PROPERTIES OF POLYPHENOLICS FROM BEARBERRY LEAVES: PROTEIN PRECIPITATING CAPACITY

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Background

There has been an increasing interest in replacing synthetic food antioxidants, because of their possible role as promoters of carcinogenesis with natural antioxidants (Ito et al., 1986). As the plant kingdom is rich in phenolic compounds, constituents of fruits, vegetables, spices, nuts, seeds, leaves, roots and barks have been targeted by many researchers as potential sources of natural antioxidants (Pratt and Hudson, 1990). However, use of natural antioxidants in foods is limited on account of the lack of knowledge concerning their molecular composition, the content of active compounds in the raw material and the availability of relevant toxicological data. Recently, a crude ethanolic extract of bearberry (*Arctostaphylos uva-ursi*) leaves was evaluated as a potential source of natural antioxidants for application to foods (Pegg et al., 2001). Characterization of this herb revealed that the main constituent was arbutin accompanied by variable amounts of methylarbutin and by small quantities of the free aglycone. Other constituents included gallic acid, galloylarbutin and a significant amount of gallotannins. From initial screening studies, it was determined that the crude bearberry extract possessed marked antioxidant properties in both model and meat systems. When added to pork at a 500 mg/kg level, the crude bearberry extract curbed oxidation in the cooked meat to a similar extent as that of 50 mg/kg of *t*-butylhydroquinone (TBHQ). Moreover, this crude extract performed better than a commercial rosemary preparation (i.e., a natural antioxidant) which was added to the meat system at a 1500 mg/kg level. One of the chief contributions to the antioxidant potential of bearberry appears to stem from its content of polyphenolics. Hagerman et al. (1998) have suggested that the antioxidant activity of polyphenolics may be lowered as a result of their binding to proteins. Therefore, an understanding of the interaction between polyphenolics of bearberry and proteins is warranted.

Objectives

The objectives of this study were to evaluate the affinity of polyphenolics from bearberry leaves for selected proteins and to assess the effect of pH and polyphenolics' concentration on the protein precipitating activity of polyphenolics.

Methods

Polyphenolics were extracted from bearberry leaves (*Arctostaphylos uva-ursi*): ground dried leaves were 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 crude extract was then separated on a Sephadex LH-20 column (30 x 700 mm) into two fractions using first 95% (v/v) ethanol (fraction I) and then 50:50 (v/v) acetone:water (fraction II) as a mobile phases. The total content of polyphenolics was assayed colorimetrically by the Folin-Denis method (Swain and Hillis, 1959), while the content of condensed tannins was estimated by the modified vanillin assay (Price et al., 1978). The protein precipitating capacity of polyphenolics from bearberry was measured using the dye-labeled protein assay (Naczk et al., 1996). The effect of pH on the formation of protein-polyphenolics complexes was evaluated according to Naczk et al. (2001). The relative affinity of bovine serum albumin (BSA), fetuin and gelatin for bearberry polyphenolics was determined according to Asquith and Butler (1987).

Results and Discussion

The total content of polyphenolics in the crude bearberry extract, expressed as catechin equivalents, was 312 mg per g extract. Calculated as a percentage of the plant material, this value represents 8,43%. The content of condensed tannins, as determined by the vanillin assay, was 53,8, 24,5 and 252 absorbance units (A_{500}) per g sample for the crude extract, fraction I and fraction II, respectively. The effect of pH on the protein precipitating capacity of polyphenolics from bearberry was evaluated in order to determine the optimum pH for precipitation of BSA by the polyphenolics present. Bovine serum albumin (BSA) was effectively precipitated by the polyphenolic constituents of bearberry between pH values of 3,8 and 4,5. A similar pH effect on the formation of polyphenolic-protein complexes was reported by Hagerman and Butler (1978) for condensed tannins isolated from sorghum and by Naczk et al. (2001) for condensed tannins isolated from beach pea, canola hulls, evening primrose and faba bean.

A statistically significant (P=0.0001) linear relationship exists between the amount of polyphenolic-dye-labeled BSA complex formed and the amount of polyphenolic extract added to the reaction mixture. The numerical values of the slope values of these lines for the crude bearberry extract, fraction I and fraction II were 135, 142 and 91.1, respectively. These values indicated that fraction I and the crude bearberry extract were the most effective dye-labeled BSA precipitants.

The relative affinities of gelatin, fetuin and BSA for polyphenolics of bearberry are shown in Table 1; results are expressed as the amount of competitor protein required to inhibit 50% of dye-labeled BSA precipitation by polyphenolics of bearberry. Gelatin, with a conformational open structure, was 3 to 7 times more of an effective precipitation inhibitor than proteins made up of a compact globular structure such as unlabeled BSA and fetuin. These results further suggest that competitor proteins displayed greater affinities for bearberry polyphenolics from low-condensed tannins' fraction I than for high-condensed tannins fraction' II. The results of our study indicate that

polyphenolic constituents of bearberry act as effective precipitants of proteins. More research is needed to determine the effect of polyphenolic-protein complex formation on their antioxidant activities using model and meat protein systems.

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Table 1. Relative Affinities of Selected Proteins for Polyphenolics from Bearberry Leaves.

Crude Polyphenolics Extract	Bovine Serum Albumin	Fetuin	Gelatin
Crude	0.66	0.34	1.86
Fraction I	0.97	0.30	2.29
Fraction II	0.30	0.36	1.26