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Extraction of condensed tannins from beach pea (*Lathyrus maritimus* L.) as affected by different solvents

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Abstract

The condensed tannins of beach pea, green pea and grass pea were extracted using methanol or acetone at different concentrations with or without acidification. Among solvents tested, 70% acetone, containing 1% concentrated HCl, extracted a maximum amount of condensed tannins from beach pea, green pea and grass pea. Condensed tannins extracted were assayed using a 0.5% vanillin solution in methanol containing 4% HCl (v/v). Beach pea contained a higher amount of condensed tannins (11.6 g/100 g) than Indian grass pea (1.54 g/100 g), Canadian grass pea (109 mg/100 g) or green pea (72.0 mg/100 g). The fresh green seeds and pod shells of beach pea had lower amounts of tannins (7.19 and 9.13 g/100 g, respectively) than mature seeds and pod shells (11.7 and 2.05 g/100 g, respectively). Branches plus stems of beach pea contained the lowest amount of condensed tannins (0.95 g/100 g) as compared to all other parts of the plant.

Keywords: Beach pea; Green pea; Condensed tannins; Extraction

1. Introduction

Legume seeds, specifically testa, contain phenolic substances, including tannins (Salunkhe, Jadhav, Kadam, & Chavan, 1982). Tannins (polyphenols) are produced via condensation of simple phenolics that are secondary metabolites and are widespread in the plant kingdom. Tannins do not constitute a unified chemical group, but have a variety of molecular structures. They are generally divided into hydrolysable (galloyl and hexahydroxydiphenoyl esters and their derivatives) and condensed proanthocyanidins (polymers of flavan-3-ols; Haslam, 1989). Tannins are biologically active compounds and may have beneficial or adverse nutritional effects. Endogenous tannins protect unharvested seeds from attack by insects, birds and herbivores, as well as certain diseases and untimely germination (Hulse, 1979). Possible harmful effects of certain biological compounds, such as phenolics, trypsin inhibitors and phytates, have received considerable attention (Deshpande, Sathe, & Salunkhe, 1984; Reddy, Sathe, & Salunkhe, 1982). These compounds occur naturally in the seeds of legumes and cereals and, if present in sufficient quantities, may lower nutritional value and biological availability of dietary proteins and minerals. Tannins form insoluble complexes with digestive enzymes and dietary proteins (Aw & Swanson, 1985; Chang, Collins, Bailey, & Coffey, 1994; Kumar & Vaithiyanathan, 1990; Laurena, Van Dean, & Mendoza, 1884; Makkar, Blummal, Borowy, & Becker, 1993, 1995). The tannin-protein complexes may be responsible for the antinutritional effects of tannin-containing feeds consumed by nonruminants (Martin-Tanguy, Guillaume, & Kossa, 1977). Earlier work suggests that phenolic substances occur primarily in the seeds of certain pigmented cultivars of sorghum, millets and legumes (Deshpande et al., 1984; Salunkhe et al., 1982). However, not all phenolics are of nutritional concern and thus the total phenolic content of foods may not offer a true index of their nutritional quality. Some methods commonly employed in tannin analysis suffer from lack of specificity, as they do not distinguish polyphenols of nutritional concern from other low-molecular-weight phenols that also occur naturally in these products. No literature data are available on tannins of beach pea; however, substantial data are available on tannins in *Phaseolus vulgaris* L. seeds (Salunkhe et al., 1982). The objective of this study was to evaluate the effects of various solvent extraction systems on the recovery of beach pea tannins.

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2. Materials and methods

2.1. Materials

The mature pods, branches plus stems and leaves of beach pea were collected from Bellevue Beach, Salmon Cove and Sandy Cove in September–October of 1995, 1996 and 1998. The seeds and pod shells were separated manually. The total fresh weight and recovery of seeds and pod shells were recorded and samples used for moisture determination immediately after harvesting and separation. Samples were dried and then kept at room temperature for further study. Seeds of green pea and grass pea were obtained from the Plant Ecology Department, University of Saskatchewan and Agriculture and Agri-Food Canada, Morden, Manitoba, respectively. The seeds of beach pea were small, round, black- and green-coloured, but green peas were round, big, green-coloured, and grass peas were irregular, dark brown and wrinkled. The seeds of beach pea, green pea and grass pea were ground using a Moulinex coffee grinder (Black and Decker Canada Inc., Brockville, ON) and subsequently sieved with a 60-mesh sieve and used immediately for subsequent analysis.

2.2. Extraction of tannins

A 1–2 g pea flour sample was extracted with different solvents as follows. Pea flours (1–2 g), together with 40 ml water, were heated in a boiling water bath for 30 min, centrifuged (4000×***g***) and the supernatant collected. The procedure was repeated two more times and the combined extracts were freeze-dried and then solubilized in absolute methanol, centrifuged (4000×***g***) and total volume made to 100 ml; this latter solution was then used for vanillin-HCl assay.

