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## Evaluation of the antioxidant potential of grape seed and bearberry extracts in raw and cooked pork

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#### **Abstract**

The effect of grape seed extract (GSE) and bearberry (BB), on lipid oxidation (TBARS, mg malondialdehyde (MDA)/kg muscle), colour (CIE 'a' redness value), pH, microbial status (log<sub>10</sub>CFU colony forming units/g pork) and sensorial properties of cooked pork patties was investigated. GSE (0–1000 µg/g muscle) and BB (0–1000 µg/g muscle) were added to raw pork (*M. longissimus dorsi*) patties which were stored in modified atmosphere packs (MAP) (75% O<sub>2</sub>:25% CO<sub>2</sub>) for up to 12 days at 4 °C. Cooked pork patties were stored in MAP (70% N<sub>2</sub>:30% CO<sub>2</sub>) for up to 4 days at 4 °C. Mesophilic plate counts and pork pH were unaffected by GSE and BB. GSE and BB addition decreased (*P* < 0.05) lipid oxidation (TBARS) in raw pork patties on days 9 and 12 of storage, relative to controls. Antioxidant activity of GSE and BB was observed in cooked pork patties demonstrating the thermal stability of GSE and BB. The 'a' redness values of raw and cooked pork patties marginally increased with increasing GSE concentration. The sensory properties of cooked pork patties were unaffected by GSE and BB addition. Results obtained demonstrate the potential for using health promoting nutraceuticals in meat and meat products.

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## 1. Introduction

In recent years, a greater emphasis has been placed on the link between diet and the prevention of chronic diseases. This "changing face" of food has given way to the rise and development of novel functional foods (Hardy, 2000). The terms functional food and nutraceutical are used interchangeably and are defined as substances which may be considered a food, or part of a food, which provides medicinal or health benefits, including the prevention and treatment of disease (Clydesdale, 1997). Nutraceuticals represent the fastest growing segment of today's food industry with increases in both consumer demand and development expanding at a rate of 12.8% per annum (Hasler, 2000).

Nutraceuticals are believed to modulate the aetiology of many chronic diseases such as cancer (Gao et al., 2003; Higdon & Frei, 2003), coronary heart disease (Donaldson, 2004; Somova, Shode, Ramnanan, & Nadar, 2003), diabetes (Maghrani et al., 2004), hypertension and osteoporosis (Position of the American Dietetic Association: functional foods, 2004). The present popularity of nutraceuticals has prompted a surge of in vitro studies examining the protective properties of physiologically active components against oxygen-induced damage. Carpenter, O'Callaghan, O'Grady, Kerry, and O'Brien (2006) investigated the antioxidant and genoprotective effects of a range of phytochemicals (resveratrol, citroflavan-3-ol) and plant extracts (grape seed, bearberry and olive leaf extracts, and *Echina*cea purpurea) under conditions of oxidative stress induced by hydrogen peroxide and tert-butylhydroperoxide, in a human monocytic cell line. Grape seed extract (GSE) and bearberry (BB) demonstrated the strongest antioxidant properties.

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Flavonoids are the most abundant and potent group of plant phenolic compounds and act as antioxidants (Rice-Evans & Miller, 1996). Grape seeds from grape juice and wine processing can be separated, extracted, dried and purified into GSE which contains phenolic compounds (Lau & King, 2003). Clinical data has shown that the antioxidant potential of grape seed is twenty and fifty fold greater than vitamins E and C, respectively (Shi, Yu, Pohorly, & Kakuda, 2003) arising from increased levels of polvphenol proanthocyanidins and oligomers of flavan-3-ol units, especially catechin and epicatechin present in GSE (Yilmaz & Toledo, 2004). The antioxidant activity of GSE has been reported in a variety of test systems (Javaprakasha, Singh, & Sakariah, 2001) including cooked beef (Ahn, Grün, & Fernando, 2002) and turkey (Lau et al., 2003; Mielnik, Olsen, Vogt, Adeline, & Skrede, 2006). The antimicrobial properties of GSE against gram positive and gram negative bacteria has been reported previously (Jayaprakasha, Selvi, & Sakariah, 2003). GSE effectively reduced the numbers of E. coli and S. typhimurium and retarded growth of L. monocytogenes and A. hydrophila in cooked ground beef (Ahn, Grün, & Mustapha, 2007). From a health perspective, GSE has been shown to act as a anticarcinogenic (Roy et al., 2002) and cardio-protective agent (Shafiee, Carbonneau, Urban, Descomps, & Leger, 2003).

