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Protein precipitating capacity of phenolics of wild blueberry leaves and fruits

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Abstract

Phenolics (PP) were extracted from blueberry leaves and fruits with 70% (v/v) acetone and 95% (v/v) ethanol. The lyophilized crude PP extract was then fractionated on a Sephadex LH-20 column using first 95% (vv) ethanol as a mobile phase for elution of fraction of phenolics low in tannins then 50% (v/v) acetone to elute fraction rich in condensed tannins. Bovine serum albumin (BSA) was effectively precipitated by crude PP extracts at pH values between 4 and 5. A statistically significant ($P < 0.05$) linear relationship exists between the amount of PP–protein complex precipitated and the amount of PP added to the reaction mixture. The slope values of these lines indicated the tannin-rich fractions of crude PP extracts to be more effective protein precipitants than the other examined fractions. Based on the amount of gelatin, fetuin and BSA required to inhibit the formation of the dye-labeled BSA-PP complex by 50%, gelatin was 4–15 times more effective as a precipitation inhibitor than unlabeled BSA and fetuin.

Keywords: Lowbush blueberry; Leaves; Fruits; Phenolics; Phenolic-protein interactions; Protein precipitating capacity; pH effect; *Vaccinium angustifolium*

1. Introduction

Polyphenols may form soluble or insoluble complexes with proteins (Baxter, Lilley, Haslam, & Williamson, 1997; Calderon, Van Buren, & Robinson, 1968; Hagerman, 1992; Siebert, 1999; Siebert, Troukhanova, & Lynn, 1996; Shahidi & Naczk, 2004). This in turn may have a detrimental effect on the in vivo bioavailability of both phenolics and proteins (Lowry, McSweeney, & Palmer, 1996; Wollgast & Anklam, 2000). Furthermore, the interaction of salivary proteins with polyphenols has been implicated in the perception of astringent flavour (Baxter et al., 1997).

The precipitation of a polyphenol–protein complex is due to the formation of sufficient hydrophobic surface on the complex (McManus, Davis, Lilley, & Haslam, 1981). Silber, Davitt, Khairutdinov, and Hurst (1998) postulated that the precipitation of polyphenol–protein complexes occurs when a critical number of polyphenol molecules are associated with a protein molecule. Later Charlton et al. (2002) suggested that a sufficient coating of protein surfaces with polyphenol molecules is needed to initiate the aggregation of protein molecules leading to the precipitation of polyphenol–protein complexes. At low concentration of proteins, the precipitation is due to the formation of a hydrophobic monolayer of polyphenols on the protein surface. At higher concentrations of proteins, on the other hand, precipitation results from the combination of complexing of phenols on the protein surface and cross-linking of different protein

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molecules with phenols (Baxter et al., 1997; Charlton et al., 2002; Papadopoulou & Frazier, 2004; Shahidi & Naczk, 2004; Siebert, 1999; Silber et al., 1998).

Polyphenol–protein interactions depend on the size, conformation and charge of protein molecules. The affinity of polyphenols for proteins with a compact globular conformational structure is much lower then that displayed for proteins rich in proline with an open and loose structure (Asquith & Butler, 1986; Hagerman & Butler, 1981). Hagerman and Butler (1978) reported that the precipitation of polyphenol–protein complexes were pH sensitive. The lowest solubility of polyphenol–protein complexes occurred at 0.3–3.1 pH units below the isoelectric point of the proteins. Polyphenol–protein interactions are also affected by the presence of surfactants as well as by the size, length and flexibility of polyphenol molecules and the number and stereospecificity of binding sites on both the polyphenol and protein molecules (de Freitas & Mateus, 2001; Hagerman, 1989; Hagerman & Butler, 1981; Hagerman, Rice, & Ritchard, 1998; Martin & Martin, 1984; Shahidi & Naczk, 2004). According to Frazier, Papadopoulou, Mueller-Harvey, Kissoon, and Green (2003) the stoichiometry of polyphenol–protein complexes, but not the mechanism of polyphenol–protein interactions, depends on the structure and flexibility of tannin molecules.

