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EXTRACTS FROM SELECTED TANNIN-RICH FOODS – A RELATION BETWEEN TANNINS CONTENT AND SENSORY ASTRINGENCY

Agnieszka Troszyńska, Olga Narolewska, Agnieszka Wołejszo

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

Key words: black chokeberry, green tea, walnut, tannins, astringency, sensory evaluation

Extracts of polyphenols were obtained from black chokeberry, green tea, and walnut using acetone-water and ethanol-water system (8:2, v/v). The extracts were subjected to sensory evaluation using the method of sensory scaling and quantitative descriptive analysis (QDA). In sensory scaling a trained panel rated the samples for astringency which was expressed as Sensation of Astringency Coefficients (SAC). The QDA was applied for quantitative and qualitative characteristics of the extracts. To determine the content of tannins three spectrophotometric methods were used (n-butanol-HCl hydrolysis, BSA precipitation assay and PVP binding assay). The results proved that both the source of tannins and the type of solvent used for extraction had significant effects on the astringency and sensory profiles of the extracts. The analysis of multiple regression demonstrated that astringency of the extracts examined was affected to the greatest extent by tannins determined with the method of their binding on polyvinylpyrrolidone (PVP) sorbent.

INTRODUCTION

Foods of plant origin are a rich source of a variety of bioactive non-nutrient compounds, including polyphenols, whose beneficial effects on human health have been revealed in the last decades. It has been reported that the consumption of food rich in phenolic compounds is associated with a reduced risk of various chronic diseases, such as certain types of cancer, cardiovascular disease and age-related functional decline [Han, 1997; Yang *et al*., 1998; Clifford & Scalbert 2000, Luo *et al*., 1997; Kris-Etherton *et al*., 2001].

As a result, the food industry tends to incorporate these bioactive components into products in order to enhance their health effects. Unfortunately, many of the polyphenols indicate bitter and astringent taste that are typically perceived as having negative hedonic value and may limit their applications [Drewnowski & Gomez-Carneros, 2000; Lesschaeve & Noble, 2005]. These negative sensory attributes are mainly demonstrated by tannins, *i.e.* catechins and their oligomeric and polymeric forms. The content and the composition of particular forms determine the intensity of bitterness and astringency in food and beverages. According to literature, lower-molecular-weight tannins are more bitter whereas the higher-molecular-weight polymers are more likely to be astringent [Peleg *et al*., 1999; Lesschaeve & Noble, 2005]. In addition, a small difference in the conformation can produce significant differences in the sensory properties. The comparison of equal weights of catechin and epicatechin, which are chiral isomers, indicated that the epicatechin was characterised by higher intensity of astringency [Kielhorn & Thorngate, 1999].

Diverse biological activity of tannins and their common occurrence in plants have prompted a widespread interest in their extraction methods. Different solvents (water, acetone, methanol, ethanol) and an aqueous solvent mixture were used in order to investigate the extraction efficiency and the composition of polyphenols present in the extracts [Alonso *et al.*, 1991; Kallithraka *et al.*, 1995; Amarowicz *et al*., 1995; Waterman & Mole 1994]. On the basis of a survey of solvents and procedures for the extraction of tannins, it was found that the acetone–water mixture is a more effective extractant than the alcoholic solvents. Whereas, their influence on the sensory astringency of extracts still remains unexplored.

The objective of the study was to compare parallel chemical and sensory characteristics of extracts obtained with two different solvent systems in order to describe the relationship between tannin contents and their astringency. The focus in this work is on food-rich tannins such as: fruits of black chokeberry, leaves of green tea and seeds of walnuts which are recommended as healthy dietary components.

MATERIAL AND METHODS

Preparation of extracts. Green tea leaves and walnuts were purchased from a local market whereas black chokeberry (*Aronia melanocarpa Elliot*) was obtained from the eco-

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

logical farm in the north-eastern Poland. All samples were ground and walnuts were defatted with hexane in a Soxhlet apparatus before grinding.

