The development of functional pork breakfast sausages containing flavonoid rich extracts: Sensory and technological impact

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Abstract— The objective of this study was to determine the influence of grape seed extract (GS) (100, 200, 300 μg/ml) and rosemary-pomegranate extract (RP) (250, 500, 1000 μg/ml) on lipid and oxymyoglobin oxidation following ferric chloride/sodium ascorbate induced oxidation in a pork muscle model system (25% M. *longissimus thoracis et lumborum* homogenates) and to investigate the effect of the addition of these flavonoid containing extracts on pH, water holding capacity, lipid oxidation, colour, texture and organoleptic properties of raw and cooked pork breakfast sausages. Following induced lipid oxidation, lipid oxidation, oxymyoglobin (OxyMb) oxidation and metmyoglobin (Met Mb) levels were measured after 24 h at 4°C. Lipid oxidation and oxymyoglobin oxidation decreased ($P<0.001$) following the addition of GS and RP at all concentrations relative to the control (C) muscle model system. Two levels of GS (100 and 200 μg/g sausage; GS100 and GS200) and of RP (250 and 500 μg/g sausage; RP250 and RP500) were selected and added to breakfast pork sausages. An analysis of the technological and sensory attributes of these sausages was then carried out. Addition of either GS or RP at both concentrations had no effect on pH, WHC, emulsion stability, colour, composition or texture profile. RP sausages had reduced cook loss ($P<0.05$) relative to the control. GS100, GS200 and RP500 had reduced lipid oxidation. GS and RP at both levels had no effect on sausage appearance, overall liking, tenderness, flavour or juiciness liking. These results demonstrate the potential of natural flavonoid containing extracts to the meat industry in the development of novel healthy functional meat products.

Keywords— **grape seed extract, rosemary/pomegranate extract, pork sausages.**

I. INTRODUCTION

Oxidative processes such as lipid oxidation and oxymyoglobin oxidation in meat is a challenging problem to the meat industry. There is increasing interest in the manufacture of healthier meat products by substituting synthetic ingredients with naturally-sourced extracts and phytochemicals. Research studies have found that the plant derived nutraceuticals may have applications in the development of nutritionally-enhanced meat products with enhanced technological quality, shelf-life characteristics and bioactivity [1, 2].

The use of phytochemicals such as rosemary (*Rosmarinus officinalis L*), pomegranate (*Punica granatum L*) and grape seed extract (*Vitis vinifera L.*) have shown antioxidant and antimicrobial properties in meat products with enhanced oxidative stability [3-5]. Clinical data has shown that the antioxidant potential of grape seed is twenty and fifty fold greater than vitamins E and C, [6] while pomegranate is known to exhibit antioxidant and antimicrobial properties [7]. Rosemary is the only spice commercially available for use as an antioxidant in Europe and the United States.

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 [8]. The objective of this study was to (A) determine the influence of GS and RP on lipid and oxymyoglobin oxidation following ferric chloride/sodium ascorbate induced oxidation in pork muscle model systems and (B) to analyse the effects of GS and RP at optimum concentrations on the technological and sensory properties of pork breakfast sausages.

II. MATERIALS AND METHODS

A. Preparation of muscle homogenates

M. longissimus thoracis et lumborum muscle homogenates (25%) were prepared [8]. GS and RP (Naturex, Avignon, France) were solubilised in distilled water and added to muscle homogenates at the following concentrations: GS 100, 200, 300 µg/ml and RP 250, 500, 1000 µg/ml. The muscle homogenates without added antioxidants were run simultaneously as controls (C). Lipid oxidation and oxymyoglobin measurements were measured initially and in samples held at 4^{o}C for 24 hours.

B. Measurement of lipid oxidation

Lipid oxidation was measured [9] and 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 in model muscle systems and [MDA]/kg product in pork breakfast sausages.

C. *Protein determination and measurement of oxymyoglobin oxidation*

The protein concentration of the pork muscle homogenates was determined [10] and the relative proportions of oxymyoglobin (OxyMb) and metmyoglobin (MetMb) in the muscle model systems were calculated [11].

