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SENSORY AND CHEMICAL APPROACH TO ASTRINGENCY OF EXTRACTS FROM SELECTED TANNIN-RICH FOODS

Agnieszka Troszyńska, Ryszard Amarowicz, Agnieszka Wołejszo

Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Division of Food Science, Olsztyn

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Extracts obtained from seeds of hazelnut, walnut, almonds, vetch, buckwheat and buckwheat grits were examined for their sensory astringency, contents of polyphenolic compounds and total antioxidant activity. The sensory astringency of this material was evaluated using the method of sensory scaling and expressed as Sensation of Astringency Indices (SAI). The contents of polyphenolic compounds and their total antioxidant activity were determined by spectrophotometric methods. The HPLC analysis was performed for phenolic acids evaluated in the extracts. The statistical analysis of the results indicated a statistically significant correlation between SAI and tannins, assayed by protein precipitation capacity, as well as SAI and total antioxidant activity. The HPLC analysis of the extracts demonstrated the presence of phenolic acids such as coffeic, *p*-coumaric, ferulic and sinapic.

INTRODUCTION

In the past decade, there has been much interest in tannins as bioactive components of food. They have been reported to exhibit anti-carcinogenic [Han, 1997; Yang *et al*., 1998; Clifford & Scalbert 2000], anti-atherogenic [Luo *et al*., 1997; Pearson *et al*., 1998; Kris-Etherton *et al*., 2001; Troszyńska & Bałasińska, 2002] and anti-microbial [Harbone & Williams, 2000; Amarowicz *et al*., 2000, 2005] effects, *etc*. The biological activity of tannins mentioned above is mainly connected with their high antioxidative properties. On the other hand, consumption of tannin-rich food and beverages is associated with the sensation described as astringency, which may limit their applications [Drewnowski & Gomez-Carneros, 2000].

Now the consumers expect both health benefits and palatable food. Sensorically, astringency is perceived as dry or rough sensation felt in the mouth [Lee & Lawless, 1991] whereas chemically astringents are defined as compounds which precipitate proteins. In world literature more and more information appear about antioxidative properties of tannins, whereas there is a lack of data on the relation between antioxidant activity of those compounds and their astringency.

Our preliminary study indicated that extracts obtained from seeds belonging to the same family *Leguminoseae* (faba bean, broad bean, pea, lentil, bean) have acted as effective astringents [Troszyńska *et al*., 2006]. The extracts showed precipitation ability of bovine serum albumin (BSA) and human salivary proteins (HSPs), which indicates that their astringency was responsible for condensed tannins. A statistically significant correlation ($p=0.05$) was found between the total antioxidant activity of extracts and their astringency [Amarowicz *et al*., 2004]. The present study is an extension to our earlier studies on astringency of tannin-rich foods. The aim of this research was to evaluate the astringency of tannins extracted from the raw materials originating from different families as well as to explore the relation between the astringency of extracts and their total antioxidant activity (TAA). The high performance liquid chromatography (HPLC) analysis of phenolic acids in the extracts was performed as well.

MATERIALS AND METHODS

Material. The materials tested included seeds of hazelnut (*Corylus avellana* L.), walnut (*Juglans regia* L.), almonds (*Amygdalus communis* L.), vetch (*Vicia sativa* L.), buckwheat (*Fagopyrum esculentum* Möench), and buckwheat grits. Hazelnut, walnut, almonds and buckwheat grits were purchased from a local market whereas the seeds of vetch and buckwheat were obtained from the Plant Breading Station in Olsztyn (Poland).

Extraction. All seeds were ground in a coffee mill. Hazelnut, walnut and almonds were defatted with hexane in a Soxhlet apparatus before grounding. Phenolic compounds were extracted from ground seeds according to Amarowicz *et al.* [1995] with 80% (v/v) aqueous acetone for 15 min at solid to solvent ratio of 1:10 (w/v). The extraction was repeated twice, supernatants combined and acetone evaporated under vacuum at 40°C in rotary evaporator. The remaining water solution was lyophilised and then evaluated for sensory astringency and by chemical analyses.

