

Qualitative and quantitative analysis of phenolic constituents in elderberry juices*

Qualitative und quantitative Analyse von phenolischen Verbindungen in Holundersäften**

M. NAGL, R. EDER, S. WENDELIN, G. REICH, G. SONTAG

Summary

Compounds like phenolic acids and flavonoids present in different plants and fruits show a considerable antioxidative activity. These substances are known for their protective effects against chronic diseases. Therefore a HPLC-UV method was developed for analysis of phenolic constituents in elderberry juices. After purifying on C-18 cartridges extracts were chromatographed on a reversed phase (C-18) column using a gradient consisting of methanol, tetrahydrofurane and sodium dihydrogenphosphate and recorded at 260, 280, 320 and 350 nm. More than sixty elderberry constituents were distinguishable by the applied chromatographic method. Protocatechuic acid, β -D-1-O-caffeoylglucose, caffeic acid, chlorogenic acid, isoquercitrin, rutin, avicularin, quercetin and kaempferol were identified, comparing the UV-spectra (220 to 450 nm) of the eluted compounds and the relative retention times with those of standard solutions. Protocatechuic acid, chlorogenic acid, rutin and avicularin were quantified at 260 nm in hundred elderberry concentrates produced in three European countries. Statistical evaluation (cluster analysis) of the contents of these characteristic compounds allowed distinction of the given sample pool into ten clusters which correlate with the origin of the samples.

Keywords:

Elderberry (*Sambucus nigra*) juices, HPLC-UV detection, analysis of phenolic compounds, cluster analysis

Zusammenfassung

Phenolsäuren und Flavonoide kommen in verschiedenen Pflanzen vor und zeigen eine beträchtliche antioxidative Aktivität. Außerdem ordnet man ihnen eine Schutzwirkung gegen chronische Krankheiten zu. Um phenolische Verbindungen in Holundersäften zu analysieren, wurde eine HPLC Methode in Kombination mit einem UV Detektor entwickelt. Nach Reinigung der Extrakte an SPE Säulchen wurden die vorliegenden Verbindungen durch Gradientenelution (Methanol, Tetrahydrofuran und Natriumdihydrogenphosphatlösung) an einer RP-18 Säule getrennt und bei 260, 280, 320 und 350 nm mehr als sechzig Verbindungen detektiert. Durch Vergleich der UV-Spektren (220-450 nm) und der relativen Retentionszeiten der eluierten Verbindungen mit jenen von Standardlösungen wurden Protocatechusäure, β -D-1-O-Caffeoylglucose, Kaffeesäure, Chlorogensäure, Isoquercitrin, Rutin, Avicularin, Quercetin und Kaempferol identifiziert. Protocatechusäure, Chlorogensäure, Rutin und Avicularin wurden bei 260 nm in hundert Holunderkonzentraten aus drei Europäischen Ländern bestimmt. Eine statistische Auswertung (Clusteranalyse) der Gehalte dieser charakteristischen Verbindungen ermöglichte eine Einteilung der vorliegenden Proben in zehn Cluster, welche mit dem Ursprung der Proben korrelieren.

Kennwörter:

Holundersäfte (*Sambucus nigra*), HPLC-UV Detektion, Analyse phenolischer Verbindungen, Clusteranalyse

Introduction

The protective effect of fruit and vegetables on cancer has been emphasized and different groups [1-3] overviewed the relation between cancer and the consumption of diets rich in fruit and vegetables. Today fruit juices are consumed frequently and can also contribute to provide the consumers with different phenolic compounds. To discuss the effect of those naturally occurring compounds on health quantitative data is necessary. Different phenolic compounds like chlorogenic acid and caffeic acid [4], rutin, isoquercitrin, *p*-coumaric acid, caffeic acid, chlorogenic acid,

quercetin, kaempferol, isorhamnetin-3-glucoside and isorhamnetin-3-rutinoside have been identified in blossoms and leaves of *Sambucus nigra* [5-9]. Stroth et al. [10] found the 1-O- β -D-glucose esters of caffeic acid and ferulic acid in blossoms and later Reschke et al. [11] detected, in addition to these compounds, the 1-O- β -D-*p*-coumaryl glucose ester in elderberries. Recently, quercetin-hexoside, kaempferol-hexoside, quercetin-3-glucoside, quercetin-3-rutinoside, kaempferol-3-rutinoside, quercetin, neochlorogenic acid and chlorogenic acid were determined quantitatively [12] in elderberry concentrates. Frequently, cyanidine glycosides [12-18] have been investigated with respect

