Influence of Tannin Addition on the Content and Composition of Polyphenolic Compounds in Wines

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Abstract: Polyphenols can greatly affect the sensorial characteristics and stability of wine. The concentration of polyphenols in wines is very low, the sample must be thus concentrated before the analytical measurement. The extraction on solid phase (SPE) is a suitable method for the isolation, purification, and concentration of polyphenols from complicated matrices. RP-HPLC with diode array detection was used for the separation and identification of polyphenols. A library of absorption spectra of standards was created and used for the identification of 14 polyphenols in wines. The contents of the individual polyphenols and their changes after the addition of four tannin preparations were determined in eight white and two rosé wine samples. The influence of the flavour profile of the applied tannin preparations on sensorial characteristics of wines was established

Keywords: wine, polyphenols; determination; HPLC-DAD; SPE; tanin

Phenolic compounds are secondary metabolites naturally present in plants. They have a great importance for the food and drink products derived from plants, since these compounds are responsible for their sensorial properties (Robbins 2003).

These compounds may be classified into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another. Distinctions are thus made between the phenolic acids, flavonoids, stilbenes, and lignans. The flavonoids, which share a common structure consisting of 2 aromatic rings that are bound together by 3 carbon atoms that form an oxygenated heterocycle, may themselves be divided into 6 subclasses as a function of the type of heterocycle involved: flavonols, flavones, isoflavones, flavanones, anthocyanidins, and flavanols (catechins and proanthocyanidins) (Manach *et al*. 2004).

Fruits and beverages (fruit juice, wine, tea, coffee, chocolate, and beer) are the main dietary source of polyphenols, and, to a lesser extent, vegetables, dry legumes, and cereals. The total intake is ~1 g/day at Western type of diet (Scalbert & Williamson 2000). Their contents in foods depend both on genetic factors (species and variety of herbage, stage of maturity) and environmental factors (light, temperature, fertilisation, pesticides, etc.) (Ribéreau-Gayon *et al*. 2000). Phenolics may help in the estimation of authenticity of regional products (Arvanitoyannis *et al*. 1999) and in the prognosis of their sensory properties (Vidal *et al*. 2004). They might be used either as markers in different technological processes or at wine ageing (Moreno & Barroso 2002).

Some polyphenols are significant natural pigments (e.g. quinones, lignans, flavonoids, xanthons), others exert aromatic (e.g. some simple phenols and derivatives of hydroxyphenolic acids, coumarins) or gustatory (e.g . condensed tannin, flavanols) functions. They are either primary components of some volatile oils, or secondary aromatic substances formed during food processing (by the action of microorganisms and thermal processes) (Velíšek 2002).

Tannins are commonly classified either as hydrolysable tannins (tannic acid) or condensed tannins; the former are composed of gallic acid and/or ellagic acid esterified with glucose, and the latter ones of flavan-3-ol subunits (termed catechins) with various degree of substitution and polymerisation (Edelmann & Lendl 2002).

In general, tannins have been associated with organoleptic characteristics of wine. Condensed tannins belong to the compounds responsible for wine astringency. Monomeric flavonoids are primarily bitter, but as the molecular weight increases by polymerisation, astringency and bitterness can increase up to 25–30 times (Noble 1994).

The intake of foods containing polyphenols can participate in the protection of the human organism from some forms of cancer, above all cancer of the digestive tract, lung, breast, and prostate (Adlercreutz 2002).

The determination of polyphenolic compounds in wine samples usually requires extraction and pre-concentration procedures prior to HPLC via SPE (solid-phase extraction), due to the fact that wine matrices are very complex and many phenolic compounds are present at very low concentrations (Guillén *et al*. 1997).

The aim of this study was to specify the contents of individual polyphenolic compounds and determine the basic differences in the content and profile composition of polyphenolic constituents in 10 wine varieties.

MATERIAL AND METHODS

Samples. Ten wines produced in the southern Moravia for the company Bohemia Sekt Inc. in 2007 were sampled. Wines of quality level (Q) were produced from grapes with the sugar content of at least 15 degrees of the standardised must meter or from late harvest (LH), which can be produced from grapes with sugar content of at least 21 degrees of the standardised must meter. The selected wines included eight white wines: Müller Thurgau-Q – (MT-Q), Welschriesling-LH (W-LH), Chardonnay-Q – (CH-Q), Chardonnay-LH – (CH-LH), Sauvignon blanc-LH – (S-LH), Rheinriesling-LH – (R-LH), Sauvignon blanc-Q – (S-Q), Pinot blanc-Q – (P-Q) and two rosé wines: Zweigeltrebe-LH – (Z-LH), and Zweigeltrebe + Lemberger-Q – (Z+L-Q).