Plant materials such as fruits, vegetables, spices, leaves, roots, and barks have been extensively evaluated as potential sources of natural antioxidants (Chyau, Tsai, Ko, & Mau, 2002; Ellnain-Wojtaszek, Kruczynski, & Kasprzak, 2002; Picerno, Mencherini, Lauro, Barbato, & Aquino, 2003). Our studies (Naczk, Amarowicz, Zadernowski, Pegg, & Shahidi, 2003) demonstrated that blueberry leaves, a commercially under-utilized by-product from mechanical harvesting of wild berries, may be considered as a excellent source of potent natural antioxidants. A number of recently published studies reported that polyphenol–protein interactions had a masking effect on the free radical scavenging activity of polyphenols (Arts, Haenen, Voss, & Bast, 2001; Arts et al., 2002; Riedl & Hagerman, 2001). Therefore, the objective of this study was to determine the relative affinity of phenolics isolated from blueberry leaves and fruits for selected model proteins. Furthermore, the effect of pH and phenolics concentration on the protein-precipitating capacity of isolated phenolics was also investigated using protein precipitation methods commonly employed for the quantification of polyphenols.

2. Materials and methods

Blueberry leaves (*Vaccinium angustifolium*), a by-product of mechanical harvesting of wild blueberries, were collected from a wild blueberry farm located in Antigonish County, Nova Scotia, Canada in July 2003. The leaves were separated from other debris by hand, dried at room temperature, and then stored in sealed polyethylene bags at −18 °C. Blueberry fruits were harvested at the commercially ripe stage and cleaned by hand to remove unripe and damaged berries, diseased fruits, stems and leaves. The berries were then packed in sealed polyethylene bags and stored at −18 °C.

Crude phenolics (crude PP) were extracted from both fruits and leaves with 95% (v/v) ethanol three times at 50 °C for 30 min at a solid-to-solvent ratio of 15:100 (w/v). The ethanolic extracts from fruits or leaves were pooled, evaporated to near dryness under vacuum at 40 °C, and lyophilized. The crude PP were also extracted from both the leaves and fruits with 70% (v/v) aqueous acetone three times at room temperature for 30 min at a solid-to-solvent ratio of 15:100 (w/v). The acetone extracts from fruits or leaves were pooled, evaporated to near dryness under vacuum at 40 °C, lyophilized and then the residue was weighed to determine the dry matter yield. The chlorophyll was removed from the lyophilized crude PP leaf extracts as described by Amarowicz, Pegg, Rahimi-Moghaddam, Barl, and Weil (2004).

The crude PP extracts of blueberry fruits and leaves were fractionated as described by Strumeyer and Malin (1975). A sample (550 mg) of crude PP extract was suspended in 5 ml of 95% (v/v) ethanol and applied onto a chromatographic column (2.3 × 40 cm) packed with Sephadex LH-20. Sephadex LH-20 was equilibrated with 95% (v/v) ethanol for over 12 h and then the column was manually packed by elution with the same solvent. The column was exhaustively washed with 95% (v/v) ethanol at a flow rate of 60 ml/h and then eluted with 50% (v/v) acetone at a flow rate of 60 ml/h. The solvent was then evaporated from the ethanol eluate (referred to as fraction A) and acetone eluate (referred to as fraction B), under vacuum at <40 °C, then each phenolic fraction was lyophilized and the residue was weighed.

The total content of phenolic compounds in the crude PP extracts and their fractions was estimated using the Folin–Denis reagent (Swain & Hillis, 1959) and expressed as gallic acid equivalents per gram of extract. The content of soluble-condensed tannins (CT) in the extracts was measured using the modified vanillin assay and expressed as absorbance units per gram of extract (Naczk, Nichols, Pink, & Sosulski, 1994; Price, van Scoyoc, & Butler, 1978).