* Dedicated to Emer. O. Univ. Prof. Dr. Gerald Kainz' 85th birthday

** Herrn Emer. O. Univ. Prof. Dr. Gerald Kainz zum 85. Geburtstag gewidmet

to their content and antioxidant capacity. Analytical methods applied to assay these substances are thin layer chromatography (TLC) [11, 18, 19], high performance liquid chromatography (HPLC) [8, 9, 11, 12, 16, 18] coupled with different detection modes, microcellular electrokinetic capillary chromatography [9, 21] and isotachopheresis [22, 23].

In the present work, hundred elderberry juice concentrates of different European origin were analyzed with respect to their content of the scarcely investigated non cyanidine phenolic compounds, since cyanidin glycosides in elderberries have been investigated exhaustively. The HPLC elution profile was optimized to reach a maximum of distinguishable components, the concentration of some components were determined and used as a data base for multivariate statistical evaluation (cluster analysis).

Experimental

Instrumentation

Qualitative and quantitative analysis were performed with two HPLC systems (Waters, Milford, MA, USA) consisting of:

- Waters 510 and 501 HPLC pumps, Waters interface modul, Waters WISP 712 autosampler; a 50 μ L sample loop, a Lichrospher 100 RP 18 column, 5 μ m, 250 x 4 mm (Merck, Darmstadt, Germany) and a UV-detector (Waters 490 E WL). This system was combined with a controlling and data processing unit (Waters Baseline 810).
- Waters 510 and 590 HPLC pumps, Waters 990 automated gradient controller, Waters WISP 712 autosampler; a 50 μ L sample loop, a Lichrospher 100 RP 18 column, 5 μ m, 250 x 4 mm (Merck, Darmstadt, Germany) and a photodiode array detector (Waters 990) with computer and software.

Chemicals and solutions

α -Resorcylic acid, protocatechuic acid, quercetin, kaempferol and rutin were purchased from Roth (Karlsruhe, Germany), chlorogenic acid, kaempferol, isoquercitrin, avicularin were from Extrasynthese (Genay, France) and genistein was from Indofine Chemical Company Inc. (Somerville, NJ, USA). Cyanidin-3-sambubioside-5-glucoside, cyanidin-3,5-diglucoside, cyanidin-3-glucoside and cyanidin-3-sambubioside were provided by Höhere Bundeslehranstalt und Bundesamt für Wein und Obstbau, Klosterneuburg. Ferulic acid, *p*-coumaric acid and caffeic acid used as starting materials for synthesis of the glucose esters were purchased from Fluka (Buchs, Switzerland).

1-O- β -D-caffeoyl-, 1-O- β -D-feruloyl- and 1-O- β -D-*p*-coumaroyl glucose esters were synthesized, the anomeric mixtures obtained by these methods were sepa-

rated by means of preparative HPLC, characterized by their NMR-spectra, and used as reference substances for identification [24-26].

Sodium dihydrogenphosphate p.A., *o*-phosphoric acid p.A. and hydrochloric acid (37 %) p.A. were delivered from Merck (Darmstadt, Germany).

HPLC solvents (methanol, acetonitrile, tetrahydrofuran) were gradient grade and bought from Riedel de Haen (Seelze, Germany). Water was purified using an Easy pure LF device (Barnstead International, Nürnberg, Germany).

Buffer solution: 30 mL of a 50 mM sodium dihydrogenphosphate solution were diluted with deionized water to 3000 mL and the pH was adjusted to 1.8 with diluted phosphoric acid (phosphoric acid/deionized water 1:5, v:v).