Polyphenols were extracted from the plant materials using acetone-water and ethanol-water system (8:2, v/v) according to Amarowicz *et al.* [1995]. The extraction was repeated twice, the supernatants combined and the organic solvent evaporated under the vacuum at 40°C in a rotary evaporator. The remaining water solution was lyophilised and the extracts obtained were evaluated using the sensory and chemical methods. Six types of samples prepared for the study were abbreviated as: CA – acetone extract of chokeberry, CE – ethanolic extract of chokeberry, TA – acetone extract of green tea, TE – ethanolic extract of green tea, WA – acetone extract of walnuts, and WE – ethanolic extract of walnuts.

Chemical analyses. Three spectrophotometric methods were used in this study. In the first one, proanthocyanidins present in the samples were hydrolysed by butyl alcohol-HCl-Fe^{3+} according to the method described by Porter *et al*. [1986]. Then absorbance of anthocyanidins liberated from tannins was read at 550 nm. In the second method, the tannins were determined by protein (bovine serum albumin – BSA) precipitation assay according to Hagerman & Butler [1978]. The results are expressed as absorbance values (A_{510}) per gram of extracts. In third method the polyvinylpyrrolidone (PVP) capacity for binding tannins was applied [Makkar *et al*., 1993]. The content of tannins was calculated as the difference between total phenolic content in the extract and that in the supernatants (non-bound by PVP). The content of total phenolics in each sample was estimated using Folin and Ciocalteau's phenol reagent [Naczk & Shahidi, 1989]. (+)Catechin was used as a standard in this study. The results were expressed as (+) catechin equivalent per gram of extract. The chemical analyses were carried out around the same time as the sensory study.

Sensory evaluation. The astringency sensation of the extracts was determined by the method of sensory scaling and expressed as Sensation of Astringency Coefficients (SAC). The intensity of astringency was measured on a linear scale, anchored "none" and "very intensive" and the results were then converted to numerical values (10 units). The concentration *vs*. intensity plot was calculated for aqueous solutions (0.2, 0.4, 0.6, 0.8, 1.0, 1.2%) for each extract. On the basis of the curves obtained, SAC values were computed for particular extracts from the following equation [Kostyra, 2003]:

$$SAC = \frac{I_{max} - I_{min}}{\log C_{max} - \log C_{min}}$$

where: I_{min} – the intensity of astringency at the lowest concentration of sample, I_{max} – the intensity of astringency at the highest concentration of sample, C_{max} – the highest concentration of samples, C_{min} – the lowest concentration of samples.

The extracts were also evaluated by the quantitative descriptive analysis (QDA) method according to ISO standard [ISO 13299:1998]. Prior to the analysis, vocabularies of the taste attributes were developed for each type of extract by the panelists together with the panel leader in a round-table session. The intensity of attributes was measured using the same type of scale as above. The attributes rated by the panelists and their definitions are presented in Table 4.

The sensory evaluation of extracts (SAC and QDA) was carried out by a sensory panel consisting of 9 members previously selected and trained according to ISO guidelines [ISO 8586-1:1993]. All had previous experience in evaluating astringency by participation in a related study [Troszyńska *et al*., 2006]. Extracts (10 mL) in coded glass cups were presented to the panelists in a random order. To minimise potential carry-over effects the panelists were required to eat unsalted crackers and rinse their mouth thoroughly with spring water. The assessments were carried out at a sensory laboratory room which fulfils the requirements of ISO standards [ISO 8589:1998]. Scores were recorded and collected using a computerised system [Baryłko-Pikielna, 1992].

Statistical analysis. ANOVA was used to test statistical differences in chemical data as well as sensory results. Treatment means were compared using the Fisher's protected least significant difference (LSD) test. Statistical significance was considered at $p<0.05$. In order to describe the relationship between results of chemical data and the sensation of astringency coefficients (SAC), a multiple regression analysis was performed. The correlation analysis between intensity of astringency evaluated by the QDA method and contents of tannins in extracts was applied as well. The Pearson's correlation coefficients were calculated. Statistical analyses were performed using software package (statSoft Inc., v. 7.1, Tulsa, OK, USA). All analyses were determined in three replications.