Sensory evaluation. Nine-member sensory panel (six women and three men) carried out astringency assessment using line scale of its intensity, anchored “none” and “very

Author’s address for correspondence: doc. Agnieszka Troszyńska, Institute of Animal Reproduction and Food Research, ul. Tuwima 10, 10-747 Olsztyn, Poland; tel.: (48 89) 523 46 26; fax: (48 89) 524 01 24; e-mail: ate@pan.olsztyn.pl

intensive". Prior to the above assessments panellists were familiarised with the method of sensory scaling of astringency during a 2-week training (nine sessions), using aqueous solutions of tannic acids of different concentration as reference of astringent material.

From each sample of a lyophilised extract of the tested material six aqueous solutions (in range of following concentrations: 0.2; 0.4; 0.6; 0.8; 1.0; 1.2%) have been prepared from stock solution of 2% in redistilled water. For testing 10-mL volume individual samples were prepared from each solution, coded and presented in random to each panel member. As astringency is quite persistent sensation, 3 min break was taken between the samples. The assessments were made in a sensory laboratory room, following general requirements for sensory testing conditions [PN-ISO 8589, 1998]. A computerised system was used for experimental setting and data collection. On the basis of obtained intensity/concentration curves sensation of astringency indices (SAI) were computed according to the formula:

$$SAI = \frac{AI_5}{EC}$$

where: AI_5 = astringency intensity of 5 units and EC = extract concentration (in %) by which the astringency is equal to 5 units (of 10 units intensity scale).

Total phenolics. The content of total phenolic compounds in each sample was estimated using Folin and Ciocalteau's reagent [Naczk & Shahidi, 1989]. (+)Catechin was used as a standard.

Protein precipitate assay. Proteins precipitation capacity of the extracts was assayed with the colorimetric method according to Hagerman & Butler [1978]. The extracts were dissolved in 1 mL of methanol and were added to protein dissolved in 2 mL of buffer (0.2 mol/L acetate, pH 5, containing 0.17 mol/L NaCl). The mixtures were vortexed, incubated for 30 min, and centrifuged at 5000 × g for 15 min. The supernatants were removed and the precipitates were rinsed with acetate buffer and centrifuged again. The protein-tannin/phenols complex was dissolved in a detergent system consisting of 1% (w/v) odium dodecyl sulfate (SDS) and 5% (v/v) triethanolamine. The tannins/phenolics present in the dissolved complex were measured at 510 nm after reaction with 1 mL of $FeCl_3$. The results were expressed as absorbance values (A_{510}) per gram of extracts.

Total antioxidant activity (TAA). The determination of the TAA was carried out using the Randox kit (Randox Laboratories Ltd., Crumlin, UK) according to the procedure provided by the supplier. The results were expressed as μmol Trolox/mg.

HPLC analysis of phenolic acids. Separation of phenolic acids was carried out according to Amarowicz & Weidner [2001]. Phenolic acids were analysed using a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan) consisting of a LC-10AD pump, SCTL 10A system controller, SPD-M 10A photo-diode array detector, and a prepacked LiChrospher 100 RP-18 column (4 × 250 mm, 5 μm; Merck, Darmstad, Germany). The mobile phase was water-acetonitrile--acetic acid (88:10:2; v/v/v) and was delivered at a rate of 1 mL/min. The detection was monitored at 320 nm.

Statistical analysis. The obtained chemical data were related to sensory evaluation and subjected to a statistical analysis. The correlation analysis between the sensation of astringency indices (SAI) and the total phenolic content, protein precipitation capacity (BSA) as well as the total antioxidant activity (TAA) was performed using Microsoft Excel software. All chemical analyses were determined in three replications.

