APPLICATION OF PLANT POLYPHENOLICS IN MODEL AND MEAT SYSTEMS

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Key words: lipid oxidation, antioxidants, polyphenolics, meat processing, bearberry

Background

During production, processing, distribution and storage, meat undergoes deterioration from chemical and microbial processes. Typically, oxidative deterioration of meat and meat products results from degradative reactions of lipids in raw and thermally-processed meat. The rate and extent of oxidative deterioration can be reduced by various means such as curing to preserve the meat tissue, vacuum packaging to remove the oxygen source, or addition of antioxidants to scavenge the oxidants. Over the past few years, antioxidants from natural sources have received an avalanche of print and media coverage on account of their alleged nutritional and health benefits. What many consumers do not know, however, is that the meat industry already utilizes, to a degree, natural antioxidants found in the binders, extenders, herbs and spices added to meat products. The best known natural antioxidants include tocopherols (vitamin E), ascorbic acid (vitamin C) and carotenoids. Moreover, the polyphenolic compounds inherent in plant material play an important role in the antioxidant story. It is well documented that extracts of green tea, and spices such as rosemary, sage, oregano, thyme and clove possess marked antioxidative activity. Recent studies have led to the identification of active compounds in green tea and rosemary: these extracts have been successfully commercialized in Japan, Europe and North America, despite higher costs in comparison to widely used synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT). Because BHA and BHT have been suspected to possess carcinogenic activity, this has resulted in an increased effort by researchers to identify antioxidants from natural sources.

Objectives

The objective of this study was to evaluate the polyphenolics extracted from several plant species as potential sources of natural antioxidants for use in meat and meat products.

Methods

The plant species investigated for their antioxidant activity included roots of narrow-leaved echinacea (*Echinacea angustifolia*), senega (*Polygala senega*), wild licorice (*Glycyrrhiza lepidota*), leaves of bearberry (*Arctostaphylos uva-ursi*) and aerial parts of 2 samples of horsetail (*Equisetum* spp). Ethanolic extracts from these plants were prepared, their content of total phenolic compounds determined and crude extracts chromatographed by TLC as described by Amarowicz et al. (1999). Antioxidant activity was assessed using a β-catorene-linoleate model system as described by Miller (1971), reducing power according to Yen and Chen (1995) and scavenging effect on DPPH radical by the method of Hatano et al. (1988). The crude bearberry extract was fractionated by Sephadex LH-20 column (30 x 700 mm) chromatography using ethanol and then methanol. Absorbance of collected fractions (15 ml) was measured at 280 nm, and eluates were pooled into major fractions. The main compound from the crude bearberry ethanolic extract was isolated on a silica gel column (30 x 250 mm) using chloroform:methanol:water (65:35:10, v/v) as the mobile phase.

Fresh pork cushions were ground twice by passing them through a 1/8″ plate using a Biro grinder/food mixer. Meat was transferred to jars and mixed with 20% (w/w) distilled water and various additives. These systems were thermal processed in an 85°C thermostated water bath with occasional stirring by a glass rod until an internal temperature of 72±1°C was reached. Systems were cooled to room temperature, homogenized in a blender for 30 s, transferred to Whirl Pak bags and refrigerated at 4°C until used. Thiobarbituric reactive substances (TBARS) were determined as described by Pegg (2001). Briefly, a 5 g portion of each sample was transferred to a stomacher bag in which 50 mL of a 20% (w/v) trichloroacetic acid (TCA) and1,6% (v/v) phosphoric acid solution were added. Each sample was stomached for 2 min and then 50 mL of cold distilled water were added. After blending for an additional 30 s, each sample was filtered through Whatman No. 1 filter paper into a 100 mL volumetric flask. Each flask was filled to mark with distilled water and its contents were mixed well. Five mL aliquots from each sample were pipetted to polypropylene conical tubes to which an equivalent volume of a 0.02 M aqueous TBA reagent was added. Tubes were capped, heated in a boiling water bath for 35 min and then cooled in ice. Absorbance measurements of the pink-coloured chromogen were made at 532 nm against a reagent blank using a spectrophotometer. The 'k' factor was determined from a calibration curve based on addition of known quantities of the malonaldehyde precursor, 1,1,3,3-tetramethoxypropane, to cooked meat samples prior to extraction.

Results and Discussion

The following numerical designations were used for the plant extracts: 1. Horsetail species (1996), aerial parts; 2. Horsetail species (1994), aerial parts; 3. Bearberry leaves; 4. Narrow-leaved echinacea root; 5. Senega root; and 6. Wild licorice root. The content of phenolic compounds in the crude extracts, expressed as catechin equivalents, varied from 58-312 mg/g. Calculated as a percent of the plant material, phenolic content varied from 8,43% (bearberry) to 0,17% (horsetail, 1994).

Antioxidative activity was observed in the crude ethanolic extracts of all 6 plant species screened, based on the coupled oxidation of β-carotene and linoleic acid. Fig. 1 illustrates the percentage of unoxidized β-carotene as a result of protection from the extracts after 60 and 120 min of incubation. The bearberry extract exhibited the greatest effect on quenching β-carotene oxidation (~98% after 120 min of heating). The greatest antioxidative efficacy was obtained for the BHA control, which completely inhibited β-carotene consumption throughout the incubation. However the relative inhibition of β-carotene consumption after 60 min of incubation by the ethanolic extracts

of bearberry, licorice root and senega root were similar: 97,5%, 91,5% and 87,4%, respectively. After a further 60 min of incubation, the inhibition of β-carotene consumption for these extracts was 95,5%, 87,2% and 80,0%, respectively. Minimal activity against β-carotene consumption was observed from both horsetail extracts which was somewhat surprising considering that material #1 contained 216 mg catechin equivalents/g. Although it is believed that the total number of hydroxyl groups present in the aromatic constituents of an extract, in part, offers better antioxidative properties to it, compounds present in ethanolic extracts belong to different classes of phenolics. These classes might possibly have varying antioxidative strengths (Shahidi et al., 1994).