RESULTS AND DISCUSSION

The results of tannin contents in the extracts are presented in Table 1. Three analytical methods have been used to quantify tannins in plant materials as there is no single assay which can assess the tannin content in a heterogeneous mixture, *i.e.* methods involving cleavage reaction (n-butanol-HCl hydrolysis), and precipitation reactions (the BSA precipitation method and PVP binding assay for tannins). The previ-

TABLE 1. Content of total phenolics and tannins in extracts.*,**

Extracts	Tannins			Total phenolics (mg/g)
	n-butanole/HCl (A_{550}/g)	BSA – precipitation (A_{510}/g)	PVP – binding (mg/g)	
CA	2744^{fB}	114^{dB}	98^{bB}	118^{aA}
CE	1749^{eA}	80^{bA}	91^{aA}	116^{aA}
TA	771^{dB}	95^{cB}	416^{fB}	428^{eB}
TE	646^{cA}	67^{aA}	349^{eA}	361^{dA}
WA	208^{aA}	132^{fB}	197^{cA}	225^{cB}
WE	277^{bB}	125^{eA}	206^{dB}	214^{bA}

* Mean chemical analysis ratings of extracts, four replicates.

** Values followed by the same letter in the same column are not significantly different (LSD test, $p<0.05$).

Small letters describe comparison between all extracts, capital letters describe comparison between acetone and ethanolic extracts.

ous one is used to determine the amount of condensed tannins (proanthocyanidins), whereas the latter measure both condensed and hydrolysable tannins [Schofield *et al*., 2001].

It was demonstrated that the content of tannins in the extracts was diversified to a statistically significant extent ($p<0.05$). It was determined by their origin and type of solvent used for extraction (Table 1). Chokeberry extracts were characterised by the highest content of proanthocyanidins (CA- 2744 A 550/g; CE – 1749 A 550/g), extracts of walnuts demonstrated the highest capacity for BSA precipitation (WA – 132 A 510/g; WE – 125 A 510/g), whereas extracts of green tea displayed the strongest binding with the PVP polymer (TA – 416mg/g; TE – 349 mg/g). It should be emphasized that the acetone-water system extracted substantially more tannins that the ethanol-water system, except for walnuts. The observed better extractivity of tannins with aqueous acetone confirms results of earlier investigations [Amarowicz *et al*., 1995].

In order to compare the chemical results and the sensory evaluation of extracts, Sensation of Astringency Coefficients (SAC) were calculated for particular extracts. Mean values of the intensity of astringency in relation to their concentrations are shown in Table 2. The results obtained served to plot an experimental curve in a semi-logarithmic plot (Figure 1), which was then used to calculate the intensity of astringency at the lowest (I_{min}) and the highest (I_{max}) concentrations of the extracts. I_{min} and I_{max} as well as SAC values (determined according to the formula) are presented in Table 3. It was shown that among all extracts analysed, TA had the highest SAC (9.32) followed by TE (9.04), CA (8.45), CE (7.49), WA (6.75) and WE (5.87). It should be stressed that the acetone extracts (richer in tannins) were characterised by higher values of I_{min}, I_{max} and SAC than the ethanolic ones.

TABLE 2. Mean values of the intensity of astringency (scale range 0–10 units).

Extracts	Concentrations of extracts (%)					
	0.2	0.4	0.6	0.8	1.0	1.2
CA	1.5	3.0	4.5	5.8	6.9	8.1
CE	1.9	2.6	4.0	5.1	6.2	7.0
TA	2.6	4.4	5.9	7.3	8.6	9.9
TE	1.8	3.5	4.9	6.2	7.8	8.9
WA	1.2	2.4	3.2	4.7	5.6	6.4
WE	0.6	1.5	2.5	3.8	4.4	5.2

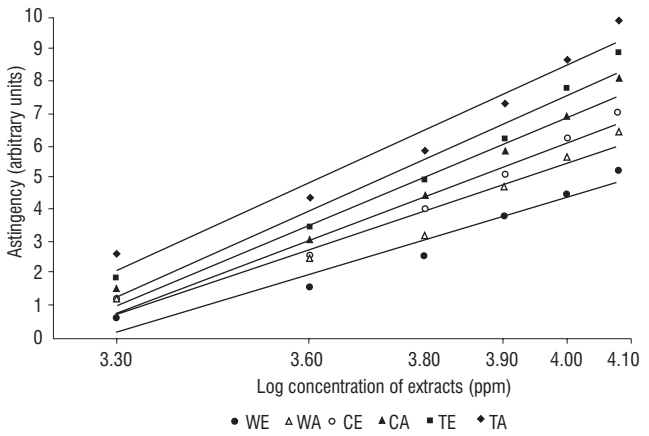

FIGURE 1. The intensity of astringency in relation to concetrations of extracts.