RESULTS AND DISCUSSION

In order to compare the astringency of extracts, sensation of astringency indices (SAI) were calculated for particular samples (Table 1). It was shown that the SAI values were diversified and varied between 4.39 and 9.62. Among all the extracts, walnut had the highest SAI value (9.62) followed by hazelnut (5.75), buckwheat (5.62), almonds (5.22), buckwheat grits (4.76) and vetch (4.39). It should be emphasised that the extract of walnut demonstrated approximately twice as high astringency as the others. The same method was used earlier to study astringency of extracts obtained from tannin--rich legume seeds [Troszyńska *et al*., 2006]. It was found that the SAI of bean, lentil and pea extracts, ranged from 5.71 to 7.93, whereas these values for the extracts from broad bean and faba bean were lower and accounted for 4.46 and 3.84, respectively.

TABLE 1. Sensation of astringency indices (SAI) of extracts and extract concentration by which the astringency intensity is equal to 5 units (EC).

Extract	SAI	EC (%)
Walnut	9.62	0.52
Hazelnut	5.75	0.87
Almonds	5.22	0.96
Vetch	4.39	1.14
Buckwheat	5.62	0.89
Buckwheat grits	4.76	1.05

In literature the availability of data on the astringency of tree nuts, cereals and legume seeds in respect of polyphenols is scant, whereas beverages such as red wine, tea, cider, juice as well as several types of fruits serve as a rich source of information [Drewnowski &Gomez-Carneros, 2000; Lesschaeve & Noble, 2005].

The results of the contents of total phenolics and tannins as well as antioxidant activity of the extracts are presented in Table 2. The total phenolic compounds, expressed in catechin equivalents, were diversified and ranged from 16.1 mg/g of extract (almonds) to 109.2 mg/g of extract (walnut). For the determination of tannins in extracts we used the method based on a biological property of those compounds, because astringency has been identified to be associated with tannin-protein interaction in the mouth and saliva [Hagerman & Butler, 1978; Wróblewski *et al*., 2001; Bennick, 2002]. The extracts were characterised by different proteins (BSA) pre-

TABLE 2. Total phenolics, protein precipitation capacity and total antioxidant activity of the extracts.

Extract	Total phenolics (mg/g)*	Protein precipitation capacity (A_{510}/g)	Total antioxidant activity (μmol Trolox/mg)
Walnut	109.2	99.54	5.25
Hazelnut	64.9	20.13	0.64
Almonds	16.1	8.31	0.24
Vetch	66.3	22.34	0.79
Buckwheat	95.1	36.97	1.89
Buckwheat grits	74.2	23.88	2.48

*as a catechin equivalent

cipitation capacity expressed as the absorbance values at 510 nm per gram of sample. The highest absorbance value was reported for walnut (99.54 A_{510}/g), while the lowest one for almonds (8.31 A_{510}/g). On the basis of the results obtained, the ability of extracts to precipitate BSA can be ordered as follows: walnut >buckwheat >buckwheat grits >vetch >hazelnut >almonds.

The total antioxidant activity (TAA) of extracts showed a similar trend as protein precipitation capacity. The highest value of TAA (5.25 μmol Trolox/mg) was noted for the extract of walnut. Buckwheat grits (2.48 μmol Trolox/mg) and buckwheat (1.89 μmol Trolox/mg) were less active. Whereas vetch, hazelnut and almonds were the least active and demonstrated TAA of 0.79 μmol Trolox/mg, 0.64 μmol Trolox/mg and 0.24 μmol Trolox/mg, respectively. Our preliminary study indicated that the extracts obtained from 7 species of legumes with coloured seed coat were characterised by TAA values ranging from 0.30 μmol Trolox/mg (pea) to 1.76 μmol Trolox/mg (bean) [Amarowicz *et al*., 2004]. Zieliński & Kozlowska [2000] reported that extracts of some varieties of cereals (wheat, barley, rye, oat) had TAA values from 0.05 μmol Trolox/mg to 0.22 μmol Trolox/mg.

