MEAT PROTEIN-TANNIN INTERACTIONS: OBSERVED ANTIOXIDANT ACTIVITY AND POTENTIAL HEALTH BENEFITS

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Background

A bearberry-leaf (*Arctostaphylos uva-ursi* L. Sprengel) extract was shown to be a good source of natural antioxidants and imparted strong antioxidant activity in model and meat systems (Pegg et al., 2001; Amarowicz et al., 2004). This observed activity stems chiefly from the polyphenolic constituents present in the extract (Amarowicz and Pegg, 2004). Polyphenolic-protein interactions are a matter of continuous research due to their importance in food: nutritional effects centre on the capacity of phenolics to bind and precipitate proteins. Tannins, which are complex secondary metabolites of plants widely distributed in foods and comprised of gallic acid esters or flavan-3-ol polymers, are most commonly responsible for such interactions with proteins. Tannins can form soluble and insoluble protein complexes; the formation depends not only on the size, conformation and charge of the protein molecules, but also on the molecular weight, length and flexibility of the tannins involved (Naczk et al., 2001a). The precipitation of a protein-tannin complex results from the development of a sufficiently hydrophobic surface on the adduct due to polyphenols binding to the protein surface and cross-linking of different protein molecules with the polyphenols. The bearberry extract is rich in tannins (~10%), which are partially responsible for the observed antioxidant activity in meat systems. Until recently, tannins have been basically considered as antinutrients in foods because they decrease the nutritional value of protein; however, it begs the question, "since the tannins function as antioxidants, do the complexes so formed in meat also do the same?"

Objectives

The objectives of this study were to verify the formation of protein-tannin complexes in meat and then to assess whether or not the antioxidant activity of the native tannin persisted in the protein-tannin complex.

Materials and methods

A crude bearberry-leaf extract was prepared according to Amarowicz et al. (2004) and then dechlorophyllized according to Pegg et al. (2003) on a silicic acid column using hexanes and 95% (v/v) ethanol as mobile phases. Dried extracts were stored at 4°C until used. The cooked pork systems were prepared and TBARS of the stored refrigerated products were determined according to the methods described by Pegg et al. (2001).

Isolation of Myosin

Myosin was isolated from chilled post-rigor knuckle muscle of hogs slaughtered at Mitchell's Gourmet Foods (Saskatoon, SK) and then shipped to the University of Saskatchewan 48 h post-mortem. A flow diagram for the isolation and purification of myosin from the muscle tissue is depicted in Fig. 1. All work was carried out in a refrigerated cabinet at 4°C. The modified Hasselbach-Schneider solution comprised 0.6 *M* KCl, 10 m*M* $Na_4P_2O_7 \cdot 10H_2O$ and 1 m*M* $MgCl_2$ in 0.1 *M* KH_2PO_4/K_2HPO_4 buffer, pH 6.4.

Production of Tannin-Protein Complexes from Porcine Myosin

Fifty milligrams of tannins (*i.e.*, obtained by Sephadex LH-20 chromatography of the crude ethanolic extract of bearberry: the first mobile phase used was 95% (v/v) ethanol to remove some polyphenolics {*i.e.*, arbutin and gallic acid} followed by the second, 1:1 (v/v) acetone:water; the acetone fraction collected was called "tannins") were dissolved in 50-mL distilled water. One hundred milligrams of isolated myosin were dissolved in 100 mL of a 0.20 M acetate buffer, pH 5.0, containing 0.17 M NaCl. The two solutions were mixed and allowed to stand at room temperature for 30 min. The reaction mixture was transferred to a 250-mL polypropylene centrifuge tube, centrifuged for 10 min at -2°C at 10,000 × *g* in a Beckman J2-HC centrifuge. The supernatant was carefully decanted. Precipitate adhering to the wall of the centrifuge tube was scrapped and the product was mixed with *ca.* 20-mL distilled water and then transferred to a small 50-

mL beaker. The contents in the beaker were lyophilized. The tannin content bound to proteins was determined by a colorimetric assay described by Hagerman and Butler (1978).

