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

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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 & Thongate, 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-
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) accord-
ing to Amarowicz et al. [1995]. The extraction was repeat-
eed 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 meth-
ods were used in this study. In the first one, proanthocyani-
dins present in the samples were hydrolysed by butyl alco-
hol-HCl-Fe³⁺ 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 & But-
ler [1978]. The results are expressed as absorbance values
(A510) per gram of extracts. In third method the polyvinyl-
pyrrolidone (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 con-
tent 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 concen-
tration 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 particu-
lar extracts from the following equation [Kostyra, 2003]:

\[
\text{SAC} = \frac{I_{\text{max}} - I_{\text{min}}}{\log C_{\text{max}} - \log C_{\text{min}}}
\]

where: \(I_{\text{min}}\) – the intensity of astringency at the lowest con-
centration of sample, \(I_{\text{max}}\) – the intensity of astringency at the
highest concentration of sample, \(C_{\text{max}}\) – the highest con-
centration of samples, \(C_{\text{min}}\) – the lowest concentration of
samples.

The extracts were also evaluated by the quantitative
descriptive analysis (QDA) method according to ISO stan-
dard [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 pan-
elists and their definitions are presented in Table 4.

The sensory evaluation of extracts (SAC and QDA) was
conducted by a sensory panel consisting of 9 members pre-
viously 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 pre-
pared and presented 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.

RESULTS AND DISCUSSION

The results of tannin contents in the extracts are present-
ed 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 mix-
ture, i.e. methods involving cleavage reaction (n-butanol-HCl
hydrolysis), and precipitation reactions (the BSA precipita-
tion method and PVP binding assay for tannins). The previ-

### Table 1. Content of total phenolics and tannins in extracts.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>n-butanol/HCl (A_{580}/g)</th>
<th>BSA – precipitation (A_{590}/g)</th>
<th>PVP binding (mg/g)</th>
<th>Total phenolics (mg/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>2744          bB</td>
<td>114          bB</td>
<td>98              bB</td>
<td>118          A</td>
</tr>
<tr>
<td>CE</td>
<td>1740          cA</td>
<td>80            aA</td>
<td>91              A</td>
<td>116          A</td>
</tr>
<tr>
<td>TA</td>
<td>771           dB</td>
<td>95            aB</td>
<td>416             bB</td>
<td>428          B</td>
</tr>
<tr>
<td>TE</td>
<td>646           aA</td>
<td>67            aA</td>
<td>349             aA</td>
<td>361          A</td>
</tr>
<tr>
<td>WA</td>
<td>208           bA</td>
<td>132           bB</td>
<td>197             aA</td>
<td>225          B</td>
</tr>
<tr>
<td>WE</td>
<td>277           bB</td>
<td>125           aA</td>
<td>206             bB</td>
<td>214          A</td>
</tr>
</tbody>
</table>

* Mean chemical analysis ratings of extracts, four replicates.
** Values followed by the same letter in the same column are not signifi-
cantly 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 than 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\text{min}) and the highest (I\text{max}) concentrations of the extracts. I\text{min} and I\text{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\text{min}, I\text{max} and SAC than the ethanolic ones.

<table>
<thead>
<tr>
<th>Extracts</th>
<th>SAC</th>
<th>I\text{min}</th>
<th>I\text{max}</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA</td>
<td>8.45</td>
<td>1.50</td>
<td>8.07</td>
</tr>
<tr>
<td>CE</td>
<td>7.49</td>
<td>1.19</td>
<td>7.01</td>
</tr>
<tr>
<td>TA</td>
<td>9.32</td>
<td>2.63</td>
<td>9.87</td>
</tr>
<tr>
<td>TE</td>
<td>9.04</td>
<td>1.81</td>
<td>8.85</td>
</tr>
<tr>
<td>WA</td>
<td>6.75</td>
<td>1.17</td>
<td>6.42</td>
</tr>
<tr>
<td>WE</td>
<td>5.85</td>
<td>0.62</td>
<td>5.17</td>
</tr>
</tbody>
</table>

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≤0.05) higher as compared to the other samples. Profiograms of the sample indicate that, apart from astringency, the samples were also characterised by other negative attributes.

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

<table>
<thead>
<tr>
<th>Extracts</th>
<th>Concentrations of extracts (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.2</td>
</tr>
<tr>
<td>CA</td>
<td>1.5</td>
</tr>
<tr>
<td>CE</td>
<td>1.9</td>
</tr>
<tr>
<td>TA</td>
<td>2.6</td>
</tr>
<tr>
<td>TE</td>
<td>1.8</td>
</tr>
<tr>
<td>WA</td>
<td>1.2</td>
</tr>
<tr>
<td>WE</td>
<td>0.6</td>
</tr>
</tbody>
</table>

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

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 bitter notes typical of the raw materials examined, by phenolic compounds. It should be emphasized that taste variability of sensory astringency of the extracts. The results have shown 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 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.

ACKNOWLEDGEMENTS

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REFERENCES

Ekstrakty z wybranych surowców bogatych w taniny – zależność pomiędzy zawartością tanin i cierpkością

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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 wykonano w 9-osobowym zespole. Zawartość tanin w badanym materiale oznaczono trzema metodami spektrofotometrycznymi. Stwierdzono, że różnica w zawartości tanin i cierpkości ekstraktów, pomimo podobnego stosowanego układu rozpuszczalników, jest istotna. Regresja wielokrotna wykazała, że największy wpływ na cierpkość ekstraktów miały taniny oznaczone przez methodę wiązania tych związków na sorbencie polivinylopyrolidonowym (PVP).