) were obtained from Sigma–Aldrich Ireland Ltd. (Airton Road, Tallaght, Dublin 24, Ireland). Lutein (4-[18-(4-hydroxy-2,6,6-trimethyl-1-cyclohexenyl)-3,7,12,16-tetramethyloctadeca-1,3,5,7,9,11,13,15,17-nonaenyl]-3,5,5-trimethylcyclohex-2-en-1-ol), sesamol (3,4-methylenedioxyphenol or 1,3-Benzodioxol-5-ol), ellagic acid (2,3,7,8-Ttetrahydroxy-chromeno[5,4,3-cde]chromene-5,10-dione) and olive leaf extract were obtained from Guinness Chemical (Ireland) Ltd. (Clonminam Industrial Estate, Portlaoise Co., Laois, Ireland). The six main phenolic compounds present in olive leaf extract were oleuropein (1151.5 μg/ml), verbascoside (68.6 μg/ml), luteolin-7-O-glucoside (25.6 μg/ml), apigenin-7-O-glucoside (15.9 μg/ml), tyrosol (15.6 μg/ml) and hydroxytyrosol (10.2 μg/ml).

### 2.2. Determination of chelating activity of natural antioxidants on $Fe^{2+}$ ions

The $Fe^{2+}$ chelating ability of lutein, sesamol, ellagic acid and olive leaf extract was measured by the ferrous iron–ferrozine complex method (Decker & Welch, 1990). EDTA was used as a positive control. The chelating ability of lutein, sesamol, ellagic acid and olive leaf extract were measured from 200 to 1000 ppm. The ability of the compounds to chelate ferrous ion was calculated as follows:

$$\text{Chelating activity } (\%) = (1 - A_{562} \text{ of sample}/A_{562} \text{ of control}) \times 100$$

### 2.3. Determination of radical scavenging activity (by DPPH)

The hydrogen atoms or electron donating ability of lutein, sesamol, ellagic acid and olive leaf extract was measured from the bleaching of purple coloured methanol solution of DPPH as described by Groupy, Hugues, Boivin and Amiot (1999). Briefly, 500 μl of diluted methanolic sample and 500 μl of the DPPH (0.238 mg/ml) working solution were added to a micro-centrifuge tube. After vortexing, the tubes were left in the dark for 30 min at room temperature after which the absorbance was measured against methanol at 515 nm using a spectrophotometer (UV-1700 Pharma Spec, Shimadzu, Milton Keynes). The scavenging activity was measured as the decrease in absorbance of the DPPH, expressed as a percentage of the absorbance of a control DPPH solution without test compounds. The inhibitory concentration, $IC_{50}$, which represents the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%, representing a parameter widely used to measure the antioxidant activity, was calculated from a calibration curve by linear regression. The lower the $IC_{50}$, the higher the antioxidant capacity. For ease of interpretation, the antiradical power (ARP) was determined as the reciprocal value of the $IC_{50}$, representing a comparable term for the effectiveness of antioxidant and radical scavenging capacity. The antioxidant activity of the samples can also be expressed in terms of micromoles of equivalents of Trolox (TE) per 100 g of samples (TE/100 g). The scavenging activity of lutein, sesamol, ellagic acid and olive leaf extract were measured from concentrations of 100–1000 ppm.

Radical scavenging activity was calculated as follows:

$$\text{Radical Scavenging activity } (\%) = (1 - A_{515} \text{ of sample}/A_{515} \text{ of control}) \times 100$$

### 2.4. Preparation of muscle homogenates

Fresh beef and pork (*M. longissimus thoracis et lumborum*) was obtained from Olhausen's Ltd. (Blanchardstown Industrial Park, Snugborough Road, Dublin 15, Ireland) and stored at 4 °C prior to use. *M. longissimus thoracis et lumborum* muscle homogenates (25%) were prepared by homogenising 80 g of bovine or porcine *M. longissimus thoracis et lumborum* in 0.12 M KCL 5 mM histidine (240 ml), pH 5.5, using an Ultra-turrax T25 homogeniser (Janke & Kunkel GmbH, IKA® Labortechnik, Staufen, Germany) at 20,462*g* for 5 min. The muscle tissue and buffer were surrounded by crushed ice during homogenisation. Lipid oxidation of muscle homogenate samples (46 g) held at 4 °C, was initiated by the addition of pro-oxidants 45 μM $FeCl_3$/sodium ascorbate (1:1). The concentrations of lutein, sesamol, ellagic acid and olive leaf extract added to muscle homogenates were based on the $IC_{50}$ values (concentration of lutein, sesamol, ellagic acid and olive leaf extract that inhibited caco-2 cell growth by 50%) determined in the Department of Food and Nutritional Sciences at University College Cork for each natural antioxidant. The $IC_{50}$ values were lutein, 265.4 μg/ml; sesamol, 1477 μg/ml, ellagic acid, 551.4 μg/ml and olive leaf extract, 177.8 μg/ml. Lutein, sesamol, olive leaf extract and ellagic acid (plus 2% NaOH) were solubilised in distilled water and added to LD homogenates at the following concentrations: lutein, 100, 200, 300 μg/ml; sesamol, 500, 1000, 2000 μg/ml; olive leaf extract, 100, 200, 300 μg/ml; ellagic acid, 300, 600, 900 μg/ml. The muscle homogenates with and without ferric chloride/sodium ascorbate and without added natural antioxidants were run simultaneously as controls with each experiment. Lipid and oxymyoglobin oxidation measurements were measured initially and in samples held at 4 °C for 24 h.

### 2.5. Measurement of lipid oxidation

Lipid oxidation was measured following a modification of the 2-thiobarbituric acid-reactive substances (TBARS) of Siu and Draper (1978). 10% TCA (4 ml) was added to muscle homogenate (4 ml) and centrifuged at 5820*g* (Beckman Optima™ XL-100 K Ultracentrifuge, Beckman Coulter Inc., 4300 N. Harbour Boulevard, Fullerton, CA 92834-3100, USA) for 15 min at 4 °C. Following centrifugation, the supernatant was filtered through Whatman No. 1 filter paper. The clear filtrate (4 ml) was added to 0.06 M TBA reagent (1 ml) in a screw capped test tube. The tubes were incubated at 80 °C for 90 min and the absorbance of the filtrate was measured spectrophotometrically at 532 nm against a blank containing buffer (2 ml, 0.12 M KCL 5 mM histidine, pH 5.5), 10% TCA (2 ml) and 0.06 M TBA reagent (1 ml). The malondialdehyde content of the