BB, also known as Uva Ursi, is a member of the evergreen heath family. Traditionally, the astringent leaves have been used in the treatment of bladder infections and other afflictions of the urinary tract. The scientific literature contains less information regarding the antioxidant potential of BB compared to GSE, however antioxidant activity of BB has been reported in cooked pork (Pegg, Amarowicz, & Barl, 2001). The antioxidant properties of BB (Amarowicz, Pegg, Rahimi-Moghaddam, Barl, & Weil, 2004) are most likely attributed to the glycoside arbutin fraction present (Annuk et al., 1999). BB extract displays potential antimicrobial benefits with respect to food-associated bacteria when used in combination with nisin (Dykes, Amarowicz, & Pegg, 2003). Furthermore, aqueous extracts of BB have been shown to alter the ability of E. coli (Türi, Türi, Anuuk, & Arak, 1999) and H. pylori (Annuk et al., 1999) to cause infection.

Lipid oxidation is a major quality deteriorative process in muscle foods resulting in a variety of breakdown products which produce off-odours and flavours (Faustman & Cassens, 1990). Muscle foods are also susceptible to microbial contamination leading to foodborne illnesses. In addition, colour changes are also an important factor influencing the quality and acceptability of meat and meat products. Currently, there is a growing interest in the use of natural antimicrobial agents and antioxidants derived from plant sources. The use of plant derived nutraceuticals may afford meat processors the opportunity to develop novel meat products with enhanced nutritional and health benefits, improved shelf-life, quality and profile. Therefore the influence of selected nutraceuticals such as

GSE and BB on meat quality parameters merits investigation.

The objective of the present study was to assess the effect of health promoting plant extracts, GSE and BB, on lipid oxidation, colour, pH, microbial status and organoleptic properties of raw and cooked pork patties during chilled storage.

#### 2. Materials and methods

#### 2.1. Reagents

All chemicals used were 'AnalaR' grade obtained from British Drug House, Poole, Dorset, UK; Sigma Chemical Co. Ltd, Poole, Dorset, UK and Rathburn Chemical Co. Ltd, Walkerburn, Peableshire, Scotland. Grape seed extract (GSE, 85% oligopolysaccharides) and bearberry (BB) extract (*Uva Ursi*, 20% arbutin) were obtained from Guinness Chemical (Ireland) Ltd, Clonminam Industrial Estate, Portlaoise, Co. Laois, Ireland. Fresh pork (*M. Longissimus dorsi*) was obtained from Ballyburden Meat Processors, Ballincollig, Co. Cork, Ireland.

## 2.2. Total polyphenol content of GSE and BB

The concentration of phenolic compounds in GSE and BB was determined by the Folin-Ciocalteau method as described by Singleton and Rossi (1965). GSE and BB were dissolved in distilled water, and to 1 ml of sample, 5 ml of Folin-Ciocalteau reagent (diluted 1:10 with distilled water) was added. After 5 mins, 4 ml of a sodium carbonate solution (7.5%) was added to each tube. The tubes were incubated for 2 h at room temperature and the absorbance determined spectrophotometrically (DU® 640 UV/Vis spectrophotometer, Beckman Coulter, CA, USA) against a reagent blank at 740 nm. The total polyphenol content was calculated using gallic acid as a standard and results were expressed as gallic acid equivalents (GAE) (g GAE/ 100 g extract).

### 2.3. Antioxidant addition and pork packaging

GSE and BB were found to be most effective against oxidative stress *in vitro* at concentrations of 50 μg/ml and 10 μg/ml, respectively (Carpenter et al., 2006). Therefore in order to evaluate the effects of GSE and BB in a meat system, a range of increasing concentrations, including the above stated values for GSE and BB, were added to minced pork. Pork samples were minced twice through a plate with 4 mm holes (Model P114L, Talsa, Valencia, Spain) following the removal of all external fat and connective tissue. Following mincing, raw pork was assigned to one of the following thirteen treatments: untreated pork (control); pork plus increasing amounts of GSE: 50 μg GSE/g muscle (GSE 50); 100 μg/g muscle (GSE 100); 200 μg/g muscle (GSE 200); 300 μg/g muscle (GSE 300); 400 μg/g muscle (GSE 400); 1000 μg/g muscle (GSE

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