The effect of plant extracts on colour and lipid stability in model bovine muscle systems

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Abstract

This study was carried out to investigate the influence of selected plant extracts, lutein (L), sesamol (S), ellagic acid (EA) and olive leaf extract (OL), on oxymyoglobin oxidation, metmyoglobin formation and lipid oxidation (TBARS) in model bovine muscle systems (25% *M. longissimus thoracis et lumborum* homogenates). Following induced lipid oxidation ($FeCl_3$/sodium ascorbate addition), lipid oxidation, oxymyoglobin oxidation and metmyoglobin formation were measured immediately and after 24 hours storage at 4°C. Lipid oxidation decreased significantly relative to the control ($P < 0.001$) following the addition of each of the plant extracts and antioxidant potency followed the order of S > OL > EA > L. EA and OL decreased oxymyoglobin oxidation ($P < 0.001$) and also inhibited metmyoglobin formation ($P < 0.001$). L had no effect on oxymyoglobin oxidation or metmyoglobin formation ($P > 0.05$). The addition of S resulted in an increase ($P < 0.001$) in oxymyoglobin oxidation but this was accompanied by an increase in metmyoglobin formation ($P < 0.001$). These health-promoting plant extracts demonstrated antioxidant activity in a model meat system by decreasing lipid oxidation in the bovine muscle systems. The results indicate that these plant extracts have potential in the development of healthier beef and beef products.

Introduction

Oxidative processes such as lipid oxidation and oxymyoglobin oxidation in meat products represents a challenging problem to the meat industry. The colour of fresh meat is the most important quality attribute influencing the consumer's decision to purchase and is generally regarded as the first limiting factor in beef shelf-life (Smith *et al.*, 2000). The beef colour is influenced by the amount and chemical state of the myoglobin pigment. The oxygenated form of myoglobin (oxymyoglobin) is responsible for the bright-red colour while the oxidised form (metmyoglobin) is responsible for browning. Many studies have provided evidence that oxidation of oxymyoglobin to metmyoglobin and lipid oxidation are closely interrelated with an increase in one resulting in a similar increase for the other (O'Grady *et al.,* 2001). Antioxidants delay auto oxidation, protect lipids from oxidation and stabilise oxymyoglobin. In order to protect lipids and avoid deterioration of appearance, meat product manufacturers in the past few decades have used several food additives with antioxidative properties. Current recommendations restrict the use of synthetic antioxidants which has prompted a huge interest in the use of natural occurring ingredients such as plant extracts. Lutein is an oxygenated carotenoid (xanthphylls) abundantly present in dark green leafy vegetables and is one of most important dietary antioxidants for eye health. Lutein is not synthesised in the body and for this reason it must be ingested via foods that contain it. Lutein significantly reduces the risk of age-related macular degeneration (O'Connell *et al.*, 2008). Ellagic acid and sesamol are polyphenol antioxidants found in numerous fruits and vegetables and have been found to exhibit anticarcinogenic activity and inhibit atherosclerosis (Decker *et al.*, 1995). Olive leaf extract, a phenolic compound derived from olive leaves, is known to have anti-oxidative properties. Incorporation of phytochemicals/nutraceuticals into 'functional' meat products offers considerable health benefit potential to consumers and would be highly beneficial to the meat industry. The aim of this research was to determine the possible protective effect of lutein, sesamol, ellagic acid olive leaf extract on oxymyoglobin and lipid oxidation in 25% *M. longissimus thoracis et lumborum* bovine muscle homogenates.

Materials and Methods

M. longissimus thoracis et lumborum muscle homogenates (25%) were prepared in 0.12M KCL 5mM histidine (240ml), pH 5.5, using an Ultra-turrax tissue homogeniser. Lipid oxidation of muscle homogenate samples (46g) held at 4°C, was initiated by the addition of pro-oxidants, 45µM $FeCl_3$/sodium ascorbate (1:1). The concentrations of plant extracts were based on the IC_{50} values (concentration of extract that inhibited caco-2 cell growth by 50%). The IC_{50} values were L, 265.4µg/ml; S, 1477µg/ml and OL, 177.8µg/ml. (IC_{50}EA not calculated). L, S, OL and EA (plus 2ml NaOH) were solubilised in distilled water and added to LD homogenates at the following concentrations: L, 100µg/ml, 200µg/ml, 300µg/ml; S, 500µg/ml,

1000μg/ml, 2000μg/ml; OL, 100μg/ml, 200μg/ml, 300μg/ml; EA, 100μg/ml, 200μg/ml, 300μg/ml. The muscle homogenates without added antioxidants were run simultaneously as controls in all experiments. Lipid oxidation and oxymyoglobin measurements were measured initially and in samples held at 4^{o}C for 24 hours.

Measurement of lipid oxidation: Lipid oxidation was measured following a modification of the 2-thiobarbituric acid-reactive substances (TBARS) of Siu and Draper (1978). The absorbance of the sample was measured spectrophotometrically at 532 nm. The malondialdehyde content of the sample was calculated using an extinction coefficient of 1.56 x 10^5 M^{-1} cm^{-1}. Results were expressed as TBARS in mg malondialdehyde (MDA)/mg protein.

Protein determination: The protein concentration of the homogenates was determined according to the method of Markwell *et al.,* (1978) using bovine serum albumin (BSA) as a standard.