Results and discussion

The bearberry-leaf extract was found to be a rich source of tannins, comprising *ca.* 10% of the crude dechlorophyllized preparation, and possessed marked antioxidant activity in model and meat systems. Previous research in our laboratory has indicated that it contains both hydrolyzable and condensed tannins. When applied to meat systems, the tannins retarded lipid oxidation of the cooked products during storage. The effect was concentration dependent as depicted in Figure 2. At a 25-ppm addition level (sample B) the tannins offered little protection against oxidation of meat lipids (*i.e.*, only 35% inhibition of lipid oxidation by day 7), but when doubled, an efficacy almost equivalent to that of the synthetic antioxidant, *tert*-butylhydroquinone (TBHQ) added at the same level, was observed (*i.e.*, > 98% inhibition). This is quite interesting considering that the tannin constituents have considerably larger molecular masses than that of TBHQ (FW=166), which on a mole basis was added to the meat system in a far greater quantity. The question is, was the free-radical scavenging capability restricted to the free tannins in the meat meatrix or were those bound to protein in the form of protein-tannin complexes also capable of exhibiting antioxidant activity?

Tannins are notorious as protein precipitants and thereby decrease the nutritional value of food proteins. It is generally assumed that the resultant complex has limited or no value; however, is this really so? Tannins from bearberry-leaf extract were found to be strong precipitants of model proteins such as bovine serum albumin and fetuin (Naczk et al., 2001b); however, we wanted to investigate their interaction with meat proteins. As myosin is the dominant protein in muscle tissue, it was isolated from fresh meat used for the TBARS studies. Electrophoretic separation of the protein from the resultant product by SDS-PAGE confirmed that the isolated protein was indeed myosin of good purity, when comparisons of the separated bands were made to commercially-available myosin. Addition of tannins to the isolated myosin dissolved in a dilute salt solution resulted in protein precipitation. The precipitate was recovered, dried and confirmed to be a complex of protein and tannin by Hagerman and Butler's colorimetric assay. It was suspected that the myosin-tannin complex so formed might have biological value as an antioxidant. When added to meat systems, which were then thermal processed, cooled and stored under refrigeration conditions for a period, TBARS data indicated that the myosin-tannin complexes imparted protection to the meat against lipid oxidation. Again, the efficacy was concentration dependent as depicted in Figure 2. At a 200-ppm addition level (sample D), the complex exhibited weak inhibition of lipid oxidation; however, when greater addition levels were incorporated in the system (*i.e.*, 500 and 750 ppm; samples E and F, respectively), an antioxidant efficacy equivalent to that of the free tannins added at 50 ppm was observed. As the myosin-tannin complex is free of tannin residues, the antioxidant activity observed in the cooked meat systems is due to either the complex itself or the release of tannin constituents therefrom. Because tannins can have strong covalent bonding interactions with protein, the latter theory seems to be more likely. The fact that protein-tannin complexes can impart a beneficial biological activity to a food system tends to negate the historical view that the protein-tannin precipitation products only reduce the nutritional value of food. This study demonstrates that the complex formed could be of a more significant value, as protein-tannin complexes consumed in food products may provide persistent antioxidant activity in the gastrointestinal tract against free-radical species.

Conclusions

The tannin constituents of the bearberry-leaf extract offered marked antioxidant activity to meat systems. Their interaction with meat proteins, such as myosin, did not result in a loss of the observed antioxidant activity. In fact, spectral data from TBARS analyses of cooked pork systems indicated that the isolated protein-tannin complexes added to meat systems survive thermal processing and give stability to meat lipids against oxidation. Thus, tannins bound to proteins may provide a sink for persistent antioxidant activity.