Reagents. The standards of gallic acid, protocatechuic acid, gentisic acid, (+)-catechin, caffeic acid, *p*-coumaric acid, ferulic acid, quercetin, *p*-hydroxybenzoic acid, vanillin, syringic acid, and rutin were purchased from Sigma-Aldrich (Germany), procyanidin B2 from Fluka (Switzerland), and resveratrol from the Department of Dairy and Fat Technology, Institute of Chemical Technology in Prague. Further reagents used were solvents such as methanol super gradient for HPLC, acetonitrile for HPLC from LabScan (Ireland), and acetic acid from Penta (Czech Republic). Tannins Castanea, Quercia, Premium UVA and Premium Limousin were obtained from O.K. Servis BioPro (Czech Republic).

Solid-phase extraction. The extraction was performed in a vacuum device SPE Vacuum Manifold Dorcus of Tessek (Czech Republic). AccuBond ODS-C18 (Agilent, UK) cartridges were used. The respective cartridge was conditioned with 5 ml of methanol followed by 10 ml of distilled water. An aliquot of the wine sample, previously acidified to pH 1.5 with hydrochloric acid (36%), was passed through the cartridge. Subsequently, phenolic compounds were eluted with 12 ml of acetone. The organic eluate was transferred into a 50 ml round-bottom flask and evaporated under

Table 1. Retention time and identification of the phenolic compounds

Compounds	t_R (min)	Wine
Gallic acid	4.1	+[a]
Protocatechuic acid	6.6	+[b]
Gentisic acid	8.6	+[a]
p-Hydroxybenzoic acid	9.7	+[c]
(+)-Catechin	14.3	+[a]
Caffeic acid	14.8	+[a]
Syringic acid	16.2	+[a]
Vanillin	16.3	+[a]
Procyanidin B2	16.5	+[a]
p-Coumaric acid	18.4	+[a]
Ferulic acid	21.0	+[a]
Rutin	26.7	+[a]
Resveratrol	30.3	+[a]
Quercetin	36.0	+[a]

+[a] detected; +[b] detected in 80% of the samples without addition of tannin; +[c] detected in 70% of the samples without an addition of tannin

vacuum at 35°C to dryness. The obtained residue was dissolved in 1 ml of mobile phase and transferred to a vial. Some samples had to be filtered through a cellulose filter (Millipore) 0.45 μm before being transferred to the vial (Dvořáková *et al*. 2007). Aliquots of 15 ml were used because this volume provided the highest yield of the determined phenolics.

HPLC analysis. A high-pressure liquid chromatography apparatus (Waters, model 2695) equipped with photo diode array detector (Waters, model 2996) was used. The Waters NovaPac C_{18} (4.6 × 250 mm) column with the particle size of 4 μm (Milford, USA) was used at 30°C. The injected volume was 10 μl. A constant flow rate of 1.2 ml/min was applied using two solvents: Solvent A, 0.7% acetic acid in water; solvent B, 20% solvent A mixed with 80% acetonitrile. For the elution program, the following proportions of solvent B were used: 0–5 min 2%; 5–10 min 6%; 10–15 min 12%; 15–30 min 22%; 30–35 min 34%; 35–40 min 100%; and 40–45 min 0% (Chamkha *et al*. 2003).

Sensory analysis. For the sensory evaluation of the wine samples, 12 panelists were employed. The panelists were 6 volunteers and 6 experts trained in the wine-tasting according to ISO standards. The samples were served at 12°C. The panelists rated the intensity of the taste and aroma using a hundred-point scale.

RESULTS

HPLC identification

Fourteen phenolic compounds were examined for their presence in wines (Table 1). The identification of the peaks was carried out by their spectra and their retention time in comparison with the standards. The results of the polyphenol content determination in the wine samples are shown in Table 2. The highest contents were found with (+)-catechin, i.e. from 1.01 to 9.59 mg/l. On the other hand, the content of protocatechuic acid ranged from not detected (ND) to 0.42 mg/l, and that of *p*-hydroxybenzoic acid from not detected to 0.08 mg/l; these compounds were found in the lowest concentrations.

