Analysis of Grape Seed Proanthocyanidins

Herbert G. Boechzelt[1], Wilhelm Haas[1], Gerald Poeltl[2], Martin Mittelbach[2]

[1]Institute of Sustainable Techniques and Systems, JOANNEUM RESEARCH, Elisabethstrasse 16/I, 8010 Graz, Austria
[2]Institute of Chemistry, Karl-Franzens-University Graz, Heinrichstrasse 28, 8010 Graz, Austria

Naturally occurring polyphenols are widespread in fruits and vegetables, and have received particular interest since recently many biological activities of these compounds on the human organism are discussed. A special group of plant polyphenols are the proanthocyanidins (PAs) as contained e.g. in grape seeds. The structural complexity of these polymeric compounds which influences their beneficial biological activities, makes their characterization a challenging task for the analytical chemist. Beside a short survey on the beneficial health effects of PAs and on current approaches to their analysis, we will present a new analytical method for a comprehensive study of these compound class.

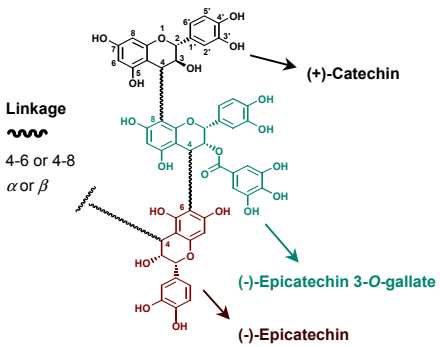

Figure 1. Chemical structure of grape seed proanthocyanidins

Proanthocyanidins: Structure, Distribution, Biological Activity, and Current Approaches to their Characterization

Proanthocyanidins (PAs), also termed as condensed tannins, are polymeric compounds built up from flavan-3-ol units as basic structural elements (see Figure 1 for the chemical structure of grape seed PAs). These compounds are widespread in the plant kingdom and have been detected in various foods and beverages including apples, chocolate, cinnamon, peanuts, grape seeds, and wine [1].
High interest is attracted by the number of beneficial health effects shown by PAs. They possess antioxidant activities, demonstrate cytotoxicity against cancer cells, ameliorate toxic effects associated with chemotherapeutic agents, and are assumed to be beneficial in lowering the incidence of atherosclerosis and coronary heart disease [2,3,4,5]. It is proposed that the physiological properties of the PAs are correlated to their chemical structure [6,7].
Grape seeds are a rich source of PAs and grape seed extracts (GSEs) are already used as active ingredients of medicinal products and nutraceuticals. As depicted in Figure 1 grape seeds PAs are composed of three individual monomer units linked to each other by different ways. Taking into account the mentioned structure-activity correlation of PAs, the structural diversity of grape seed PAs complicates the assessment of the quality of GSEs.
A number of analytical methods including photometric measurements and specific degradation have been proposed for the characterization of mixtures of PA oligomers [8]. Applying modern mass spectrometric (MS) techniques affords information on the composition and degree of polymerization (DP) of even highly polymerized PAs which were only partly accessible to analysis before the implementation of these methods [9,10,11]. A very interesting approach to the characterization of PAs is the use of normal-phase high-performance liquid chromatography coupled to MS (NP HPLC/MS). PAs are separated regarding their DP and subsequently analyzed by mass spectrometry [11]. The method presented here is based on this technique.

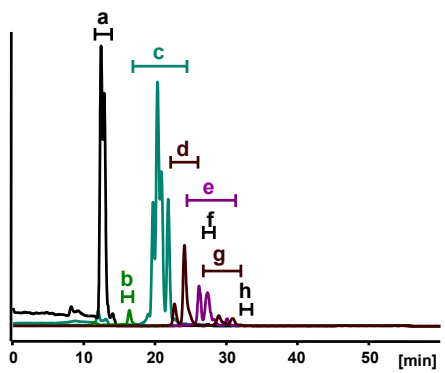

Figure 2. Analysis of a Zweigelt grape seed extract using selected ion monitoring to detect proanthocyanidin monomers, dimers, and trimers (a-h, see Table 1)

The Use of PBP-HPLC ESI MS for Comprehensive Characterization of Grape Seed Proanthocyanidins