Stock solution I for qualitative analysis contained 2.5 mg protocatechuic acid, 12.4 mg α -resorcylic acid, 2.7 mg caffeic acid, 17.4 mg chlorogenic acid, 2.4 mg cyanidin-3-sambubioside-5-glucoside, 2.6 mg cyanidin-3,5-diglucoside, 2.8 mg cyanidin-3-glucoside, 2.1 mg cyanidin-3-sambubioside, 5.8 mg quercitrin, 12.7 mg rutin, 1.6 mg avicularin, 4.8 mg genistein, 5.0 mg quercetin and 5.4 mg kaempferol in 50 mL methanol/water (6:4, v: v). The flask was stored under exclusion of light at + 4 °C. This solution was diluted with methanol/water (6:4, v:v) to the appropriate concentrations.

Stock solution II for identification of cinnamic glucose esters was prepared by dissolving 14.8 mg 1-O- β -D-caffeoyl-, 14.9 mg 1-O- β -D-feruloyl- and 12.5 mg 1-O- β -D-*p*-coumaroyl glucose esters in 100 mL methanol/water (6:4, v:v).

Stock solution III for calibration and standard addition contained 12.5 mg protocatechuic acid, 62 mg α -resorcylic acid, 87 mg chlorogenic acid, 63.5 mg rutin, 8 mg avicularin, 24 mg genistein and 13.5 mg caffeic acid in 50 mL methanol/water (6:4, v: v). It was stored at + 4 °C under exclusion of light.

Internal standard solution consisted of 62 mg α -resorcylic acid and 24 mg genistein dissolved in 50 mL methanol/water (6:4, v: v) stored as described above.

Samples

Elderberries were harvested in different regions of Austria and stored at +4 °C. Within two days 50 g batches of these berry samples were squeezed in a fruit press and then the juices were stored in 2 mL Eppendorf vials at -78 °C in a deep freezer.

Hundred frozen elderberry concentrates were delivered from the Danish Institute for Agriculture in Aarslev (Denmark). They were allowed to thaw at room temperature, homogenized and diluted (1:3, v:v) with deionized water and stored in 2 mL Eppendorf vials at -78 °C in a deep freezer.

Qualitative and quantitative analysis

Sample preparation

1500 µL of the diluted (1:3, v:v) juice concentrate or self produced juice were transferred into a 10 mL centrifugation tube and 60 µL of internal standard solution were added. After homogenisation the sample was centrifuged for 10 min at 5000 rpm in order to remove insoluble matrix compounds. 1000 µL of the supernatant were transferred on to a preconditioned (with methanol and afterwards with water) C-18 Bondelut cartridge (500 mg, 1 mL; Varian, Vösendorf, Austria). After washing out the highly polar components with 5 mL deionized water, phenolic compounds were eluted with 750 µL of a solution containing conc. hydrochloric acid and methanol (1:999, v:v) and the eluate was filled up to 1000 µL with the same solution.

Chromatographic separation and detection

50 µL of extract or standard solution, respectively, were injected and the compounds were separated (flow rate: 0.8 mL/min) on a LiChrospher 100 RP18 column using the following gradient profile: 0-32 min: 3-9 %, 32-55 min: 9 % (isocratic), 55-68 min: 9-12 %, 68-145 min: 12-65 %, 145-160 min: 65 % methanol/tetrahydrofuran (99:1, v:v) in buffer solution (isocratic).

The chromatograms were recorded at 260, 280, 320 and 350 nm, the spectra of the eluted compounds were measured between 220 and 450 nm and compared with those of standard compounds eluted at the same capacity factors. Additionally the peak height ratios measured at the wavelengths mentioned above were compared with those of standard compounds to

exclude co-elution of the identified compounds with other sample components. Quantitative analysis was performed using the above mentioned gradient.

Calibration and limits of detection

Calibration curves of protocatechuic acid (0.5 to 60 mg/L), chlorogenic acid (3.5 to 417.6 mg/L), rutin (2.5 to 304.8 mg/L), avicularin (0.3 to 38.4 mg/L) and for the internal standards α -resorcylic acid (2.5 to 297.6 mg/L) and genistein (1 to 115.2 mg/L) were established by diluting 10, 20, 40, 80, 120 and 240 µL of stock solution III with methanol to 1000 µL. The limits of detection of the analytes (at 260 nm) were evaluated making use of the corresponding calibration curve considering a signal/noise ratio of 3.

