THE EFFECT OF PLANT EXTRACTS ON LIPID OXIDATION AND OXYMYOGLOBIN OXIDATION IN BEEF MUSCLE MODEL SYSTEMS

L. McGovern*1, M.N. O'Grady1, S.A. Aherne1, E. Ryan1, N.M. O'Brien1 and J.P. Kerry1

¹Department of Food & Nutritional Sciences, University College Cork, Cork, Ireland. *Email: lr_McGovern@yahoo.com

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Introduction

Colour and lipid oxidation are important factors affecting the quality of fresh beef. The muscle pigment myoglobin is responsible for the colour of fresh beef. Lipid oxidation is a major quality deteriorative process in muscle foods resulting in a variety of breakdown products which produce off-odours and flavours. The processes of colour and lipid oxidation in beef are linked (O'Grady et al., 2001). In recent years, concerns regarding safety and toxicity of synthetic antioxidants prompted a surge of interest in the use of health promoting natural antioxidants derived from plant sources. The functional properties of many plant extracts have been investigated for their potential use as novel nutraceuticals. Nutraceuticals are believed to modulate the aetiology of many chronic diseases such as coronary heart disease, cancer and diabetes. Rosemary (Rosmarinus officinalis L.), oregano (Origanum vulgare L.) and sage (Salvia officinalis L.) are members of the Lamiaceae family and contain antioxidant compounds such as carnosol, carnosic acid and rosmarinic acid (Capecka et al., 2005). Compounds present in rosemary and sage possess anticancer (Cheung and Tai, 2007) and memory boosting properties, respectively. Extracts of oregano inhibited the growth of H. pylori a stomach ulcer inducing bacterium (Chun et al., 2005). Echinacea (Echinacea purpurea L.), a member of the Asteraceae family, contains compounds such as chlorogenic acid, cynarin, caffeic acid and cichoric acid. Echinacea has immuno-stimulatory and free radical scavenging properties (Facino et al., 1995). The aim of this study was to assess the influence of rosemary (R), oregano (O), sage (S) and echinacea (E) on oxymyoglobin and lipid oxidation in 25% M. longissimus dorsi (LD) in order to determine plant extract levels suitable for inclusion in beef and beef products.

Materials and Methods

Longissimus dorsi (LD) homogenates (25%) were prepared in 0.12 M KCl 5mM histidine buffer pH 5.5 using an Ultra Turrax tissue homogeniser. Lipid oxidation in 50 ml LD homogenate samples, held at 4°C, was initiated by the addition of 45µM FeCl₃/sodium ascorbate (1:1). The concentrations of R, O, S and E added to the LD homogenates were based on the IC₅₀ value (concentration of plant extract that inhibited caco-2 cell growth by 50%) for each plant extract. The IC₅₀ values were R, 123 µg/ml; O, 318 µg/ml; S, 214 µg/ml and E, 1421 µg/ml (Aherne et al., 2007). R, O, S and E (solubilised in distilled water) were added to LD homogenates at the following concentrations: R, 100 µg/ml, 200 µg/ml, 300 µg/ml; O, 200 µg/ml, 400 µg/ml, 600 µg/ml; S, 200 µg/ml, 400 µg/ml, 600 µg/ml; E, 500 µg/ml, 1000 µg/ml, 2000 µg/ml. LD homogenates with and without FeCl₃/ascorbate were run simultaneously as controls. Lipid oxidation and oxymyoglobin (oxyMb) oxidation was measured in samples held at 4°C for up to 24 hours. Lipid oxidation in LD homogenates was measured following a modification of the 2-thiobarbituric acid reactive substances (TBARS) procedure of Siu and Draper (1978). Lipid oxidation was expressed in units of absorbance at 532 nm (Abs₅₃₂). OxyMb oxidation in 25% LD homogenates was measured in the supernatant obtained after centrifuging, filtering and re-centrifuging 7 ml of LD homogenate at 13,000 rpm for 15 minutes at 4°C. The relative portion of oxyMb (% total myoglobin) in LD homogenates was calculated using absorbance measurements at selected wavelengths (730, 572, 565, 545 and 525 nm) as described by Krzywicki (1982). Each experiment was performed three times and all analysis was carried out in duplicate.

Results and Discussion

Following FeCl₃/ascorbate addition, lipid oxidation increased over the 24 hour storage period (Table 1). The increase in lipid oxidation was accompanied by an increase in oxyMb oxidation. The relationship between oxyMb oxidation and lipid oxidation in 25% LD homogenates has been previously reported by O'Grady et al. (2001). Graded addition of each plant extract reduced lipid oxidation relative to controls. Lipid oxidation decreased with increasing concentration of each plant extract and antioxidant potency followed the order: S > R > O > E. The antioxidant efficacy of R, O, S and E correlated well with their corresponding IC₅₀ values. The antioxidant properties of R has been previously demonstrated in beef (Formanek et al., 2001). OxyMb oxidation decreased with increasing plant extract concentration and trends observed were similar to those for lipid oxidation.

Incubate		µg/ml	Time, hours					
				0			24	
			¹ OxyMb, %		² TBARS	OxyMb, %	TBARS	
H ³			86.78 ± 3.95		0.124 ± 0.026	78.71 ± 4.87	0.539 ± 0.097	
H+P ⁴ H+P+			73.67 ± 4.24		0.143 ± 0.025	36.32 ± 4.41	1.690 ± 0.061	
11 1	R	100	85.87 ± 1.82		0.164 ± 0.066	64.67 ± 11.23	1.043 ± 0.056	
		200	87.07 ± 5.88		0.169 ± 0.060	79.23 ± 6.43	0.703 ± 0.057	
		300	87.37 ± 4.80		0.155 ± 0.044	82.77 ± 5.35	0.487 ± 0.074	
H+P+								
	0	200	76.70 ± 2.16		0.143 ± 0.024	47.83 ± 1.55	1.144 ± 0.030	
		400	80.87 ± 3.80		0.142 ± 0.028	60.37 ± 1.50	0.814 ± 0.017	
		600	82.77 ± 1.08		0.149 ± 0.024	67.57 ± 2.76	0.601 ± 0.024	
H+P+								
	S	200	78.17 ± 5.96		0.102 ± 0.003	57.67 ± 5.44	0.598 ± 0.043	
		400	78.85 ± 6.72		0.100 ± 0.004	66.38 ± 6.14	0.280 ± 0.061	
		600	76.57 ± 4.51		0.105 ± 0.007	66.89 ± 5.68	0.199 ± 0.056	
H+P+								
	Е	500	75.90 ± 0.71		0.223 ± 0.047	43.75 ± 1.06	1.483 ± 0.020	
		1000	77.20 ± 3.96		0.224 ± 0.061	51.10 ± 1.84	1.243 ± 0.012	
		2000	74.80 ± 3.68		0.269 ± 0.079	62.95 ± 2.19	0.853 ± 0.096	

Table 1. OxyMb oxidation and lipid oxidation in 25% M. *longissimus dorsi* homogenates following the addition of R ($100 - 300 \mu g/ml$), O ($200 - 600 \mu g/ml$), S ($200 - 600 \mu g/ml$) and E ($500 - 2000 \mu g/ml$) and storage at 4°C.

¹Oxymyoglobin, % of total. ²Abs₅₃₂. ³25% LD homogenate. ⁴FeCl₃/ascorbate.

Conclusions

Health promoting plant extracts demonstrated antioxidant activity in beef muscle model systems. Lipid oxidation decreased with increasing concentrations of all plant extracts and antioxidant potency followed the order: S > R > O > E. Based on the results obtained, suitable plant extract levels were determined for inclusion in beef and beef products.

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