The effect of the phenolic extract (crude PP as well as fractions A and B of both leaves and fruits) concentrations on the formation of insoluble PP–protein complex was assayed by the protein precipitation method of Hagerman and Butler (1978) (at 1 mg BSA per ml) and by the dye-labeled BSA assay of Asquith and Butler (1987) (at 2 mg dye-labeled BSA per ml) with pH modifications as described by Naczk, Oickle, Pink, and Shahidi (1996). A series of methanolic solutions of

extracts (0.1–2.0 mg/ml) were prepared. The protein precipitating potential of phenolic extracts was expressed as the slope of the line reflecting the amount of PP–protein complex precipitated vs. the amount of total phenols added (Naczk, Amarowicz, Zadernowski, & Shahidi, 2001a). The effect of pH on the formation of PP–protein complexes was monitored as described by Naczk et al. (1996).

Relative affinities of the crude PP extracts for BSA, fetuin and gelatin were measured according to the procedure described by Asquith and Butler (1987) with the following modification. One millilitre of dye-labeled BSA solution, containing 2 mg of dye-labeled BSA instead of 1 mg recommended by the authors, was mixed with 0.6 ml of a solution containing 100–6000 μg of protein competitors (BSA or gelatin). To this mixture was added 0.4 ml of methanol containing 0.4 mg of PP extract. The relative affinity was calculated as the ratio between the weight of dye-labeled BSA present and the weight of competitor which prevented 50% of dye-labeled BSA from precipitating.

All assays were conducted at room temperature (about 22 °C) using appropriate samples and blanks. Results presented in tables and figures are mean values of at least four determinations. The bars in the figures represent standard deviations from mean values.

3. Results and discussion

The solvents 70% (v/v) aqueous acetone and 95% (v/v) ethanol were used for extraction of phenolics from wild blueberry leaves and fruits, as these solvents systems are commonly utilized for the extraction of condensed tannins (70% v/v acetone) and other classes of phenolics (95% v/v) from plant materials (Antonolovich, Prenzler, Robbards, & Ryan, 2000; Naczk & Shahidi, 2004; Rohr, Meier, & Sticher, 2000). The crude PP extraction yields ranged from 10.1% to 14.5%. Leaves yielded a higher amount of crude PP extract than fruits (Table 1). Fraction A was always the predominant one and amounted to 86–97% of the total crude PP extract weight. In addition, ethanol crude PP extracts yielded somewhat more fraction B than acetone extracts (Table 1).

Total phenols content (TP) in the crude PP extracts and fractions A and B from wild blueberry leaves and fruits was expressed as gallic acid equiv./g of extracts (Table 1). Gallic acid is a common standard for the quantification of phenolics present in plant extracts. The TP of ethanolic crude PP extracts was slightly higher than those of acetone extracts. The crude PP extracts from leaves contained approximately 10-fold more TP than the corresponding fruits extracts (Table 1). This is in a good agreement with data published by Ehlenfeldt and Prior (2001). Furthermore, TP in the fruits is within the range of previously published results (Moyer, Hummer, Finn, Frei, & Wrolsdat, 2002; Sellappan, Akoh, & Krewer, 2002; Zadernowski, Naczk, & Nesterowicz, 2005). It should be noted that TP content may be affected by such factors as varietals and regional differences (Häkkinen & Törrönnen, 2000), the degree of berry ripeness (Mosel & Hermann, 1974), harvest time and also the analytical procedure used for extraction and quantification of phenolics (Antonolovich et al., 2000; Naczk & Shahidi, 2004; Robbins, 2003; Shahidi & Naczk, 2004).