Standard addition

In addition to two unspiked samples, four 1500 µL aliquots of the diluted or self produced juice were transferred into 10 mL centrifugation tubes and spiked with 60, 120, 180 or 240 µL of stock solution III, respectively. Further treatment was as described above.

These solutions were injected into the chromatographic systems and the chromatograms were evaluated. The calibration functions were obtained by linear regression of the peak areas on standard concentrations (*Tab. 1*) or added amounts. The ratio of the slopes of calibration and the standard addition curve give the recovery for each compound. Using the calibration curves (peak area ratios/concentration) for quantitative evaluation of samples, the determined values have to be corrected considering the recovery.

Compound	Calibration curve	Correlation coefficient R	Recovery (%)	Detection limit (mg/L)
Protocatechuic acid	$y = 0.001155.x + 0.000138$	0.9999	92.4 ± 1.41	0.25
Chlorogenic acid	$y = 0.000247.x + 0.001388$	0.9998	96.3 ± 3.57	2.80
Rutin	$y = 0.000608.x + 0.000194$	0.9999	92.6 ± 0.82	0.75
Avicularin	$y = 0.00081.x - 0.000215$	0.9999	97.4 ± 1.67	0.50

Tab. 1: Calibration curves, correlation coefficients, recovery and detection limits of the investigated compounds

Cluster analysis

A multivariate statistical method was applied to estimate the similarity of objects. Each object is described by properties building the base data set for calculation. These properties are used to calculate a so called similarity measure. In this example the similarity measure is based on the Euclidean Distance. In the context of this work, the objects are the elderberry juices and the

properties are the measured concentrations of the phenolic compounds. The resulting clusters are defined by a similarity threshold which has to be adjusted to give an appropriate separation. If the properties are of different magnitudes, the similarities are influenced to the advantage of the larger numerical values. Normalization of the data is necessary to prohibit this influence. Additional information can be found in [27].

Results and discussion

Identification of phenolic elderberry constituents

Self prepared elderberry juices were investigated in preliminary HPLC separation experiments to optimize the chromatographic method and to obtain qualitative information about their chemical composition (substance profile). The different polarity of the compounds present in these juices required different gradient steps for their elution. Addition of 1% tetrahydrofuran significantly improved chromatographic separation (peak resolution) allowing the discrimination of more than sixty components in the elderberry juice extracts. Based on this method the investigation of one hundred elderberry samples (nine samples from the United Kingdom, 51 samples from Denmark and forty samples from Austrian plants) was carried out. Each peak

in the chromatogram was characterized by its relative retention time and its UV-spectrum. Additional information was obtained by analyzing samples spiked with aliquots of supposed compounds. Comparing the spectra and the relative retention times of elderberry juice constituents with those of the standard substances (with exception of the four cyanidine-glycosides) the following phenolic compounds were identified: protocatechuic acid, 1-O- β -D-caffeoyl glucose, caffeic acid, chlorogenic acid, quercitrin, rutin, and avicularin (Fig. 1). Other elderberry constituents that are known [10, 11, 12], like 1-O- β -D-caffeoyl glucose, quercitrin, quercetin and kaempferol were only present in traces in twenty of the self prepared juices.

Two juice components, protocatechuic acid and avicularin, were shown to be present in the berries of *sambucus nigra* for the first time. 1-O- β -D-feruloyl- and 1-O- β -D-p-coumaroyl glucose esters were not identified in the juice extracts.

