Saskatchewan Herbs as Potential Sources of Natural Antioxidants Used in Meat and Meat Products

Ronald B. Pegg[1] and Ryszard Amarowicz[2], [1]Saskatchewan Food Product Innovation Program, Department of Applied Microbiology and Food Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, S7N 5A8, Canada and [2]Institute of Animal Reproduction and Food Research of Polish Academy of Sciences, 10-718 Olsztyn-Kortowo, ul. Tuwima 10, P.O. Box 55, Poland.

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 5 years, antioxidants from natural sources have received an avalanche of print and media coverage due to their alleged nutritional and health benefits. Yet, the use of natural antioxidants is limited due to a lack of knowledge about their molecular composition, the amount of active ingredients in the source material and the availability of relevant toxicity data (Shahidi *et al.*, 1994). What many consumers do not know, however, is that the meat industry already utilizes, to a degree, natural antioxidants found inherent in the binders, extenders, herbs and spices added to meat products. There are a number of Saskatchewan herbs which contain phenolic compounds; these herbs or their ethanolic extracts have a potential for use to extend the shelf-life of fabricated meat products by retarding lipid oxidation.

Objectives. The objectives of this study were to evaluate the potential of several indigenous Saskatchewan herbs as sources of natural antioxidants for use in meat and meat products.

Methods

Preparation of Herbal Extracts. Saskatchewan herbs, namely bearberry (*Arctostaphylos uva-ursi*), echinacea root (*Echinacea angustifolia*), seneca root (*Polygala senega*), licorice root (*Glycyrrhiza glabra*) and 2 varieties of horsetail (*Equisetum arvense L.* and *Equisetum hyemale*), were cut-up and ground using a Moulinex coffee mill. Prepared material was transferred to dark coloured-flasks, mixed with 95% (v/v) ethanol at a material to solvent ratio of 15:100 (m/v) and placed in a shaking Magni Whirl constant temperature bath at 50°C for 30 min. The extraction was repeated two more times and supernatants were combined. The resultant ethanolic extracts were evaporated to dryness using a rotavapor under vacuum at 40°C. The extracts so obtained were stored at 4°C until further used.

Determination of Total Phenolics. The content of total phenolic compounds in each extract was determined according to Swain and Hillis (1959) as modified by Naczk and Shahidi (1989). Instead of 3,5-dimethoxy-4-hydroxycinnamic acid (*trans*-sinapinic acid) as a standard, (+)-catechin was used. Hence, results are expressed as catechin equivalents.

Evaluation of Antioxidant Activity by Determination of β-Carotene Consumption. The antioxidative activity of crude extracts was evaluated using a β-carotene-linoleate model system (Miller, 1971). Briefly, 2 mg quantities of β-carotene were dissolved in 10 mL of chloroform. One millilitre of this solution was then pipetted into a small round-bottom flask. After removing the chloroform using a rotary evaporator, 20 mg of purified linoleic acid, 200 mg of Tween 40 emulsifier and 50 mL of aearted, distilled water were added to the flask with vigorous stirring. Aliquots (5 mL) of prepared emulsion were transferred to a series of tubes containing 2 mg of extract or 0.5 mg of butylated hydroxyanisole. As soon as the emulsion was added to each tube, the zero time absorbance was read at 470 nm against a reagent blank using a Milton Roy Spectronic Genesys 5 spectrophotometer. Subsequent absorbance readings were recorded at 15 min intervals by keeping the samples in a water bath at 50°C until the colour of β-carotene disappeared.

Preparation of Meat Systems. Fresh boneless picnic pork was obtained from Intercontinental Packers 1997 Ltd. (Saskatoon, SK) and its subcutaneous fat was trimmed. The meat was comminuted through a 1/8 in plate using a Biro grinder/food mixer and then homogenized by passing it two times through a 1/8 in plate on a Hobart meat grinder. Pork from the meat block was transferred to Mason jars and mixed with 20% (w/w) distilled water and various additives. Meat 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 an Osterizer blender for 30 s, transferred to Whirl Pak bags and refrigerated at 4 °C until used.