TABLE 3. Sensory astringency coefficients and the intensity of astringency at the lowest and the highest concentrations of extracts.

Extracts	SAC	$I_{min.}$	$I_{max.}$
CA	8.45	1.50	8.07
CE	7.49	1.19	7.01
TA	9.32	2.63	9.87
TE	9.04	1.81	8.85
WA	6.75	1.17	6.42
WE	5.85	0.62	5.17

The QDA analysis demonstrated that the type of solvent used (water solution of acetone and ethanol) and the source of tannins were the factors differentiating sensory profiles of the samples (Figure 2). Astringency of acetone extracts from chokeberry (CA) and walnuts was statistically significantly ($p\leq0.05$) higher as compared to the other samples. Profilograms of the sample indicate that, apart from astringency, the samples were also characterised by other negative attributes,

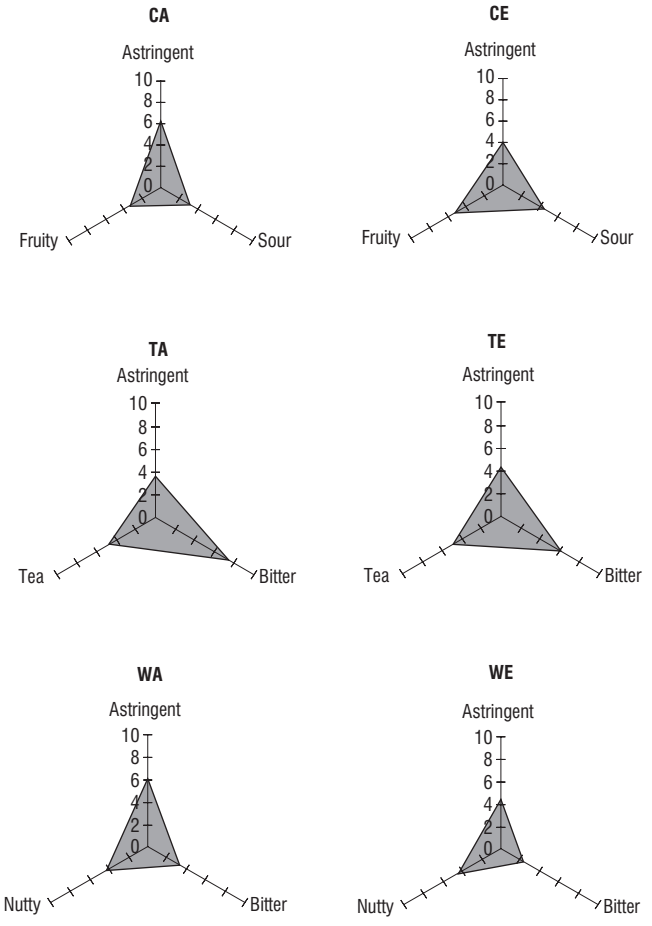

FIGURE 2. Spider diagrams of sensory profiling of extracts: CA – acetone extract of chokeberry; CE – ethanolic extract of chokebery; TA – acetone extract of green tea; TE – ethanolic extract of green tea; WA – acetone extract of walnuts; WE – ethanolic extract of walnut.

TABLE 4. Taste attributes definitions for extracts profiling.

Taste attributes	Definition of attribute
Astringent	The intensity of dryness, roughness and puckerness in the mouth (reference sample: tannic acid in water 0.2%)
Bitter	Basic taste (reference sample: caffeine in water 0.2%)
Sour	Sour note characteristic for citric acid (reference sample: citric acid in water 0.15%)
Fruity	Typically associated with berry fruits
Tea	Taste characteristic to infusion of green tea
Nutty	Taste related to notes such as walnuts, hazelnuts, almonds