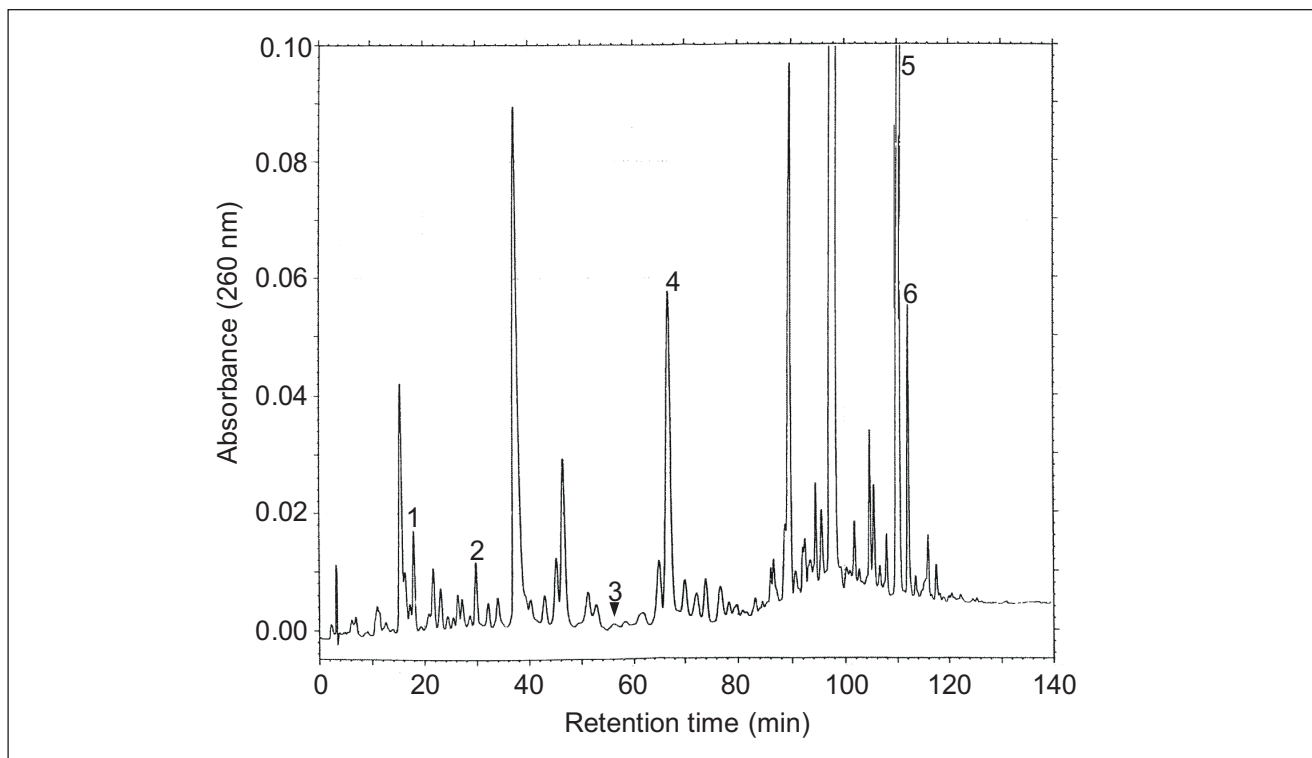


Fig. 1: Chromatogram of a self produced elderberry juice. 1: protocatechuic acid, 2: β -D-1-O-caffeoylglucose, 3: caffeic acid, 4: chlorogenic acid, 5: rutin, 6: avicularin

Apart from the two peaks of the four elderberry cyanidine-glycosides [t_R = 89 min and 97 min] and three unidentified peaks [t_R = 15 min, t_R = 36 min, t_R = 46 min] (Fig. 1), the HPLC profiles of all juice samples investigated are dominated by the peaks of four compounds (protocatechuic acid, chlorogenic acid, rutin and avicularin, Fig. 1), which were quantified by their peak areas.

Quantitative analysis

The calibration curve of each of the substances was established daily by injecting different levels of standard solutions [2.5–300 mg/L], the detection limits (S/N=3) were estimated and the recovery was determined by analyzing the standard solution III five times. This data is summarized in Table 1.

Due to the length of the chromatogram, two internal

Number of cluster [Number of samples]	Protocatechuic acid mg/kg	Chlorogenic acid mg/kg	Rutin mg/kg	Avicularin mg/kg
1 [7]	6.1 ± 1.9	230 ± 38	805 ± 80	29.3 ± 5.4
2 [6]	8.0 ± 3.1	178 ± 101	677 ± 343	62.3 ± 13.6
3 [15]	4.7 ± 3.4	429 ± 72	704 ± 122	24.2 ± 10.3
4 [9]	10.8 ± 3.3	313 ± 85	1141 ± 239	26.5 ± 8.6
5 [3]	5.9 ± 1.5	740 ± 150	1340 ± 294	63.2 ± 8.5
6 [16]	8.5 ± 2.4	159 ± 58	470 ± 156	15.5 ± 8.8
7 [10]	5.6 ± 1.5	286 ± 49	511 ± 129	14.2 ± 6.7
8 [9]	17.9 ± 2.5	228 ± 71	684 ± 153	18.3 ± 6.0
9 [13]	8.1 ± 2.3	297 ± 48	901 ± 114	5.9 ± 3.0
10 [12]	17.5 ± 2.9	309 ± 40	1056 ± 166	6.8 ± 2.6