Blueberry fruits are a rich source of phenolic acids, catechins, flavonols, anthocyanins and condensed tannins (proanthocyanidins) (Naczk & Shahidi, 2003; Shahidi & Naczk, 2004). Phenolic acids liberated from soluble esters were the predominant phenolic acids in the fruits (Zadernowski et al., 2005). Moreover, over 10 anthocyanins have been identified in lowbush berries (Mazza & Miniati, 1993). Catechin, myrecitin, quercetin and kaempferol were also detected in fruits (Sellappan et al., 2002). The presence of oligomeric and polymeric proanthocyanidins in fruits has been reported (Gu et al., 2002; Prior, Lazarus, Cao, Muccitelli, & Hammerstone, 2001). Of these, the polymeric proanthocyanidins amounted for over 76% of total proanthocyanidins in fruits and were mixture of polymers with a degree of polymerization from 14.4 to 114.1 (Gu et al., 2002). It should also be stated here that the chemical structures of blueberry leaf phenolics are still unknown and more detailed chemical analyses of these phenolics are still needed.

Condensed tannins (CT) isolated from plant materials are mixtures of polymeric compounds that differ in their sensitivity towards the reagents used for their determination. The longer the polymer chain the less of the red condensation product of vanillin reaction is formed due to an increased steric hindrance towards the vanillin reagent (Mole & Waterman, 1987). The contents of CT in blueberry phenolic extracts, shown in Table 1, are expressed in absorbance (A_{500}) units per gram of extract (A_{500}/g) due to lack of an appropriate standard for CT quantification. The data were presented in Table 1 indicate that acetone was a somewhat more efficient solvent for extraction of CT from blueberry leaves and fruits than ethanol. Condensed tannins were predominantly found in fraction B from both leaves and fruits. Small quantities of CT detected in fraction A from leaves are probably composed of lower molecular weight CT. This is in accordance with the finding of Gu et al. (2002) that only monomers, dimers and trimers may be eluted from Sephadex columns with aqueous alcohol.

A number of methods are available for the determination of protein-precipitating capacity of CT (Asquith & Butler, 1987; Bacon & Rhodes, 1998; Charlton et al., 2002; Frazier et al., 2003; Hagerman & Butler, 1978;

Table 1
Yield and total phenols content of various phenolic fractions extracted from blueberry leaves and fruits

Sample	Solvent system	Phenolic fraction	Extract yield[1]	Total phenols content[2]	Total condensed tannins[3]
Leaves	Acetone 70% (v/v)	Crude	14.2	218.0 ± 3.2	128.0 ± 2.6
		Fraction A	12.8	189.0 ± 2.6	26.2 ± 4.7
		Fraction B	1.1	523.0 ± 9.0[a]	651.0 ± 5.8
Fruits	Acetone 70% (v/v)	Crude	10.7	31.3 ± 0.4	71.4 ± 6.0
		Fraction A	10.2	18.8 ± 0.2	ND
		Fraction B	0.2	348.0 ± 1.6	742.0 ± 8.6
Leaves	Ethanol 95% (v/v)	Crude	14.5	227.0 ± 2.5	109.0 ± 1.9
		Fraction A	12.5	202.0 ± 6.3	12.4 ± 0.6
		Fraction B	1.9	519.0 ± 7.4[a]	673.0 ± 5.9[a]
Fruits	Ethanol 95% (v/v)	Crude	10.1	23.6 ± 0.9	39.3 ± 10
		Fraction A	9.8	18.3 ± 0.1	ND
		Fraction B	0.2	341.0 ± 18.6	669.0 ± 6.1[a]

Values in each column sharing the same superscript letters are not significantly different (*t*-test; $P > 0.05$).
ND – not detected.
[1] Mean value of $n = 2$; % of fresh sample weight.
[2] Mean value ($n = 4$) ± standard deviation; expressed in mg gallic acid equivalents per gram of extract.
[3] Mean value ($n = 4$) ± standard deviation; expressed in absorbance units per gram of extract.