Measurement of oxymyoglobin oxidation: The relative proportions of oxymyoglobin (OxyMb) and metmyoglobin (MetMb) were calculated as described by Krzywicki (1982) using absorbance measurements at selected wavelengths of 525, 545, 565, 572 and 730nm.

Statistical analysis: Data was analysed using one-way analysis of variance (ANOVA) and the F-protected LSD test in GenStat Release (10.1). Each experiment was preformed three times and carried out in duplicate.

Results and discussion

Following induced lipid oxidation ($FeCl_3$/sodium ascorbate addition), lipid oxidation, oxymyoglobin oxidation and metmyoglobin formation was measured immediately and after 24 h storage at 4^{o}C. In model bovine muscle systems, lipid oxidation was reduced ($P < 0.001$) at time zero following the addition of OL and S in comparison to the control which contained no plant extracts. Following incubation for 24 h, lipid oxidation decreased ($P < 0.001$) relative to the control following the addition of all of the plant extracts. Lipid oxidation decreased with increasing concentration of L and OL. Since a number of polyphenolic compounds such as oleuropein, and luteolin are present in the olive leaf extract, the antioxidant potential demonstrated in the present study may also be due to the additive and synergistic effects of the individual compounds present. Increasing concentrations of EA had no effect on reducing lipid oxidation further as lipid oxidation was significantly reduced to a similar level ($P > 0.05$) at all concentrations. None of the plant extracts at concentrations employed in this study exerted any prooxidant activity in bovine muscle homogenates. The antioxidant potency followed the order of S > OL > EA > L. The antioxidant efficacy of L, S and OL correlated well with their corresponding IC_{50} values.

The initial level of oxymyoglobin reduced significantly ($P < 0.001$) with increasing levels of OL. Increasing levels of S and EA resulted in a reduced level of oxymyoglobin ($P < 0.001$). Following incubation for 24 h, oxymyoglobin oxidation was significantly ($P < 0.001$) reduced following the addition of EA and OL at all concentrations. OL was the most effective plant extract in reducing oxymyoglobin oxidation. L had no effect on reducing oxymyoglobin oxidation relative to the control. The level of oxymyoglobin increased by 5% ($P < 0.05$) following addition of S at a concentration of 500μg/ml; however the addition of S at higher concentrations resulted in an increase ($P < 0.001$) in oxymyoglobin oxidation and an increase in the formation of metmyoglobin. This is a negative effect as an increase in metmyoglobin (i.e. browning) would have a detrimental effect on overall meat colour. Metmyoglobin formation was reduced ($P < 0.001$) with increasing EA and OL concentrations, with higher concentrations of OL resulting in <1% metmyoglobin.

Table 1. Oxymyoglobin oxidation in 25% bovine *M. longissimus thoracis et lumborum* homogenates following the addition of L (200-600μg/ml), S (500 – 2000 μg/ml) EA (100-300 μg/ml) and O (100-300 μg/ml)

		Storage time at 4°C (hours)					
		0			24		
Incubate	μg/ml	OxyMb (%)	MetMb (%)	[MDA[1]]/ mg protein	OxyMb (%)	MetMb (%)	[MDA]/ mg protein
H[2]+P[3]		79.93[a]	1.15[a]	0.050[a]	13.67[a]	77.71[a]	1.246[a]
H+P+L	200	77.75[a]	0.99[a]	0.042[a]	19.52[a]	70.74[b]	0.719[b]
H+P+L	400	77.21[a]	-0.24[a]	0.050[a]	15.23[a]	75.21[ac]	0.697[b]
H+P+L	600	78.55[a]	4.45[b]	0.033[a]	14.22[a]	74.18[c]	0.665[b]
H+P+S	500	54.59[b]	29.68[b]	0.034[b]	18.33[b]	72.75[a]	0.022[b]
H+P+S	1000	42.30[c]	44.04[c]	0.025[b]	8.42[c]	77.74[a]	0.039[c]
H+P+S	2000	25.65[d]	62.75[d]	0.025[b]	4.19[d]	88.75[b]	0.020[b]
H+P+EA	300	63.05[b]	15.67[b]	0.081[b]	68.72[b]	15.43[b]	0.103[b]
H+P+EA	600	58.53[c]	20.06[c]	0.082[b]	62.89[b]	19.75[bc]	0.101[b]
H+P+EA	900	53.39[d]	24.15[d]	0.078[b]	61.41[b]	21.82[c]	0.103[b]
H+P+OL	100	93.89[b]	-10.65[b]	0.011[b]	83.50[b]	7.82[b]	0.171[b]
H+P+OL	200	94.30[b]	-10.92[b]	0.011[b]	83.76[b]	-0.36[b]	0.090[c]
H+P+OL	300	93.30[b]	-10.07[b]	0.019[b]	83.74[b]	0.90[b]	0.041[d]

[1][Malondialdehyde]/mg protein; [2]25% *M. longissimus thoracis et lumborum* homogenates; [3]$FeCl_3$/ascorbate; [abcd] Mean values in the same column with different superscripts are significantly different, $P \leq 0.05$. (Each antioxidant type was compared to the control)

Conclusions

The health-promoting plant extracts lutein (L), sesamol (S), ellagic acid (EA) and olive leaf extract (OL) demonstrated antioxidant activity in a model meat system by reducing lipid and oxymyoglobin oxidation in the bovine model muscle systems. The results indicate that these plant extracts have potential in the development of healthier beef and beef products.

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