Tab. 2: Concentration ranges of protocatechuic acid, chlorogenic acid, rutin and avicularin in different elderberry juices and resulting clusters

standards, one at the beginning (α -resorcylic acid) and the other in the last part (genistein) of the chromatogram were selected. The recoveries determined by dividing the slope of the standard addition curve through the slope of the calibration curve of each compound resulted in $75.2 \pm 4.6\%$ for protocatechuic acid, $75.9 \pm 5.8\%$ for chlorogenic acid, $89.6 \pm 4.4\%$ for rutin, $91.5 \pm 6.5\%$ for avicularin, $88.1 \pm 2.7\%$ for α -resorcylic acid, and $96.2 \pm 0.6\%$ for genistein (N=3). The reduced recovery in comparison to standard solutions is due to the matrix of the juices. The concentration of protocatechuic acid varied between 4.7 and 17.9 mg/kg, of

chlorogenic acid between 159 and 740 mg/kg, for rutin and avicularin a range between 470 and 1340 mg/kg, and 5.9 up to 63.2 mg/kg was found, respectively. Applying the cluster analysis to the quantitative results of hundred European samples ten clusters were identified (Fig. 2). The highest concentration of rutin, chlorogenic acid and avicularin were found in UK samples (cluster 4 and 5). In cluster 5 only UK samples were present. Austrian samples were mainly found in cluster 6, 9 and 10, and for Danish samples low concentrations of rutin and chlorogenic acid (cluster 3, 6 and 7) were characteristic.