Addition of tannin

Addition of tannin. Each of four different tannins was added in the same two doses of 1 and 5 g/hl into each wine. The addition resulted in a considerable increase of polyphenolic substances

Table 2. Polyphenol content (mean value in mg/l) in wine samples

Compounds	MT-Q	W-LH	CH-Q	CH-LH	S-LH	R-LH	S-Q	P-Q	Z-LH	Z+L-Q
Gallic acid	0.11	0.06	0.17	0.02	0.05	0.12	0.29	0.15	0.18	0.21
Protocatechuic acid	n.d.	0.42	0.03	n.d.	0.07	0.35	0.27	0.13	0.26	0.38
p-Hydroxybenzoic acid	0.05	n.d.	0.06	0.02	0.03	0.02	n.d	0.06	0.08	n.d
(+)-Catechin	1.01	1.90	1.78	6.28	9.59	1.76	4.35	1.14	3.48	1.82
Caffeic acid	0.10	0.08	0.09	1.16	0.16	0.10	0.27	0.13	0.14	0.15
Vanillin	0.75	0.66	0.29	0.37	0.36	0.62	0.55	0.77	1.29	0.88
p-Coumaric acid	0.14	0.07	0.11	1.38	0.17	0.09	0.35	0.13	0.15	0.17
Ferulic acid	0.22	0.49	0.18	0.25	0.23	0.21	0.33	0.16	0.28	0.33
Quercetin	0.17	0.31	0.31	0.41	0.32	0.34	0.30	0.19	0.29	0.35
Resveratrol	0.15	0.20	0.19	0.20	0.13	0.21	0.25	0.21	0.42	0.27
Gentisic acid	0.70	1.08	0.27	0.24	0.46	1.07	0.59	0.51	0.99	0.36
Syringic acid	0.33	0.21	0.05	0.24	0.07	0.26	0.67	0.04	2.91	1.99
Rutin	0.23	0.11	0.15	0.04	0.39	0.12	0.11	0.12	0.10	0.11
Procyanidin B2	0.19	0.42	0.24	0.18	0.16	0.33	0.71	0.73	0.45	0.38

n.d. (not detected); MT-Q (Müller Thurgau-quality), W-LH (Welschriesling-late harvest, CH-Q (Chardonnay-quality), CH-LH (Chardonnay-late harvest), S-LH (Sauvignon blanc-late harvest), R-LH (Rheinriesling-late harvest), S-Q (Sauvignon blanc-quality), P-Q (Pinot blanc-quality), Z-LH (Zweigeltrebe-late harvest), Z+L-Q (Zweigeltrebe + Lemberger-quality)

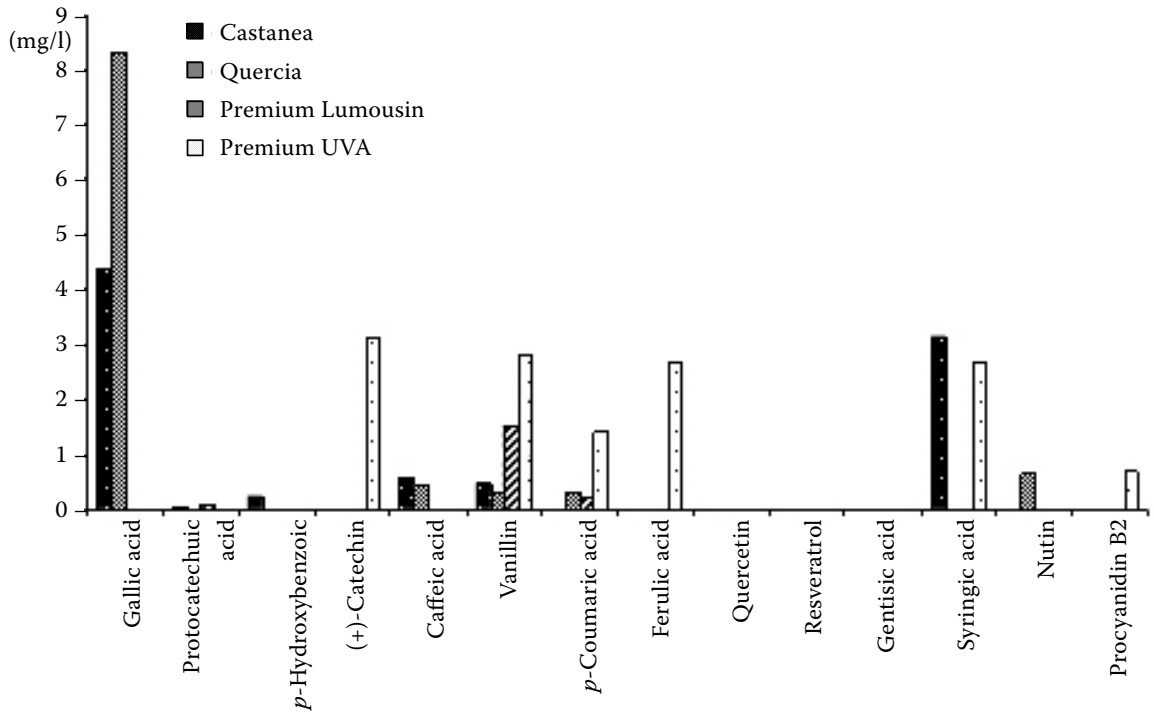

Figure 1. Substitution choices of polyphenols in elect tannins

content. This increase mostly correlated with the addition of tannin into wines. Each of tannins has a different composition of polyphenolic substances (Figure 1) and gives diverse sensorial characteristics to wines.