Sample preparation of the grape seed extracts was done by applying a two step solid-phase extraction (SPE) method. By using of a MCX SPE cartridge (mixed mode, reversed phase/cation exchange) even anthocyanins could be removed from the sample. Semiquantitaive analysis of selected PAs (monomers to trimers, pentamers, decamers, and the corresponding galloylated compounds) was done with polar-bonded-phase high-performance liquid chromatography electrospray ionization mass spectrometry (PBP-HPLC ESI MS). Detection of the analytes was done by selected ion monitoring (SIM, see Table 1). As the analyzed compounds extremely differed in their amount two chromatographic runs using differing injection volumes and mobile phase gradients had to be performed to facilitate quantification [Figures 2 and 3 show results from analyzing a PAs mixture obtained from Zweigelt (Austrian grape variety) grape seed press cake]. The method affords information on the degree of polymerization as well as of galloylation of the PAs in the analyzed sample (see Figures 2 and 3, and Table 1). As these parameters are described to influence the biological activity of PAs, the method is an important tool for assessing the quality of products containing these compounds.

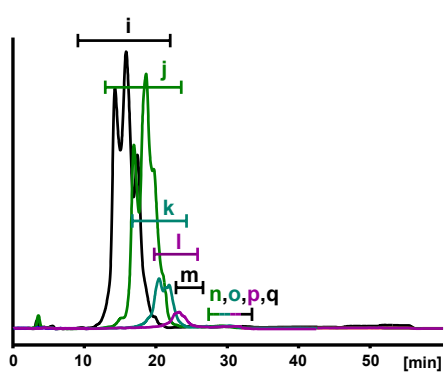

Figure 3. Analysis of a Zweigelt grape seed extract using selected ion monitoring to detect proanthocyanidin pentamers and decamers (i-q, see Table 1)

Materials and Methods

The grape seed extract was obtained from Zweigelt grape seed press cake by extraction with ethyl acetate and subsequent precipitation of the PAs by adding petroleum ether. Sample preparation was done by two-step solid phase extraction over Oasis® MCX and HLB SPE cartridges. HPLC ESI MS was done on a Hewlett Packard 1100 LC/MSD system using a polar-bonded-phase column (LiChrospher® Diol, Merck), and a mobile phase from dichloromethane, methanol, and methanol/water/acetic acid (78:11:11, v/v/v). Gradients of 0.45 M methanolic ammonium hydroxide, methanol, and dichloromethane were added postcolumn. The mass selective detector was performed in negative ion mode. Monitored ions are listed in Table 1.

Constituents			Monitored Ions
Labels	DP	DG	[m/z]
a	1	0	288.9 $[M-H]^-$
b	1	1	441.0 $[M-H]^-$
c	2	0	577.2 $[M-H]^-$
d	2	1	729.1 $[M-H]^-$
e	3	0	865.2 $[M-H]^-$
f	2	2	881.1 $[M-H]^-$
g	3	1	1017.3 $[M-H]^-$
h	3	2	1169.1 $[M-H]^-$
i	5	0	720.3 $[M-2H]^{2-}$
j	5	1	796.5 $[M-2H]^{2-}$
k	5	2	872.1 $[M-2H]^{2-}$
l	5	3	948.3 $[M-2H]^{2-}$
m	5	4	1024.7 $[M-2H]^{2-}$
n	10	0	960.0 $[M-3H]^{3-}$
o	10	1	1010.7 $[M-3H]^{3-}$
p	10	2	1061.7 $[M-3H]^{3-}$
q	10	3	1112.6 $[M-3H]^{3-}$

Table 1. Assignment of a-q from Figures 2 and 3 (DP, Degree of polymerization; DG, Degree of galloylation)

References

[1] Lazarus, S. A.et al. *J. Agric. Food Chem.* **1999**, *47*, 3693-3701.
[2] Hagerman, A. E. et al. *J. Agric. Food Chem.* **1998**, *46*, 1887-1892.
[3] Bagchi, D. et al. *Toxicology* **2000**, *148*, 187-197.
[4] Joshi, S. S. et al. *Toxicology* **2000**, *155*, 83-90.
[5] Yamakoshi, J. et al. *Atherosclerosis* **1999**, *142*, 139-149.
[6] Zhao, J. et al. *Carcinogenesis* **1999**, *20*, 1737-1745.
[7] Lotito, S. B. et al. *Biochem. Biophys. Res. Commun.* **2000**, *276*, 945-951.
[8] Schofield, P. et al. *Anim. Feed Sci. Tech.* **2001**, *91*, 21-40.
[9] Hammerstone, J. F. et al. *J. Agric. Food Chem.* **1999**, *47*, 490-496.
[10] Behrens, A. et al. *Phytochemistry* **2003**, *62*, 1159-1170.
[11] Hayasaka, Y. et al. *Rapid Commun. Mass Spectrom.* **2003**, *17*, 9-16.