The results of the correlation analysis between total phenolics, proteins precipitation capacity, total antioxidant activity and SAI are presented in Figures 1a, b and c. A statistically significant linear correlation ($p \leq 0.05$) was found for proteins precipitation capacity *versus* SAI and total antioxidant activity *versus* SAI. The correlation coefficients between those parameters were $r^2=0.8797$ and $r^2=0.7118$, respectively. Our earlier study indicated the statistically significant correlation between the astringency of extracts from legume seeds and BSA as well as human salivary proteins precipitation [Troszyńska *et al*., 2006]. No satisfactory correlation has been found between the total phenolics and SAI ($r^2=0.3421$; $p \leq 0.05$). It can be explained by the fact that Folin-Ciocalteu reagent used in the total phenolic content method detects all phenolic groups present in the samples. The extracts, apart from astringents, could also contain varied phenolic compounds sensorically inactive. It is well known that sensory activity of polyphenols as well as their antioxidant properties are dependant not only on their relative concentration, but are also strictly connected with chemical structure (such as spatial conformation and degree of polymerization). According to literature, astringency of tannins increases with their degree of polymerization [Peleg *et al*.,

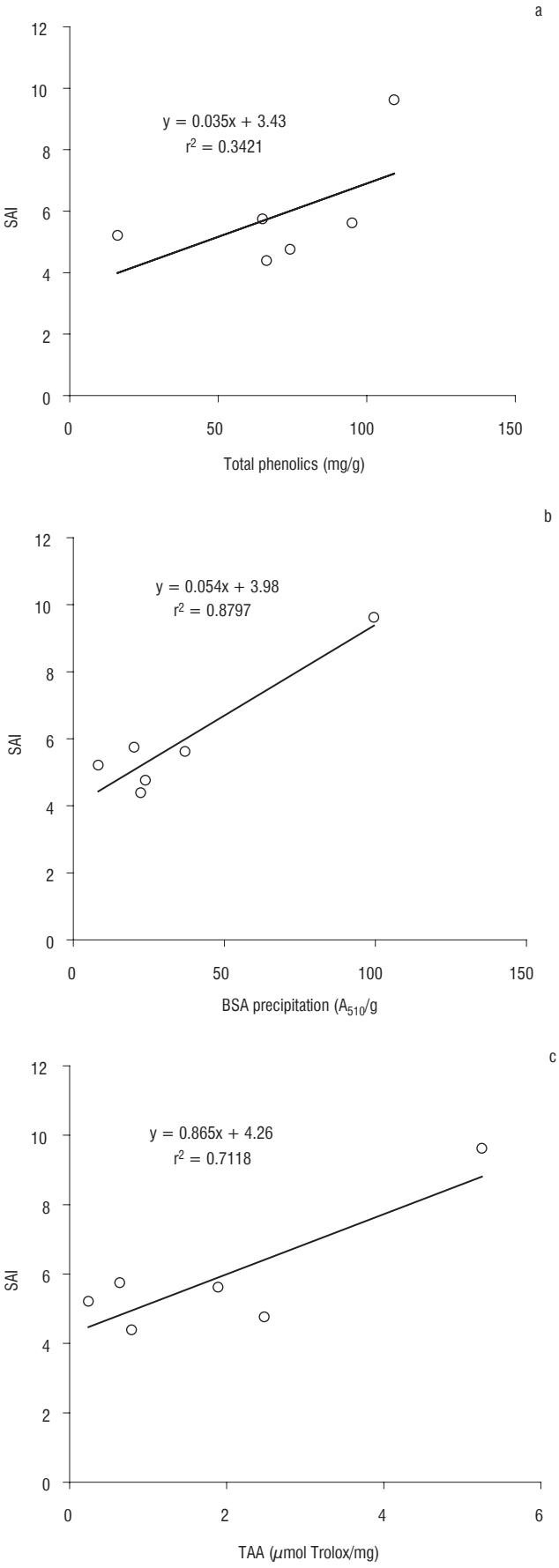

FIGURE 1. Correlations between sensation of astringency indices (SAI) and (a) total phenolics content, (b) BSA precipitation capacity, (c) total antioxidant activity.