2-Thiobarbituric Acid Test. Thiobarbituric reactive substances (TBARS) were determined according to Witte *et al.* (1970) as modified by Bedinghaus and Ockerman (1995). Briefly, a 5 g portion of each sample was transferred to a stomacher bag in which 50 mL of a 20% (w/v) 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 #1 filter paper into a 100 mL volumetric flask. Each flask was filled to mark with 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 and heated in a boiling water bath for 35 min, and then cooled in an ice bath. 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. After scrutinizing some background information concerning various indigenous Saskatchewan herbs from the Saskatchewan herb database (Barl *et al.*, 1996), six herbs, namely bearberry (*Arctostaphylos uva-ursi*), echinacea root (*Echinacea angustifolia*), seneca root (*Polygala senega*), licorice root (*Glycyrrhiza glabra*) and 2 varieties of horsetail (*Equisetum arvense L.* and *Equisetum hyemale*), were selected and examined more carefully. Table 1 summarizes the percentage of ethanolic extract obtained from each herb as well as their total phenolic content (reported as catechin equivalents). Of the Saskatchewan herbs examined, the ethanolic

extract of bearberry contained the greatest amount of phenolics and its yield was the second largest of all the material investigated. Although the extract from one of the horsetail species (#1) contained the second greatest percentage of total phenolics, it had a very low yield (*i.e.*, only 3%). On the other hand, the ethanolic extract from senega root afforded the greatest yield (*ca.* 33%), but its total phenolic content was only 23% of that found in the bearberry extract.

The antioxidative activity of each crude extract was assessed based on the coupled oxidation of β-carotene and linoleic acid. Figure 1 illustrates the percentage of unoxidized β-carotene as a result of protection from the extracts after 60 and 120 min of incubation. The greatest antioxidative efficacy was obtained for the BHA control, which completely inhibited β-carotene consumption throughout the incubation. However the relative inhibitions of β-carotene consumption after 60 min of incubation by the ethanolic extracts of bearberry, licorice root and senega root (Material # 3, 6, 5) 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).

The antioxidant activity of selected herb extracts, reported as percent inhibition formation of TBARS during 7 days of storage at 4°C, incorporated in meat systems at levels ranging between 100 and 2000 ppm were compared to that of BHA- and TBHQ-treated samples (50 ppm). In many cases, the TBARS values of pork model systems which had been treated with herbal extracts were much lower than those of the control, thereby indicating protection to the meat from these additives against autoxidation. The greatest degree of protection, which was concentration dependent, was afforded by the ethanolic extract of bearberry. At a concentration of 200 ppm, the inhibition of TBARS production after 7 days of storage offered by this extract was greater than 95% and at a 500 ppm addition level, the degree of protection was not different from that of the BHA and TBHQ-treated samples. Further work is underway to isolate and elucidate the active component(s) from bearberry as well as some of the other Saskatchewan herbs.

Pertinent Literature

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Table 1. Percentage of Extracts Recovered from Saskatchewan Herbs and Their Content of Phenolics

Material	Extract from Raw Material (%)	Total phenolics in Extracts (mg catechin eq. / g)
1	3.0	216
2	2.9	58
3	27.0	312
4	10.9	62
5	32.8	72
6	6.7	63

Materials are: (1) Horsetail *(Equisetum arvense L.)*; (2) Horsetail (*Equisetum hyemale*); (3) bearberry (*Arctostaphylos uva-ursi*); (4) Echinacea root (*Echinacea angustifolia*); (5) Seneca root (*Polygala senega*); and (6) Licorice root (*Glycyrrhiza glabra*). Captions also apply to Figure 1.

Figure 1. Antioxidant Activity of Herbal Extracts in a β-Carotene-Linoleate Model System