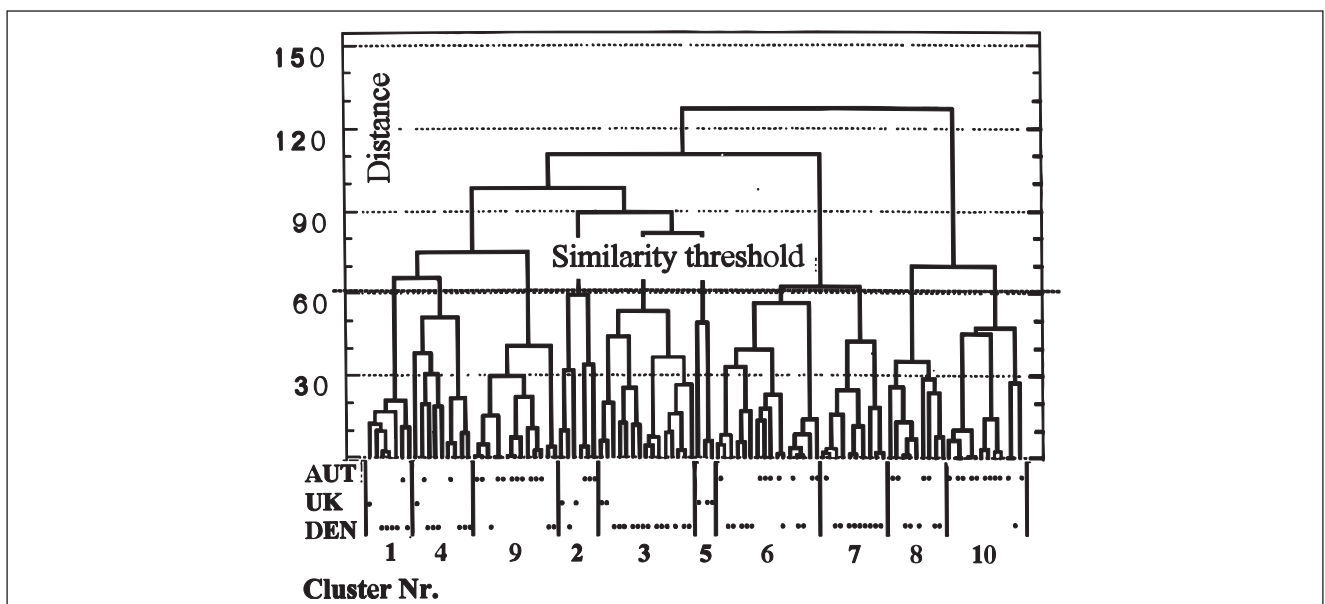


Fig. 2: Dendrogram of cluster analysis of hundred elderberry samples (juice concentrates) of three different origins (Austria, United Kingdom, and Denmark): The contents of quantified substances were used as database. The ten clusters (at a similarity threshold of sixty units on the distance axis) are given by cluster numbers (1 to 10). Each sample is represented by a dot (correlated to each of the vertical sample lines of the dendrogram). The significance of the clusters for each sample group is given by the distribution of sample dots over the columns of cluster numbers.

The presence of protocatechuic acid and *p*-hydroxybenzoic acid in juice samples was shown to be connected with the decomposition of flavonoids in the course of acid hydrolysis. The analysis of such hydrolysates reveals a significant increase in the ratio of protocatechuic acid and quercetin and in the ratio of *p*-hydroxybenzoic acid and kaempferol, respectively, which is furthermore associated with a significant decrease of the two mentioned flavon-3-ol aglyca. These results are correlating with the findings of food technological experiments, which show a connection between the content of protocatechuic acid and the extent of thermal exposition of elderberry juice samples [28].

Conclusion

This method has made it possible to gain an overview over the complete spectrum of UV active compounds within the range of polarity limited by the most polar hydroxyl benzoic acids and the flavonol aglycons. The UV-profiles (fingerprints) of the juice extracts enables one to recognize deviant substance profiles of non elderberry juices. It could also be shown for the first time that protocatechuic acid and avicularin are present in berries of *Sambucus nigra*.

Cluster analysis of the analytical data reveals that quantification of four characteristic phenolic compounds is a potential tool for assignment of an individual sample to a sample group. In our case, a sample pool of hundred elderberry juice concentrates belonging to three groups of samples of different origin (Austria, Denmark, United Kingdom) are distributed over ten sample clusters (Fig. 2). Austrian samples are found to a percentage of 92 % in cluster 10 and 77 % in cluster 9. Danish samples are predominating in cluster 3 (87 %) and in cluster 7 (90 %). Cluster 5, showing by far the highest average contents of phenolic constituents of all clusters, consists totally of samples of British origin. These clusters are significant for a certain sample origin.

Acknowledgment

We thank the European Union for supporting the project "Optimisation of raw material characteristics for the production of beverages" (Contr. Nr. FAIR-CT98-9653).

References

[1] Steinmetz K.A., Potter J.D.: Vegetables, fruit, and cancer. II. Mechanisms. *Cancer Causes Control* 1991a; 2: 325-357.