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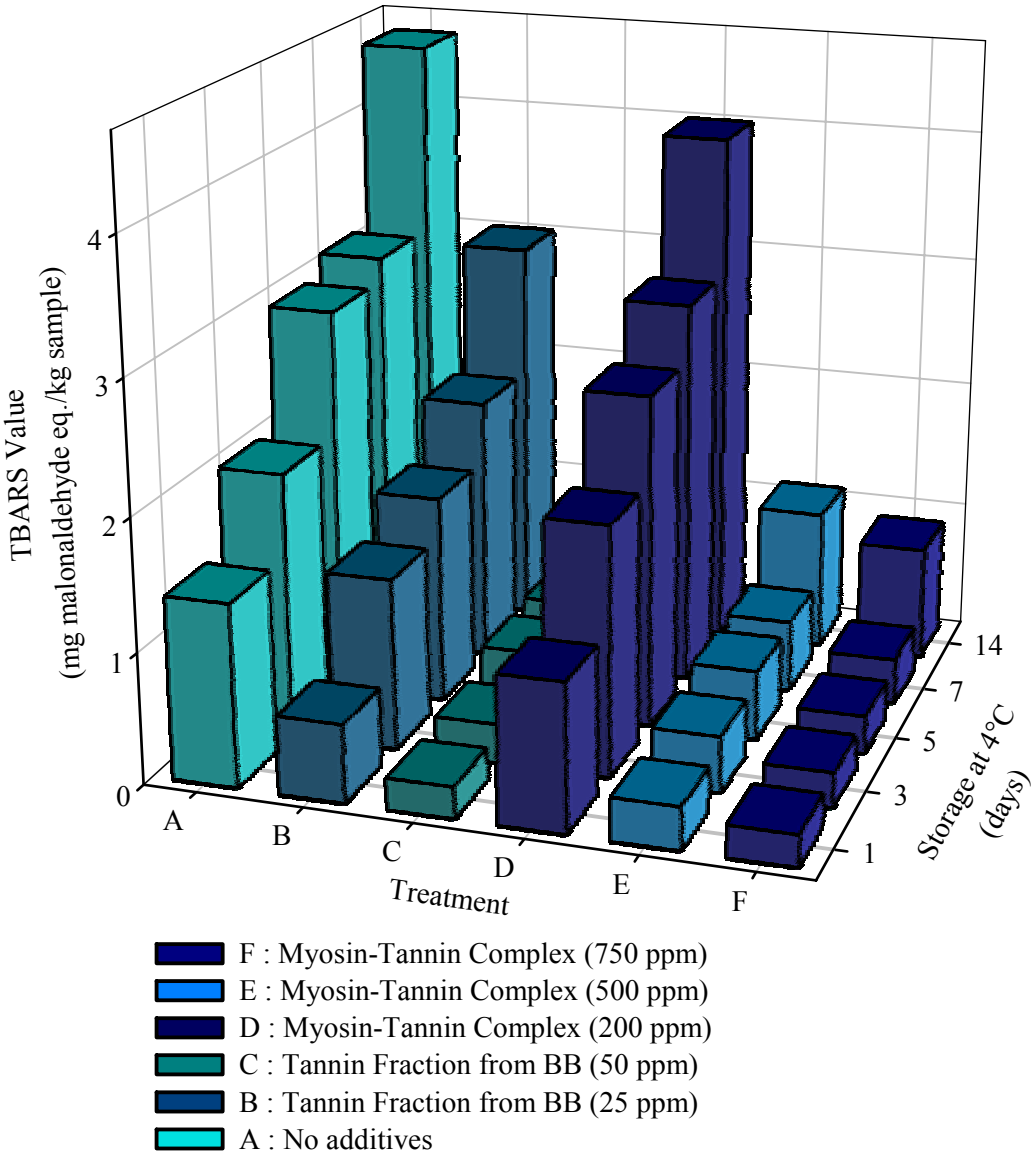

Fig. 2. TBARS values of cooked pork systems as affected by polyphenolics from the bearberry-leaf (BB) extract and a myosin-tannin complex.