Makkar, 1989; Papadopoulou & Frazier, 2004; Shahidi & Naczk, 2004). Of these methods, the dye-labeled BSA assay developed by Asquith and Butler (1987) and the protein precipitation assay developed by Hagerman and Butler (1978) were selected for the quantification of the protein-precipitating potential of phenolic extracts isolated from wild blueberry leaves and fruits. The dye-labeled BSA assay (DPLA) measures the amount of protein precipitated by phenolics, while the protein-precipitation assay (PPA) estimates the amount of phenolics precipitated as protein-phenolic complex.

The effect of pH on the protein precipitating capacity of the crude PP extracts isolated from both wild blueberry leaves and fruits was evaluated in order to determine the optimum pH for BSA precipitation by the examined extracts. The optimum pH is defined as the pH at which the maximum precipitation of a tannin–protein complex occurs. Fig. 1 shows the effect of pH on the precipitation of BSA by crude PP as determined by the protein precipitation assay. BSA was effectively precipitated by crude PP extracts at pH values between 4.0 and 5.0 (Fig. 1). A similar effect of pH on the formation of PP-BSA complexes was reported by Hagerman and Butler (1978) for crude PP isolated from sorghum grains (*Sorghum bicolor* L. Moench), by Naczk et al. (1996) for crude PP extracts of Westar, Cyclone and Excel canola hulls (*Brassica* oilseeds), and by Naczk, Amarowicz, Zadernowski, and Shahidi (2001b) for crude PP extracts obtained from beach pea (*Lathyrus maritimus* L.), Cyclone canola hulls (*Brassica* oilseeds), evening primrose seeds (*Oenothera biennis* L.) and faba beans (*Vicia faba*).

Figs. 2 and 3 show the graphs (here referred to as titration curves) depicting the amount of proteins or phenolics precipitated as a PP–protein complex with

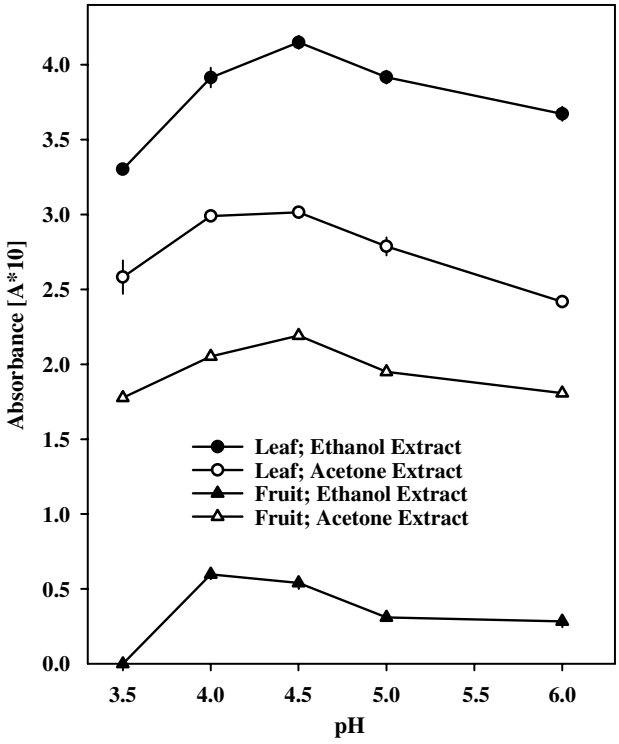

Fig. 1. The pH dependence of complex formation of crude PP of blueberry leaves and fruits with BSA determined by the protein precipitation assay (Hagerman and Butler, 1978).

increasing quantities of total phenols added to a reaction mixture containing a known amount of protein (1/ml for the protein precipitation assay and 2 mg/ml for the dye-labeled BSA assay). The acetone crude PP extract from leaves and corresponding fraction B were selected to illustrate this relationship. A statistically significant ($P < 0.05$) linear relationship existed between

