PE4.104 The Effect of Functional Ingredients on Colour and Lipid Stability in Model Porcine Muscle Systems 376.00

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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 pork and pork products.

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I. 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 meat shelf-life (Smith *et al.,* 2000). The pork 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 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. 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, 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 is a phenolic compounds derived from olive leaves, 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* porcine muscle homogenates.

II. MATERIALS AND METHODS

Materials and chemicals: 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 functional ingredients was based on the IC_{50} value (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 (C) in all experiments. Lipid oxidation and oxymyoglobin measurements were measured initially and in samples held at 4°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 1.56×10^5 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

III. 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 hours storage at 4°C. In model bovine muscle systems lipid oxidation decreased ($P < 0.001$) relative to the control following the addition of all of the plant extracts.

Table 1. Lipid oxidation in 25% porcine *M. longissimus thoracis et lumborum* homogenates following the addition of lutein (200-600 µg/ml), sesamol (500 – 2000 µg/ml), ellagic acid (300-900 µg/ml) and olive leaf extract (100-300 µg/ml)

[abcd] Mean values in the same column with different superscripts are significantly different, $P \leq 0.05$. (Each treatment type was compared to the control)

		Storage Time at 4°C (hr)	
		0	24
Incubate	Level (µg/ml)	[MDA[1]]/mg protein	[MDA]/mg protein
C[3]		0.028 ± 0.002^a	0.395 ± 0.071^a
C + L[4]	200	0.017 ± 0.004^a	0.400 ± 0.046^a
C + L	400	0.017 ± 0.004^a	0.411 ± 0.051^a
C + L	600	0.014 ± 0.003^a	0.356 ± 0.049^a
C		0.026 ± 0.004^a	0.389 ± 0.086^a
C + S[5]	500	0.024 ± 0.002^a	0.019 ± 0.002^b
C + S	1000	0.019 ± 0.002^a	0.016 ± 0.005^b
C + S	2000	0.017 ± 0.007^a	0.011 ± 0.003^b
C		0.029 ± 0.003^a	0.384 ± 0.031^a
C + EA[6]	300	0.031 ± 0.008^a	0.027 ± 0.003^b
C + EA	600	0.025 ± 0.004^a	0.016 ± 0.003^b
C + EA	900	0.027 ± 0.006^a	0.015 ± 0.003^b
C		0.021 ± 0.004^a	0.448 ± 0.024^a
C + OL[7]	100	0.019 ± 0.006^a	0.209 ± 0.013^b
C + OL	200	0.015 ± 0.006^a	0.132 ± 0.018^c
C + OL	300	0.018 ± 0.003^a	0.087 ± 0.010^d

Lipid oxidation decreased with increasing concentration of L and OL. 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. 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.

Table 2: Oxymyoglobin oxidation in 25% porcine *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 (hr)	
	Level	0	24
Incubate	(µg/ml)	[2]OxyMb (%)	OxyMb (%)
C[3]		39.74 ± 0.64[a]	14.30 ± 0.25[a]
C + L[4]	200	33.19 ± 1.56[a]	19.05 ± 1.94[a]
C + L	400	34.77 ± 5.82[a]	19.04 ± 1.49[a]
C + L	600	35.40 ± 3.74[a]	18.69 ± 1.87[a]
C		41.44 ± 2.46[a]	19.77 ± 1.55[a]
C + S[5]	500	36.29 ± 0.43[a]	12.36 ± 2.12[b]
C + S	1000	27.38 ± 2.49[b]	7.28 ± 1.76[b]
C + S	2000	18.67 ± 0.56[c]	9.55 ± 1.45[b]
C		22.40 ± 3.14[a]	19.25 ± 0.42[a]
C + EA[6]	300	26.01 ± 2.00[a]	31.63 ± 2.66[b]
C + EA	600	24.33 ± 0.18[a]	26.89 ± 2.49[c]
C + EA	900	21.25 ± 1.26[a]	23.94 ± 1.63[c]
C		49.87 ± 0.54[a]	18.93 ± 2.24[a]
C + OL[7]	100	49.13 ± 2.36[a]	24.83 ± 1.02[b]
C + OL	200	47.99 ± 2.30[a]	28.19 ± 1.57[c]
C + OL	300	47.95 ± 0.94[a]	31.93 ± 1.64[d]

[abcd] Mean values in the same column with different superscripts are significantly different, $P \leq 0.05$. (Each treatment type was compared to the control)

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 S; however the addition of S at higher concentrations resulted in an increase ($P < 0.001$) in oxymyoglobin oxidation and an increased in 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.

IV. CONCLUSION

These health-promoting nutraceuticals demonstrated antioxidant activity in a model meat system by reducing lipid and OxyMb oxidation in the bovine model muscle systems. The results indicate that these nutraceuticals have potential in the development of healthier pork products.

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