Sensory analysis

In the dose of 1g/hl, tannin Castanea added to wine MT-Q, supplemented an indistinct aroma of wine with an interesting tone and the wine became fully impressive. On the contrary, tannin-enriched W-LH acquired a woody flavour inappropriate for the fruit character of wine.

It was interesting that added tannin Quercia caused in 70% wine samples a sensorial improvement although this tannin is characteristic for its woody flavour. E.g., for W-LH, CH-Q, and CH-LH is this type of tannin completely unsuitable, sensed very astringently in taste. On the contrary, in S-LH the variety character of wine was preserved and at both the concentrations of 1 g/hl and 5 g/hl

Table 3. Optimal and inappropriate addition of different tannin preparations to wine samples

Wine	Optimal tannin (concentration)	Inappropriate tannin (concentration)
MT-Q	Premium UVA (5 g/hl)	Castanea (5 g/hl)
W-Q	Premium UVA (5 g/hl)	Quercia (5 g/hl)
CH-Q	Castanea (5 g/hl)	Premium Limousin (5 g/hl)
S-LH	Premium Limousin + P. UVA (1 g/hl and 5 g/hl)	Premium Limousin (5 g/hl)
R-LH	Premium Limousin + P. UVA (5 g/hl)	Castanea (5 g/hl)
P-Q	Premium UVA (1 g/hl)	Castanea (5 g/hl)
CH-LH	Premium UVA (5 g/hl)	Quercia (1 g/hl)
ZW-LH	Premium UVA (5 g/hl)	Premium Limousin (5 g/hl)
ZW+FR-LH	Premium UVA (1 g/hl)	Quercia (5 g/hl)
S-Q	Quercia (1 g/hl)	Castanea (5 g/hl)

For the abbreviations of wines see the Table 2

the taste was very becomingly completed with a gentle oak flavour.

Tannin Premium Limousin gave wines fine astringency and vanilla flavour, but at the higher concentration, it induced an amplified milky and acidulous flavour in white wines. Nevertheless, in P-Q the addition of this tannin at dose 1 g/hl gave the wine nice aroma of fruit to vanilla, the taste being harmonic with higher persistence. In the concentration of 5 g/hl, the taste gained fine foxiness, with which, however, the wine became completed.

In 8 wine samples, tannin Premium UVA resulted in an expressive improvement in the sensorial profile of wines as well as punctual valuation up to 8 points, in the hundred-point system. Wines treated with this tannin had characteristic complex bouquet and full taste, as well as sensations of sweetish tones.

We tested also the influence of a combination of tannins Premium Limousin and Premium UVA (concentrations 0.5 + 0.5 g/hl and 2.5 + 2.5 g/hl, respectively). In W-LH, the combination of tannins affected disturbingly. The effect of both tannins was partly mixed as to the taste and aroma, which was unsuitable. CH-Q also lost its harmonic quality in the taste. On the contrary, S-LH appeared to acquire a delicate aroma of unripe fruit and a persistent rise in tastes with minimum loss of the fruit character of wine.

In Table 3 is given the survey of tannins which proved to be optimal or unsuitable for the tested wine samples.

CONCLUSIONS

Among polyphenolic substances, catechin was found to be present in the highest concentration of 9.6 mg/l in Sauvignon-LH. However, catechin was found in higher levels in all wine samples. In higher amounts there were found also syringic acid, in Zweigeltrebe-LH (3 mg/l) and *p*-coumaric acid in Chardonnay-LH.

As regards sensorial analyses, for the majority of wines appeared to be optimal tannin Premium UVA in concentrations 1 g/hl and 5 g/hl, on the contrary Premium Limousin proved to be better for red wines.

In our study, the addition of all tannins was helpful for Sauvignon-LH wine, wine without the addition of tannins correspond to a typical character of variety, it was very rounded, however up to addition tannin the wine was roundness without expressive losses of fruity in smell. On the contrary, with Chardonnay-LH the addition of tannin was inappropriate, because, the wine was first-rate and its sensorial characteristics could not be expressively improved, by the tannin addition, each addition incurring a decrease in the sample fruity flavour.

Thus, for the enhancement of the content of polyphenolic substances and improvement of the sensorial profile of wines, a commercial tannin preparation can be added. However, the use of tannin cannot be generalised due to a different effect of various tannin preparations on sensorial quality of wines.

Acknowledgement. The authors thank Bohemia Sekt Inc. and O.K. Servis BioPro, Ltd., for material support and consultations in our research.

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