Pea flours (1–2 g) together with 40 ml acidified water (1%, v/v, HCl in water) were heated in a boiling water bath for 30 min, centrifuged (4000×***g***) and the supernatant collected in a clean beaker. This procedure was repeated two more times; the combined extracts were freeze-dried and, after solubilization in absolute methanol, centrifuged (4000×***g***) and the volume made to 100 ml prior to performing the vanillin-HCl assay.

Pea flours (1–2 g) were extracted three times with 10–20 ml absolute methanol, absolute acetone, 90, 80 and 70% methanol or 90, 80 and 70% acetone. In another experiment, 100, 90, 80, and 70% methanol, as well as similar acetone solutions, acidified with 1% concentrated HCl, respectively, were used as extraction solvents. Samples were homogenized using a PT 3000 Polytron homogenizer (Brinkman Instruments, Rexdale, ON) for 1 min at 10,000 rpm and subsequently centrifuged (4000×***g***) and supernatants collected in a clean flask. This procedure was repeated two more times and combined extracts were evaporated using a rotary evaporator at 40 °C to dryness and the dry residue was then dissolved in 25 ml absolute methanol prior to performing the vanillin-HCl assay.

2.3. Determination of condensed tannins

The condensed tannins were assayed colorimetrically by the method of Price, Hagerman, and Butler (1978). To 0.2–1 ml of methanolic solution of condensed tannins, 5 ml of 0.5% vanillin reagent were added; a 5 ml volume of 4% concentrated HCl in methanol was used as a blank. The absorbances of samples and blank were read at 500 nm after standing for 20 min at room temperature. Catechin (+; 3.5 moles of water per mole of catechin, Sigma Chemical Co., St. Louis, MO) was used as a standard in these experiments. The content of condensed tannins in the meal was expressed as mg or g catechin equivalents per 100-g sample.

2.4. Statistical analysis

Statistical analysis (ANOVA and *t*-test) of data was carried out using the statistical software (SigmaStat).

3. Results and discussion

In previous studies we have demonstrated that beach pea served as an excellent source of condensed tannins (CT; Shahidi, Chavan, Naczk, & Amarowicz, 2001) that are both effective protein precipitants (Naczk, Amarowicz, Zadernowski, & Shahidi, 2001) and scavengers of DPPH radical (Amarowicz, Naczk, & Shahidi, 2000). In the literature, different solvent systems have been used for extraction of CT from plant materials as the extraction efficacy of CT depends on their chemical nature, solvent system used and extraction conditions employed (Shahidi & Naczk, 1995). The crude extracts of CT contain low-molecular-weight phenolics as well as CT. The low- molecular-weight phenolics may include phenolic acids, as well as several subclasses of flavonoids (Merken & Beecher, 2000). In addition the extracted CT consist of a series of oligomeric and polymeric compounds (Salunkhe, Chavan, & Kadam, 1990) that differ in their sensitivity toward the reagents used for their determination (Shahidi & Naczk, 1995). This makes the selection of appropriate methods for quantitation of phenolics a difficult task. Recently, Merken and Beecher (2000) reviewed HPLC methodologies for measurement of food flavonoids. According to these authors the existing HPLC methods can only separate a limited number of flavonoids and other phenolics and a method for simultaneous determinations of all prominent flavonoids is still needed. The molecular composition of crude beach pea CT extracts is still unknown and therefore it is difficult, based on available data, to select

Table 1
Effect of different solvents on extraction of condensed tannins from different peas[a]

Solvent	Beach pea (g/100 g meal)	Grass pea[b] (g/100 g meal)	Green pea (mg/100 g meal)	Grass pea[c] (mg/100 g meal)
Water	0.20±0.01	0.04±0.02	15.2±0.82	19.1±0.51
Water + HCl	0.46±0.08	0.12±0.03	40.0±0.10	53.0±0.49
Methanol				
100%	2.20±0.21a	0.17±0.12e	83.5±0.54	71.0±0.48
90%	2.20±0.12a	0.12±0.04e	34.0±0.03k	66.9±0.47p
80%	1.58±0.12	0.11±0.02e	25.7±0.34	56.2±0.72n
70%	0.92±0.07	0.21±0.05e	52.7±0.96	50.8±0.25o
Acidified methanol[d]				
100%	2.46±0.07b	0.23±0.12e	58.5±0.16l	49.3±0.50o
90%	2.85±0.13b	0.43±0.18e,f	66.0±0.27m	90.5±0.21
80%	4.40±0.13c	0.57±0.03f	69.0±0.19j	77.9±0.61
70%	4.54±0.67c	0.65±0.04f	69.8±0.24j	75.1±0.13
Acetone				
100%	0.23±0.11	0.14±0.05e	33.4±0.88k	34.8±0.89
90%	4.12±0.22c	0.75±0.20f,g	32.5±0.61k	59.1±0.54
80%	8.47±0.20	0.99±0.50f,g	43.0±0.80	57.2±0.91n
70%	10.2±0.88	1.04±0.30g	58.8±0.59l	45.1±0.37
Acidified acetone[d]				
100%	0.35±0.14	0.39±0.07f	57.4±0.76l	43.4±0.37
90%	6.59±0.43	0.93±0.53g	65.8±0.59m	64.9±0.17p
80%	10.7±0.58d	1.43±0.24h	69.7±0.74	67.0±0.47p
70%	11.6±0.19d	1.54±0.15h	72.0±0.12	109±0.73