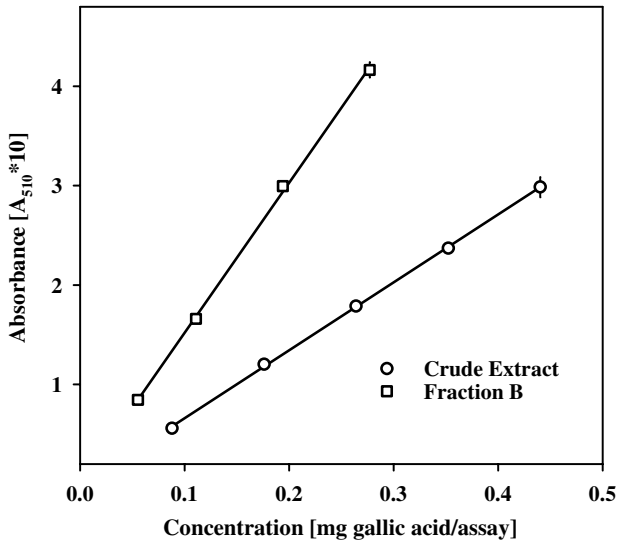

Fig. 2. Titration curves of a known amount of protein with increasing amounts of total phenols of crude PP extract from leaves (70% (v/v) acetone) and its corresponding fraction B as determined by the protein precipitation assay (Hagerman and Butler, 1978).

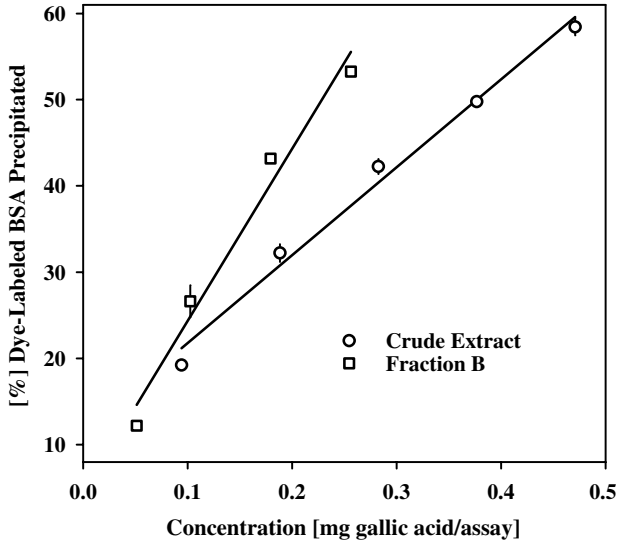

Fig. 3. Titration curves of a known amount of protein with increasing amounts of total phenols of crude PP extract from leaves (70% (v/v) acetone) and its corresponding fraction B as determined by the dye-labeled BSA assay (Asquith and Butler, 1987).

the amount of PP–protein complex precipitated and the amount of total phenols added to the reaction mixture for up to 0.3 mg gallic acid equiv./ml for all fraction B tested and up to 0.5 mg gallic acid equiv./ml for all crude PP extracts examined (Figs. 2 and 3). It should be noted that fraction A from fruits did not exhibit any significant protein precipitating potential. The numerical values of slopes of the titration curves obtained using the precipitation assay (PPA) ranged from 5.5 for ethanolic crude PP extract of leaves to 59.5 for acetone crude PP extract of fruits. On the other hand, the slope values of titration curves obtained using dye-labeled BSA assay were between 42.6 for ethanolic crude PP extract of fruits and 337.1 for fraction B of ethanolic crude PP extract of fruits (Table 2). Thus, the observed differences between the slope values of the titration curves obtained for phenolic extracts from blueberry leaves and fruits may be due to differences in their affinities for proteins. Similar differences in the slopes were reported by Naczk et al. (2001a, 2001b) for crude phenolic extracts isolated from low- and high-tannin hulls of canola and rapeseed (*Brassica* oilseeds), beach pea (*Lathyrus maritimus* L.), evening primrose seeds (*Oenothera biennis* L.), and faba beans (*Vicia faba*). According to Porter and Woodruffe (1984) the ability of phenolics to precipitate proteins depends upon their molecular weights. Asquith and Butler (1987) have also suggested that precipitation of dye-labeled BSA was affected by the degree of condensed tannin polymerization. However, the chemical structures, polymer chain lengths, and molecular weights of phenolics are still unknown and more detailed chemical analyses of these phenolics are still needed.

