51st International Congress of Meat Science and Technology
August 7-12, 2005 – Baltimore, Maryland USA

ASSESSMENT OF HEALTH PROMOTING PLANT DERIVED NEUTRACEUTICALS WITH POTENTIAL ANTIOXIDANT PROPERTIES FOR USE IN MEAT SYSTEMS

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Key Words: phytochemicals, plant extracts, oxidative stress, meat quality

Introduction

Phytochemicals and plant extracts, present in fruit, vegetables, plants, herbs and beverages exhibit a wide range of biological activities in vitro, attributed in part, to the presence of polyphenolic compounds with antioxidant and free radical scavenging properties (Beattie et al., 2005). In general, there is now a greater demand than ever by consumers for foods perceived as natural, fresh tasting, healthy and more nutritious. Concerns over the safety of synthetic compounds have resulted in renewed interest in the incorporation of natural compounds into foods as a means of improving product quality and shelf life. Lipid oxidation is one of the major causes of quality deterioration in muscle foods, resulting in adverse effects on flavour, colour, texture and nutritional value. The antioxidant activity of plant-based extracts has been reported in beef (Mansour and Khalil, 2000), turkey (Botsoglou et al., 2003) and pork (Rey et al., 2005; Nissen et al., 2004; McCarthy et al., 2001). In light of the current dietary recommendations for increased consumption of fruit and vegetables, the dramatic rise in the use of plant derived extracts and phytochemicals as supplements in the human diet, and consumer demands for quality enhanced meat products serving as neutraceutical sources for consumer health, studies on the biological effects of such compounds merits investigation.

Objectives

The objective of the present study was, to investigate the biological activity of two phytochemicals, resveratrol (RES) and citroflavan-3-ol (C3ol) and four plant derived extracts, grapeseed polyphenols (GSP), olive leaf extract (OLE), bearberry (BB) and echinacea purpurea (ECH) under conditions of oxidative stress in a human monocytic blood cell line, U937 cells. The effect of direct addition of selected plant extracts, GSP and BB, on colour, lipid stability, pH and microbial status of minced pork was also investigated.

Methodology

The compounds were solubilised in methanol, with the exception of echinacea purpurea in distilled water and added to U937 cells at incremental concentrations.

Samples were incubated for 24 h at 37ºC and cell viability was assessed by the fluorescein diacetate-ethidium bromide (FDA/EtBr) assay, a microscopic staining assay (Ryan et al., 2004). The concentration of compound that inhibited cell growth by 50% (IC_{50}) was determined. To examine the antioxidant and genoprotective effects, cells were pretreated with each compound at levels below the IC_{50}. U937 cells were then exposed to oxidants: 0.5μM etoposide or 100μM hydrogen peroxide (H_20_2) or 400μM *tert*-butylhydroperoxide (tBOOH). Cellular glutathione (GSH) levels were measured as an indicator of etoposide induced oxidative stress (O'Callaghan et., 2002). H_20_2 and tBOOH induced DNA damage was assessed by the alkaline single-cell gel electrophoresis (ASCGE) assay or comet assay (Woods et al.,1999) and results expressed as olive tail moment (OTM).

Pork loin (*M. longissimus dorsi* (LD)) was minced through a plate with 4mm holes. Following mincing, GSP and BB, solubilised in distilled water, were added at incremental levels of, 0, 50, 100, 200, 300 and 400μg/g muscle and 0, 10, 20, 40, 60 and 80μg/g muscle respectively and subsequently the minced LD was formed into pork patties. Trays containing LD patties were stored under modified atmosphere conditions (75% O_2 : 25% CO_2) for up to 12 days at 4ºC.

Colour measurements were made at 3 day intervals using a Cr-300 Chromameter (Minolta Co.Ltd. Japan) set on the LAB colour scale and results were reported as the 'L' lightness, 'a' redness and 'b' yellowness values.

Lipid oxidation was measured by the distillation method of Taradgis et al. (1960) as modified by Ke et al., (1977) and results expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg malondialdehyde/kg muscle.