[a] Results are means of six determinations, on a dry weight basis, ±standard deviation. Values in each column and row carrying the same superscripts are not different ($P > 0.05$) from each other.
[b] Indian grass pea.
[c] Canadian grass pea.
[d] Ninety nine millilitres of methanol or acetone at different concentrations (i.e. 100, 90, 80, or 70%, v/v) plus 1 ml of concentrated HCl.

both appropriate standards and HPLC methodologies for separation and quantitation of phenolics involved. Since the aim of this study was to evaluate the effectiveness of solvent systems for extraction of CT from beach pea, we selected the vanillin assay for quantification of CT crude extract. This method is commonly used for quantification of CT due to its specificity toward flavanols and dihydrochalcones (Shahidi & Naczk, 1995). Moreover, according to Oszmainski and Bourzeix (1996), the vanillin method provides the most accurate estimate of the content of CT. Methanol is usually used for carrying out the vanillin assay because, in methanol, the vanillin reaction is more sensitive toward polymeric CT than monomeric flavanols (Price, Van Scoyoc, & Butler, 1978). However, it may still lead to overestimation of CT content of crude extracts that are rich in monomeric components.

The content of condensed tannins of beach pea seeds ranged from 7.19% in fresh green seeds to 11.7% in fully mature dark green seeds (Table 1). The contents in green pea, Canadian grass pea and Indian grass pea were 0.07, 0.11, and 1.54%, respectively. The content of condensed tannins in beach pea was nearly 100 times more than that in green pea and Canadian grass pea and 7.5 times that of Indian grass pea. This might be due to the very thick coat of beach pea seeds. The cotyledons to seed coat ratio of beach pea was also higher than other peas examined. The content of condensed tannins in different plant parts of beach pea was significantly different ($P < 0.05$); the highest amount was present in dark green seeds (11.7%), followed by leaves (2.68%), mature pod shells (2.05%) and branches plus stems (0.90%). The synthesis of tannins in different plant parts may depend on the metabolic rate of tannin synthesis at a particular site. Another reason may be higher polymerization of existing polyphenolic compounds in the seed coat to high-molecular-weight compounds during maturation. The proportion of condensed tannins in beach pea seeds increased from 7.19% (fresh green seeds) to 11.7% (mature seeds), but in the case of pod shells, the reverse was observed; condensed tannins decreased from 9.13% (fresh green pod shells) to 2.05% (mature pod shells). These results indicate that, as the maturity progressed, delocalization of condensed tannins from pod shells to seeds occurred and this was followed by their polymerization into high-molecular-weight compounds. Price, Hagerman, and Butler (1980) analyzed 10 varieties each of cowpea,

Table 2
Condensed tannins in different plant parts of beach pea[a]

Plant part	Condensed tannins (g/100 g meal)
Fresh green seeds	7.19±0.26
Immature seeds	9.29±0.48
Mature seeds	11.7±0.39
Leaves	2.68±0.20
Branches plus stems	0.95±0.07
Premature pod shells	9.13±0.25
Mature pod shells	2.05±0.08

[a] Results are means of six determinations, on a dry weight basis, ±standard deviation. Seventy percent acetone, containing 1% concentrated HCl, was used as a solvent for extraction of tannins.

chickpea, pigeonpeas, and mung beans for their tannin content by vanillin assay and reported values ranging from zero to 0.7%. Several factors, such as plant type, cultivar, age of the plant or plant parts, stage of development, and environmental conditions, govern the tannin content in plants. The changes observed during development or maturation were mostly due to metabolism of polyphenolic compounds or polymerization of existing phenolic compounds.

The condensed tannins in different plant parts of beach pea were extracted into 70% acidified acetone; results are presented in Table 2. Mature seeds of beach pea had a higher tannin content than physiologically immature seeds (light in weight, light green colour, relatively smaller size). The fresh green seeds and mature pod shells had a lower amount of tannins than the premature pod shells and immature seed samples. Thus, as the maturity stage progressed, the concentration of beach pea tannins increased. Branches plus stems had a lower content of tannins (0.95%) than all other plant parts. Similar results were observed by Chang et al. (1994) for cowpea, but tannin content in cowpea was lower than that in beach pea. This increase in tannin content may be due to a higher polymerization of existing polyphenolic compounds in the seed coat to high-molecular-weight compounds during maturation. Similar results and conditions have been reported by earlier workers for beans (Deshpande & Cheryan, 1985; Salunkhe et al., 1982).

In conclusion, dark green coloured, mature seeds and premature pod shells of beach pea contained higher amounts of tannins than their immature counterparts. Acidified acetone-water served as an efficient system for recovery of a maximum amount of condensed tannins from different peas and plant parts of beach pea.

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