BSA, gelatin and fetuin have been selected as model proteins for the evaluation of the affinity of proteins for PP isolated from both wild blueberry leaves and fruits. BSA, a globular protein, and gelatin, a proline-rich protein with an open random coil conformation are commonly used as model proteins for the elucidation of polyphenol–protein interactions (Asquith & Butler, 1986; Feldman et al., 1999; Frazier et al., 2003; Hagerman & Butler, 1978, 1981; Makkar, 1989). Various binding mechanisms have been proposed to describe the interactions between BSA and tannins and gelatin and tannins (Frazier et al., 2003). Fetuin, a globular glycoprotein, has been also used as a model protein for examining the tannin–protein interactions (Asquith & Butler, 1986; Hagerman & Butler, 1981), but the binding mechanism has not been yet elucidated. Fig. 4 shows the effect of the competitors (gelatin, BSA and fetuin) on the precipitation of dye-labeled BSA by acetone crude PP extract isolated from blueberry leaves. Similar plots were reported by Hagerman and Butler (1981) and Asquith and Butler (1986) for sorghum tannins (*Sorghum bicolor*, L. Moench), and Naczk et al. (2001b) for crude PP of beach pea (*Lathyrus maritimus* L.), evening primrose seeds (*Oenothera biennis*, L.), Cyclone canola hulls (*Brassica* oilseeds) and faba beans (*Vicia faba*). The plot for gelatin is not parallel to the plots for BSA and fetuin. This, according to Creighton (1980), may be explained by co-operative binding between CT and protein or the heterogeneity of binding sites. The relative affinities of the examined crude PP extracts and their corresponding tannin fraction for BSA, fetuin and gelatin, calculated as the ratio between the weight of dye-labeled BSA present and the weight of competitor which prevented 50% of dye-labeled BSA from precipitating, are shown in Table 3. Based on the amount of gelatin, fetuin

Table 2
Protein precipitating potential of various fractions of phenolics extracted from blueberry leaves and fruits[1] as determined by the protein precipitation (PPA) and dye-labeled protein (DLPA) assays

Sample	Phenolic Fraction	Ethanol Extract		Acetone Extract	
		PPA[2]	DLPA[3]	PPA	DLPA
Fruits	Crude	25.6 ± 1.8	42.6 ± 1.9	59.5 ± 2.5	128.5 ± 11.1
	Fraction A	ND[4]	ND	ND	ND
	Fraction B	NA[5]	337.1 ± 48.0[a]	12.9 ± 0.7	311.4 ± 5.0[a]
Leaves	Crude	5.5 ± 0.1	85.1 ± 1.6	6.8 ± 0.1	101.9 ± 1.4
	Fraction A	ND	ND	ND	ND
	Fraction B	13.0 ± 0.1	211.2 ± 24.3[a]	15.1 ± 0.3	199.6 ± 21.8[a]

Values in each row and column sharing the same superscript letters are not significantly different ($n = 4$; t-test; $P > 0.05$).
[1] Expressed as a slope of line reflecting the amount polyphenol–protein complex precipitated vs. the amount of phenolic fraction added to the reaction mixture.
[2] Absorbance units/mg gallic acid.
[3] % precipitated protein/mg extract.
[4] ND – not detected.
[5] NA – not assayed due to insufficient amount of sample.