Table 3. Presence of phenolic acids in the extracts.

Extract	Vanillic	Coffeic	p-Coumaric	Ferulic	Sinapic
Walnut	-	+	+	+	+
Hazelnut	-	-	+	+	+
Almonds	-	+	+	+	-
Vetch	-	+	+	-	+
Buckwheat	-	+	+	-	+
Buckwheat grits	-	+	+	+	-

1999; Lesschaeve & Noble, 2005]. In addition, a small difference in conformation can produce significant differences in sensory properties. A comparison of equal weights of catechin and epicatechin, which are chiral isomers, indicated that epicatechin was characterised by a higher intensity of astringency [Kielhorn & Thorngate, 1999].

Because the astringent sensation can also be elicited by small molecules the high performance liquid chromatography (HPLC) analysis of phenolic acids in the extracts was performed. The following phenolic acids have been identified in the samples: coffeic, *p*-coumaric, ferulic and sinapic (Table 3). Despite the inability of low molecular weight of phenols to precipitate proteins in chemical assays those compounds can participate in evoking the astringent sensation throughout the formation of soluble complexes with proteins [Charlton *et al*., 2002; Bartolomé *et al*., 2000]. More detailed studies are requested to find out whether these phenolic acids identified in the extracts can cause astringency. According to Peleg & Noble [1995], benzoic acid derivatives in equimolar concentrations elicited astringency but the intensity of this persistent attribute was significantly different. Salicylic and gentisic acids were the highest in astringency.

CONCLUSIONS

In conclusion, all extracts have been reported to be astringents in aqueous solution. The potency of astringency of extracts (SAI) decreased in the following order: walnut > hazelnut > buckwheat > almonds > buckwheat grits > vetch. We also found that SAI values were positively correlated with BSA precipitation and total antioxidant activity (TAA). In this context more extensive studies, both in sensorial and chemical area, are required to explore the relationship between the antioxidant activity and astringency of various polyphenols co-existing in food. It is caused by the fact that food-choice process is mainly based on sensory quality.

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SENSORYCZNE I CHEMICZNE ASPEKTY CIERPKOŚCI EKSTRAKTÓW Z WYBRANYCH ROŚLIN BOGATYCH W TANINY

Agnieszka Troszyńska, Ryszard Amarowicz, Agnieszka Wołejszo

Instytut Rozrodu Zwierząt i Badań Żywności Polskiej Akademii Nauk, Oddział Nauki o Żywności, Olsztyn

W pracy zbadano zależność pomiędzy cierpkością sensoryczną ekstraktów uzyskanych z orzechów włoskich, orzechów laskowych, migdałów, nasion wyki, gryki i kaszy gryczanej, a zawartością w nich związków fenolowych i ich aktywnością przeciwutleniającą. Cierpkość sensoryczną wyrażono w wartościach Indeksów, charakteryzujących zależność pomiędzy stężeniem ekstraktów, a ich intensywnością wrażenia cierpkości. Zawartość związków fenolowych w ekstraktach i ich całkowitą aktywność przeciwutleniającą, oznaczono metodami spektrofotometrycznymi. Stwierdzono istotną statystycznie korelację: (1) pomiędzy sensoryczną cierpkością ekstraktów, a zawartością w nich tanin, oznaczonych metodą precypitacji albuminy surowicy wołowej oraz (2) pomiędzy sensoryczną cierpkością, a aktywnością przeciwutleniającą ekstraktów. Analiza HPLC wykazała obecność w ekstraktach fenolokwasów takich jak: kawowy, *p*-kumarowy, ferulowy i synapowy, które mogły również wpływać na cierpkość badanych ekstraktów.