The pH of minced LD homogenates (10%) were measured using a pHm 201 portable pH meter (Radiometer, Copenhagen) at 20ºC. Total plate counts were determined at 3 day intervals, and samples were incubated at 30ºC for 72 h. Results were expressed in log colony forming units/g (cfu/g).

All analysis was performed in triplicate. Data was analysed by ANOVA using the Prism statistical package followed by Dunnett's test ($P<0.05$).

Results & Discussion

RES was the most toxic compound in U937 cells demonstrating the highest IC_{50} value of 13.7μg/ml. ECH was the least toxic plant extract with the lowest IC_{50} at 9400μg/ml (Data not shown). Exposure of cells to etoposide alone resulted in a significant decrease in glutathione content to 52% of control value (Table 1). GSP, C3ol, ECH and RES increased glutathione levels to a range of 64% to 76% of the control value. OLE and BB resulted in a significant ($P<0.05$) increase in glutathione content to 94% and 112% of the control, respectively following the trend: BB> OLE > RES > GSP> C3ol > ECH.

None of the compounds alone increased DNA damage as measured by the olive tail moment (OTM). Exposure of U937 cells to H_2O_2 alone increased DNA damage, resulting in a 13-fold increase in DNA OTM versus that of the untreated sample (Table 1). C3ol, ECH and RES had a slight but nonsignificant effect on reducing H_2O_2 induced DNA damage. GSP and BB significantly ($P<0.05$) decreased the formation of DNA single strand breaks. The order of effectiveness in reducing H_2O_2 induced DNA damage was as follows: BB > GSP > C3ol > ECH > RES > OLE. Exposure of U937 cells to tBOOH

alone also increased DNA damage, resulting in a 4-fold increase in DNA OTM versus the untreated sample (Table 1). The two plant extracts, GSP and BB, lead to a reduction in DNA single strand breaks caused by tBOOH treatment following the trend: GSP > BB > C3ol > ECH > OLE > RES.

The antioxidant effectiveness of GSP is believed to be due to the presence of oligomer procyanidins within grapeseed, which possess a greater protective effect than monomer components (Llopiz et al., 2004). Furthermore, this protection is also related to the closeness of catechol moieties in monomer components and the resulting reduction in oxidation potential of grapeseed, found to be twenty times greater than vitamin E and fifty times that of vitamin C (Shi et al., 2003). The observed antioxidant effect of BB may be explained by the vast array of hydroquinone derivatives present in this extract, such as arbutin, tannins, flavonoids, triterpenes and phenolcarboxylic acids (Tziveleka et al., 2002).

From this screening process, the direct addition of the selected extracts, GSP (0, 50, 100, 200, 300, 400µg/g) and BB (0, 10, 20, 40, 60, 80µg/g), resulted in greater lipid stability, relative to controls, in LD patties stored under modified atmosphere conditions at 4ºC (Table 2). After 6, 9 and 12 days of storage, lipid oxidation in LD patties decreased with increasing concentrations of GSP and BB. The antioxidant activity of GSP (200µg/g) (Nissen et al., 2004) and BB extract (100, 200 and 500µg/g) (Pegg et al., 2001) in cooked pork has been previously reported. The surface redness 'a' values of LD patties increased with GSP and BB concentration, relative to controls (Table 2). The lightness 'L' values (range=58.27-62.19) and yellowness 'b' values (range=8.88-10.23) were essentially unaffected by the incorporation of GSP and BB until 9 and 12 days of storage. The pH (5.6-5.78) and total plate count (log 3.3-3.6 cfu/g) of LD patties were unaffected by the presence of GSP and BB.

Conclusions

In conclusion, the overall data support the view that non-nutrient dietary constituents may act as significant bioactive compounds in vitro and that plant extracts may play a role in the modulation of oxidative processes in vivo. Our results also support the view that plant based extracts may act as neutraceuticals in "true terms", having the potential to improve oxidative stability, and thus eliminate the need for synthetic antioxidants. Further research is necessary to ascertain the effects of plant extracts under different packaging conditions. The influence of dietary supplementation with health promoting neutraceuticals such as GSP and BB as a means of enhancing pork quality also needs to be investigated.