As shown in Fig. 2, the reducing power of bearberry was far superior to any of the other extracts examined (2,26 at a 1 mg dose) and is comparable to that of green tea catechins. The scavenging effect of extracts on DPPH radical (Fig. 3) decreased in the order of bearberry >> horsetail (1996) > wild licorice > senega > echinacea > horsetail (1994). Having the highest phenolic content (312 mg/g) and the second highest extract percentage (27%), bearberry was deemed to be a most promising source of natural antioxidants. An attempt to separate the phenolics from bearberry by Sephadex LH-20 chromatography resulted in 7 fractions, of which some possessed antioxidant activity comparable to that of BHA.

TBARS values of cooked pork treated with a synthetic (i.e., *t*-butylhydroquinone, TBHQ) and a commercial natural antioxidant (i.e., rosemary) were compared to those of systems treated with the bearberry extract at various concentrations. The TBARS values of pork model systems that had been treated with the bearberry sample were much lower than those of the control with no additive, thereby indicating protection to the meat from constituents of the extract against autoxidation; complementary research supports the view that the compounds providing antioxidant activity are polyphenolics. Although an antioxidative efficacy was noticeable at 100 and 200 ppm levels, use of the bearberry extract at a 500 ppm addition level protected the meat against oxidation during storage at 4°C to a similar extent as that of TBHQ (100 ppm). While some of the plant extracts tested showed antioxidant potential in model systems, their efficacy was lost when applied to meat systems. Partly, this is attributable to poor carry through properties of active constituents during thermal processing. However, this was not the case for the bearberry extract which showed excellent thermal stability in cooked meat systems.

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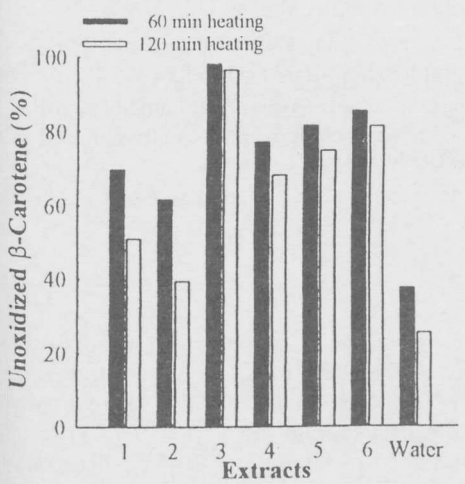


Fig 1. Antioxidant activity of plant extracts using a β-carotene-linoleate model system.

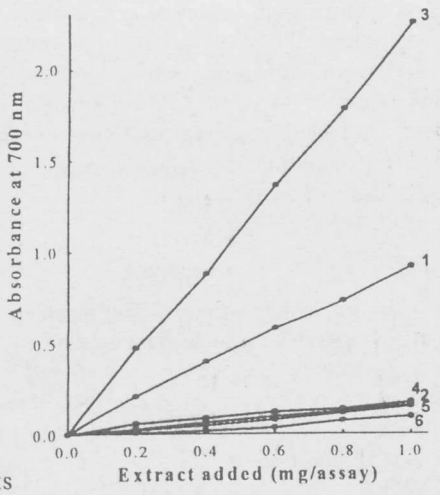


Fig 2. Reducing power of plant extracts.

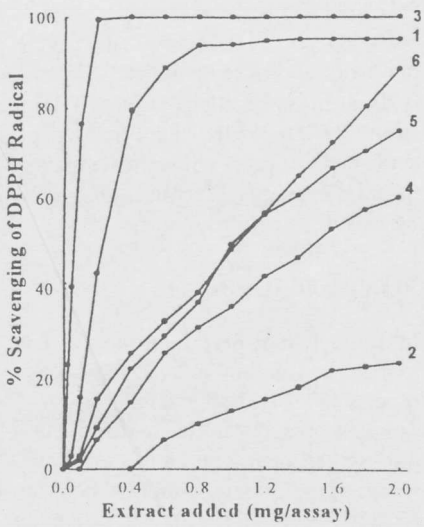
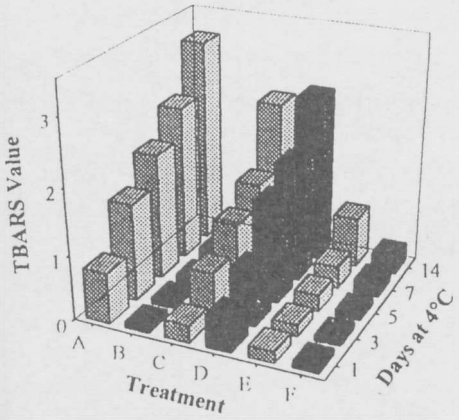


Fig 3. Scavenging effect of plant extracts on DPPH radical.



A - No additive
B - TBHQ, 100 ppm
C - Rosemary extract, 1000 ppm
D - Bearberry extract, 100 ppm
E - Bearberry extract, 200 ppm
F - Bearberry extract, 500 ppm

Fig 4. TBARS values of cooked pork systems as affected by synthetic and natural antioxidants.