D. Sausage preparation

Pork breakfast sausages were prepared [1]. Following mincing, raw materials were assigned to one of five treatments: control (no flavonoid extract added), sausage formulations with added GS at level of 200 µg/g and 300µg/g (GS200, GS300, respectively) and RP at level of 250 µg/g and 500 µg/g (RP 250, RP500, respectively). 5kg batches were manufactured in triplicate. All plant extracts were dissolved or dispersed in cold water prior to addition to sausage formulations. Sausages were formulated to contain 1.4% salt. All analyses were carried out following 1 day of refrigerated storage at 4^{o}C.

E. Compositional analysis

The moisture, fat and protein content of the pork sausages was determined [1].

F. WHC and Emulsion stability

The WHC [1] and the emulsion stability of the pork breakfast sausages was determined [12].

G. Colour evaluation

Lightness (L^*), redness (a^*) and yellowness (b^*) values of raw sausages were measured by the CIE LAB system using a dual beam xenon flash spectrophotometer (Ultra Scan XE, Hunter lab, Virginia, USA).

H. Texture profile analysis and sensory evaluation of cooked pork sausages

TPA was applied to the cooked pork sausages [13]. Samples of freshly cooked pork breakfast sausages were evaluated by a 60 member non-trained panel of laboratory co-workers. Pork sausages were cooked by grilling on a conventional oven with turning every 3 min until cooked to an internal temperature of 71^{o}C. Sausages were divided into 25 mm pieces, wrapped in aluminium foil and placed in a bain-marie (50^{o}C) until serving (< 15 min). Samples were labelled with 3 digit random numbers and served in William Latin square order to panellists. Panellists were asked to evaluate tenderness, flavour, juiciness liking, overall appearance, overall liking and purchase intent on an eight point hedonic scale.

I. Statistical analysis

Significance of differences among treatments were determined by analysis of variance (ANOVA) using the Least Square Difference method of GenStat (Release 10.1 Copyright 2007, Lawes Agricultural Trust, Rothamsted Experimental Station, Hertfordshire, UK). Differences were considered significant at the $P < 0.05$ level. Following rescaling, the effect of the panellists in the sensorial responses was analysed by ANOVA according to a complete randomised block experimental design. The entire experiment was replicated three times.

III. RESULTS AND DISCUSSION

A. Porcine muscle model systems

Following induced lipid oxidation ($FeCl_3$/sodium ascorbate), lipid oxidation was measured immediately and after 24 h refrigerated storage.

Table 1 Lipid and Oxymb oxidation in porcine muscle model systems following addition of GS (100-300 µg/ml) and RP (250-1000 µg/ml).

Treatment	Level (µg/ml)	[MDA]/ mg protein	% Met Mb	% Oxy Mb
C		0.406[a]	37.87[a]	13.66[c]
C+GS	100	0.015[b]	23.39[b]	28.11[b]
C+GS	200	0.014[b]	17.42[b]	39.34[a]
C+GS	300	0.016[b]	15.84[b]	47.30[a]
C		0.416[a]	33.01[a]	14.52[a]
C+RP	250	0.017[b]	20.64[b]	22.94[a]
C+RP	500	0.011[b]	19.06[b]	24.16[a]
C+RP	1000	0.011[b]	20.88[b]	23.36[a]

[abc]Mean values in the same column bearing different superscripts are significantly different (P<0.05).

In porcine model muscle systems lipid oxidation decreased (P < 0.001) relative to the control following the addition of GS and RP at all concentrations (Table 1). Increasing concentrations of both flavonoid extracts had no effect on reducing lipid oxidation further as lipid oxidation was reduced to a similar level at all concentrations. GS and RP had similar antioxidant efficiency.