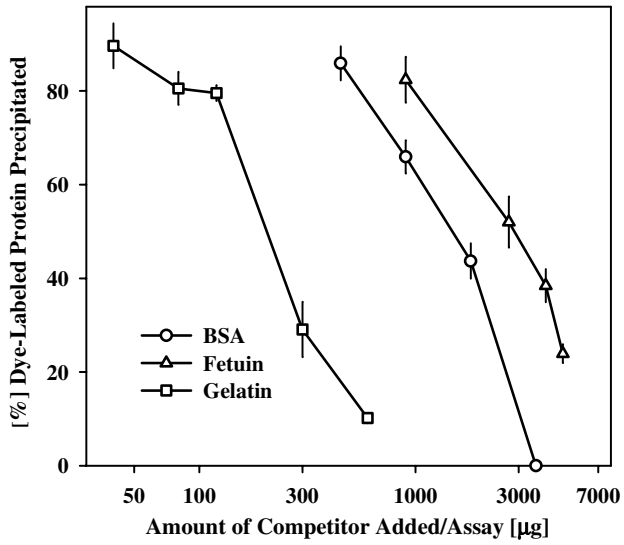

Fig. 4. Competition assays between dye-labeled BSA and standard proteins (BSA, fetuin and gelatin) for crude PP extract (leaves; 70% acetone).

Table 3
Relative affinity of selected proteins for crude phenolic extracts of blueberry fruits and leaves

Sample	Solvent	BSA[1]	Gelatin	Fetuin
Fruits	Ethanol	1.29	6.16	0.47
	Acetone	0.89	5.78	0.57
Leaves	Ethanol	0.91	3.97	0.49
	Acetone	0.81	5.78	0.36

[1] Bovine serum albumin.

and BSA needed to inhibit 50% of dye-labeled BSA precipitation by PP extracts, gelatin was 4–7 times more effective inhibitor than unlabeled BSA, while unlabeled BSA was twice as effective as fetuin. This suggests that the protein-polyphenol interactions may be affected by the nature of proteins involved. The relative affinities of the tested proteins for PP extracts were similar to those reported by Asquith and Butler (1987) for sorghum (*Sorghum bicolor* L. Moench) and quebracho (*Scinopsis balansae*) tannins and crude PP extracts of beach pea (*Lathyrus maritimus* L.), evening primrose seeds (*Oenothera biennis* L.), canola hulls (*Brassica* oilseeds) and faba beans (*Vicia faba*) (Naczk et al., 2001b).

4. Conclusion

The results of our study indicate that crude PP extracts and the corresponding fraction B isolated from both blueberry leaves and fruits were very effective precipitants of both unlabeled and dye-labeled BSA. The optimum pH for precipitation of unlabeled BSA by crude PP extracts was in the pH range similar to that reported by Hagerman and Butler (1978) for sorghum CT (*Sorghum bicolor* L. Moench), by Naczk et al. (1996) for crude canola hulls (*Brassica* oilseeds) PP, and by Naczk et al. (2001b) for crude PP extracts of beach pea (*Lathyrus maritimus* L.), primrose evening seeds (*Oenothera biennis* L.) and faba beans (*Vicia faba*). The tested PP extracts exhibited a greater affinity for gelatin, which has a conformational open structure than that known for BSA and fetuin with a compact globular structures. The differences in the affinities of BSA and fetuin for PP are probably due to the existing differences in the availability of binding sites for PP adsorption as well as in the net charge of these proteins in the model system used here.

Future research should be focused on: (i) chemical analysis of blueberry leaf phenolics in order to determine their chemical structures, polymer chain lengths, and molecular weights; (ii) elucidation of the mechanism governing the blueberry PP–protein interactions; (iii) contribution of various classes of blueberry phenolics

to overall protein precipitating potential. The evaluation of the masking effect of polyphenol–protein interactions on antioxidant activity of blueberry PP is in progress.

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