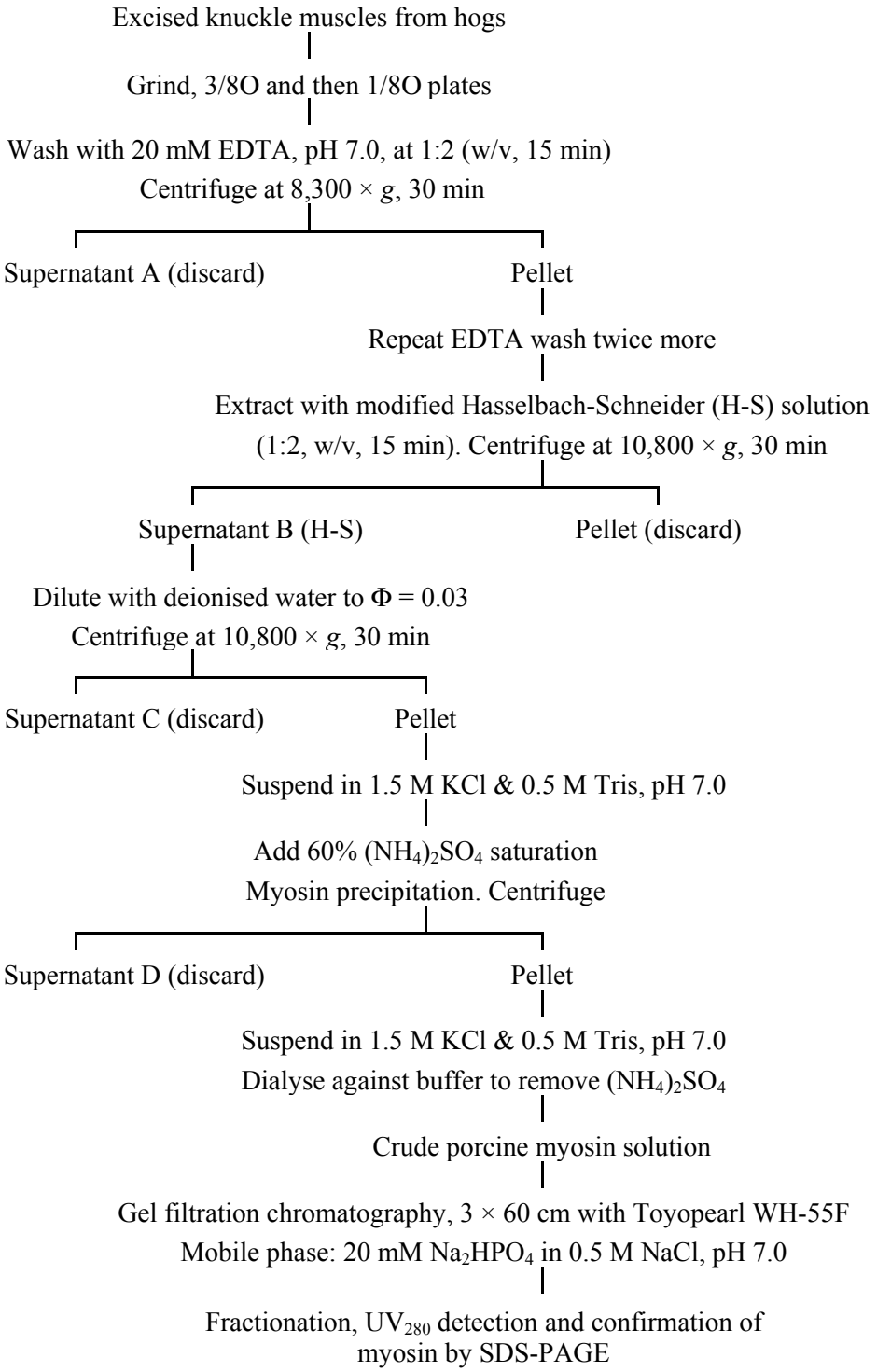

Figure 1. Flow diagram for the isolation and purification of myosin from porcine knuckle muscles.