Acknowledgements

This research was part funded by the Department of Agriculture under the National Development Plan through the FIRM research programme.

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Table 1. Effect of resveratrol, citroflavan-3-ol and plant derived extracts on etoposide induced oxidative stress, H_20_2 or tBOOH induced DNA damage in U937 human blood cells.

Treatment	Glutathione (% control) 0.5µM Etoposide	DNA damage (OTM)[x] 100µM H_20_2	DNA damage (OTM)[x] 400µm tBOOH
Control	100 ± 1.21	0.39 ± 0.09	0.39 ± 0.09
Etoposide	52 ± 1.18[a]	—	—
H_20_2	—	5.36 ± 0.59[a]	—
tBOOH	—	—	1.60 ± 0.44[a]
GSP 50µg/ml	72 ± 0.29[a]	1.70 ± 0.06[b]	0.57 ± 0.07[b]
BB 10µg/ml	112 ± 0.19[b]	1.64 ± 0.46[b]	0.66 ± 0.04[b]
OLE 50µg/ml	94 ± 1.46[b]	6.96 ± 0.36[a]	2.01± 0.67[a]
C3ol 100µg/ml	65 ± 0.94[a]	3.82 ± 0.33[a]	1.63 ± 0.36[a]
ECH 1mg/ml	64 ± 0.67[a]	4.12 ± 0.40[a]	1.83 ± 0.47[a]
RES 2µg/ml	76 ± 0.81[a]	4.59 ± 0.28[a]	3.44 ± 0.68[a]

All six compounds were co-incubated with etoposide or H_20_2 or tBOOH. [ab] Mean values (± SEM) in the same column bearing different superscripts are significantly different, $P<0.05$. [X] OTM= olive tail moment.

Table 2 Effect of the direct addition of GSP and BB on lipid oxidation and colour in minced *M. Longissimus dorsi* (LD) stored in modified atmosphere packs (75% 0_2 : 25% $C0_2$) at 4°C

	Storage time, days									
	0		3		6		9		12	
	TBARS[b]	'a' value[c]	TBARS[b]	'a' value[c]	TBARS[b]	'a' value[c]	TBARS[b]	'a' value[c]	TBARS[b]	a' value[c]
Control	0.44	10.92	0.4	8.86	0.75	7.79	0.9	7.67	2.02	6.25
GSP 50µg/g	0.46	10.96	0.31	8.85	0.32	8.6	0.34	8.11	0.58	7.03
GSP 100µg/g	0.29	10.9	0.39	9.19	0.42	9.21	0.31	8.12	0.37	7.28
GSP 200µg/g	0.12	10.91	0.54	9.51	0.2	8.89	0.23	8.54	0.32	7.75
GSP 300µg/g	0.13	11.21	0.32	9.7	0.12	8.87	0.22	8.13	0.19	8.04
GSP 400µg/g	0.1	11.2	0.22	9.58	0.15	9.55	0.19	8.21	0.2	7.93
Control	0.44	10.92	0.4	8.86	0.75	7.79	0.9	7.67	2.02	6.25
BB 10µg/g	0.24	10	0.63	9.4	0.61	7.94	0.65	7.76	1.27	6.5
BB 20µg/g	0.09	10.87	0.6	9	0.4	8.37	0.41	7.79	0.46	7.05
BB 40µg/g	0.08	10.99	0.3	8.52	0.27	8.38	0.33	7.84	0.37	7.96
BB 60µg/g	0.08	10.62	0.16	9.13	0.16	8.04	0.18	7.79	0.27	7.45
BB 80µg/g	0.09	10.49	0.1	8.73	0.18	8.47	0.16	7.21	0.3	7.29

[b] TBARS, mg MDA/kg muscle, [c] 'a' value denotes redness of meat