including bitterness (TA, TE, WA, WE) and sourness (CA, CE). High intensity of bitter taste was reported for the green tea extracts (TA, TE). That negative attribute predominated over other quality attributes of the extracts, especially in the acetone extract (Table 4). The bitter taste of green tea can be evoked by phenolic compounds, including: epicatechin, epicatechin gallate, epigallocatechin, epigallocatechin gallate, catechin as well as caffeine. An HPLC analysis demonstrated that the content of catechin in the acetone and ethanolic extracts accounted for 68.8 mg/g and 74.1 mg/g, respectively (paper in preparation). The lower content of catechin in the TA extract and simultaneously higher intensity of its bitterness suggest that this attribute was affected to a great extent by phenolic compounds. It should be emphasized that taste notes typical of the raw materials examined, *i.e.* fruity (CA, CE), tea (TA, TE), and nutty (WA, WE), were clearly distinguished on sensory profiles of all extracts examined.

A linear multiple regression analysis was used to determine the relationships between the content of tannins and sensory astringency of extracts. The results have shown that the SAC values were correlated by tannins determined using the PVP-binding assay. The equation of the fitted model was as follows: $y=3.8913 + 0.0013x_1 = 0.011x_2$, where: y – SAC values, x_1 and x_2 independent variables expressed by content of tannins (x_1 – n-butanol/HCl; x_2 – PVP-binding assay). The R^2 statistics indicates that the equation explains 89.67% of the variability of sensory astringency of the extracts.

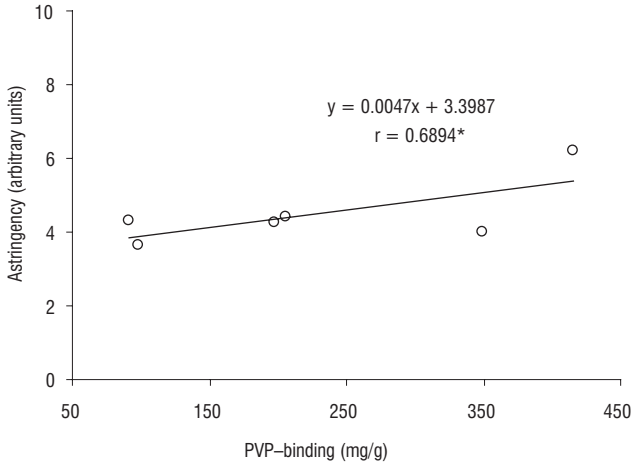

FIGURE 3. Correlation between astringency of extracts and PVP-binding assay for tannins (* significant at p≤0.1).

The correlation analysis was also used to determine the relationships between the content of tannins in the extracts and intensity of their astringency evaluated with the QDA method (Figure 3). A statistically significant correlation (p≤0.1) was found between the astringency and tannins determined with the PVP-binding assay. The correlation coefficient between those parameters was r =0.689.

CONCLUSIONS

In conclusion, we found that astringency of the extracts was affected by both the source of tannins and the type of solvent used for their extraction. On the basis of the results obtained, astringency of the extracts can be ordered as follows: TA > TE > CA > CE > WA > WE. A statistically significant correlation was found between SAC values and tannins content determined by PVP-binding assay. These results may be useful for producers to design health-promoting food products.

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EKSTRAKTY Z WYBRANYCH SUROWCÓW BOGATYCH W TANINY – ZALEŻNOŚĆ POMIĘDZY ZAWARTOŚCIĄ TANIN I CIERPKOŚCIĄ

Agnieszka Troszyńska, Olga Narolewska, Agnieszka Wołejszo

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

Celem pracy było porównanie cierpkości ekstraktów uzyskanych z owoców aronii, liści zielonej herbaty i orzechów włoskich stosując do ekstrakcji tanin wodny aceton i wodny etanol (8:2, v/v). Badania sensoryczne wykonano metodą skalowania sensorycznego i profilowania smakowitości ekstraktów. Oceny sensoryczne wykonał wyszkolony zespół 9-osobowy. Zawartość tanin w badanym materiale oznaczono trzema metodami spektrofotometrycznymi. Stwierdzono, zróżnicowanie cierpkości i profili sensorycznych badanych ekstraktów na co miał wpływ zarówno zastosowany układ rozpuszczalników jak i źródło tanin. Analiza regresji wielokrotnej wykazała, że największy wpływ na cierpkość ekstraktów miały taniny oznaczonych metodą wiązania tych związków na sorbencie polivinylopyrolidonowym (PVP).