The level of OxyMb in the control muscle model systems after 24 h ranged from 13.7 to 14.5%. OxyMb oxidation was reduced (P<0.001) following the addition of GS with OxyMb% tending to increase with increasing levels of GS. RP at all concentrations increased OxyMb concentration but there was no tendency for the effect to increase at higher inclusion levels. The level of antioxidant potency and changes in oxymyoglobin levels are attributed to compounds suppressing lipid oxidation thereby stabilising oxymyoglobin. These results indicate that GS and RP have potent antioxidant activity and levels of GS (100 and 200 µg/ml) and RP (250 and 500 µg/ml) were selected for analysis in pork breakfast sausages.

B. Analysis of GS and RP on the technological and sensory properties of pork sausages.

The addition of GS and RP extracts had no effect (P>0.05) on moisture (57.5-58.6%) or fat (18.7-20.3%) content of raw pork sausages. Similar moisture (54.7-56.1%) and fat (20.0-21.4%) levels were recorded for all cooked pork sausages. The protein content increased following cooking and ranged from 11.48 to 11.98 g/100g for fresh sausages and from 13.59 to 14.93 g/100g in cooked sausages.

Table 2 Effect of GS and RP on pH, WHC, cookloss and lipid oxidation of raw pork sausages

Treatment	pH	WHC	Cookloss	TBARS
Control	6.3[a]	79.5[a]	7.9[ab]	0.51[a]
GS 100	6.2[a]	81.7[a]	8.3[a]	0.40[a]
GS 200	6.2[a]	79.9[a]	8.0[ab]	0.39[a]
RP 250	6.2[a]	79.3[a]	7.5[ab]	0.41[a]
RP 500	6.2[a]	80.4[a]	7.2[b]	0.39[a]

[ab]Mean values in the same column bearing different superscripts are significantly different (P<0.05).

The addition of GS and RP had no effect on the pH, WHC and TBARS of the raw pork breakfast sausages (Table 2). The addition of RP at both concentrations reduced the cook loss in the pork breakfast sausages relative to the control.

Table 3 Effect of GS and RP on the colour of raw and cooked pork sausages

	Raw			Cooked		
	L*	a*	b*	L*	a*	b*
Control	69.4[a]	9.7[a]	17.6[a]	67.3[a]	4.2[a]	16.3[a]
GS 100	69.3[a]	8.6[ab]	17.2[a]	66.6[a]	4.5[a]	15.2[bc]
GS 200	70.4[a]	7.9[bc]	16.6[a]	67.0[a]	4.2[a]	14.8[c]
RP 250	68.8[a]	8.8[bc]	17.0[a]	64.7[a]	4.6[a]	15.7[ab]
RP 500	69.1[a]	8.1[c]	17.1[a]	65.1[a]	4.4[a]	15.9[ab]

[abc]Mean values in the same column bearing different superscripts are significantly different (P<0.05).

No major colour differences were observed in the raw and cooked pork sausages however small differences (P<0.05) were recorded resulting in slight reduction in a* redness values following the addition

of RP at concentrations of 250 and 500 μg/g and GS at 200 μg/g possibly due to the colour of the extracts. In cooked pork sausages GS reduced b* yellowness values relative to the control (Table 3).

The addition of GS and RP at both concentrations had no effect (P>0.05) on hardness, chewiness, cohesiveness, springiness and gumminess of the cooked pork breakfast sausages.

The untrained panel (n=60) found the addition of RP and GS had no detrimental effect on the appearance liking, overall liking, tenderness, flavour or juiciness liking of the pork breakfast sausages.

IV. CONCLUSIONS

Natural flavonoid extracts such as GS and RP were found to exhibit potent lipid antioxidant activity in porcine muscle model systems.

GS and RP had no detrimental effect on the sensory, technological properties and improved lipid stability in raw and cooked pork sausages. These results demonstrate the potential of natural flavonoid extracts to the meat industry in the development of novel healthier meat products. In addition, from a nutritional point of view, the addition of natural functional ingredients such GS and RP to pork sausages could provide bioactive components and also addresses consumer demands for healthier functional meat products.

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