- [2] Block G., Patterson B., Subar, A.: Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr. Cancer* 1992; 18: 1-29.
- [3] Boehm H., Boeing H., Hempel J., Raab B., Kroke A.: Flavonols, flavones, and anthocyanins as native antioxidants of food and their possible role in the prevention of chronic diseases. *Z Ernährungswiss.* 1998; 37: 147-163.
- [4] Baerheim S.: A paper chromatography in phytochemical analyses. II. Occurrence of chlorogenic and caffeic acids in the Umbelliferae. *Pharm Acta Helv* 1951; 26: 253-258.
- [5] Inoue T., Sato K.: Constituents of *Sambucus* species. Triterpenoids of *Sambucus nigra* and *S. canadensis*. *Phytochem* 1975; 14: 1871-1872.
- [6] Stroh H.H.: Distribution of γ -pyronine dyes and hydroxycinnamic acids of elderberries during growth. *Naturwissensch* 1958; 45: 547-548.
- [7] Schmersahl K.J.: Active principles of diaphoretic drugs from DAB 6 [elderberry]. *Naturwissensch* 1964; 51: 361.
- [8] Petitjean-Freytet C., Carnat A., Lamaison J.L.: Flavonoids and hydroxycinnamic acid derivatives in *Sambucus nigra* L. flowers. *J Pharm. Belg* 1991; 46: 241-246.
- [9] Pietta P., Bruno A., Mauri P., Rava A.: Separation of flavonol-2-O-glycosides from *Calendula officinalis* and *Sambucus nigra* by high-performance liquid and micellar electrokinetic capillary chromatography. *J. Chromatogr* 1992; 593: 165-170.
- [10] Stroh H., Schaefer H., Haschke E.: D-Glucose esters of hydroxycinnamic acids in *Sambucus nigra*. *Z Chemie* 1962; 2: 373-374.
- [11] Reschke A., Herrmann K.: Occurrence of 1-O-hydroxycinnamyl- β -D-glucoses in fruits. 15 Phenolics of fruits. *Z Lebensm Unters Forsch*, 1981; 173: 458-463.
- [12] Bermudez-Soto M.J., Tomas-Barberan F. A.: Evaluation of commercial red fruit juice concentrates as ingredients for antioxidant functional juices. *Eur Food Res Techn* 2004; 219: 133-141.
- [13] Reichel L., Stroh H.H., Reichwald W.: Pigment of the berries of the common elder. *Naturwissensch* 1957; 44: 468.
- [14] Reichel L., Reichwald W.: Pigment of the black elderberry. *Naturwissensch* 1960; 47: 40-41.
- [15] Pfannhauser W., Riedel O.: Anthocyanins: formation, extraction and analysis of natural food dyes. *Ernährung* 1983; 7: 560-564.

- [16] *Wrolstad R.E., Hong V.*: Characterization of anthocyanin-containing colorants and fruit juices by HPLC/photodiode array detection. *J Agric Food Chem* 1990; 38: 698-708.
- [17] *Bridle P., Garcia-Viguera C.*: Analysis of anthocyanins in strawberries and elderberries. A comparison of capillary zone electrophoresis and HPLC. *Food Chem* 1997; 59: 299-304.
- [18] *Brønnum-Hansen K., Hansen S.H.*: High-performance liquid chromatographic separation of anthocyanins of *Sambucus nigra* L. *J Chromatogr* 1983; 262: 385-392.
- [19] *Males Z., Medic-Saric M.*: Investigation of the flavonoids and phenolic acids of Sambuci flos by thin-layer chromatography. *J Planar Chromatogr - Modern TLC* 1999; 12: 345-349.
- [20] *Hawryl M.A., Hawryl A., Soczewinski E.*: Application of normal- and reversed-phase 2D TLC on a cyanopropyl-bonded polar stationary phase for separation of phenolic compounds from the flowers of *Sambucus nigra* L. *J Planar Chromatogr-Modern TLC*, 2002; 15: 4-10.
- [21] *Watanabe T., Yamamoto A., Nagai S., Terabe S.*: Analysis of elderberry pigments in commercial food samples by micellar electrokinetic chromatography. *Anal Sci* 1998; 14: 839-844.
- [22] *Seitz U., Bonn G., Oefner P., Popp M.*: Isotachophoretic analysis of flavonoids and phenolcarboxylic acids of relevance to phytopharmaceutical industry. *J Chromatogr* 1991; 559: 499-504.
- [23] *Urbanek M., Pospisilova M., Polasek M.*: On-line coupling of capillary isotachopheresis and zone electrophoresis for the assay of phenolic compounds in plant extracts. *Electrophoresis* 2002; 23: 1045-1052.
- [24] *Birkofer L., Kaiser C., Nouvertne W., Thomas U.*: Naturally occurring sugar esters of phenolcarboxylic acids. *Z Naturforsch* 1961; 16b: 249-251.
- [25] *Birkofer L., Kaiser C., Kosmol H., Romussi G., Donike M., Michaelis G.*: Sugar esters. III. D-Glucose and L-rhamnose esters of p-coumaric and ferulic acids. *Liebigs Ann d Chem* 1966; 699: 223-231.
- [26] *Nagl M.*: Analyse phenolischer Inhaltsstoffe in Beeren von Holunder (*Sambucus nigra*) Doctoral Thesis, University of Vienna, Austria, 2002.
- [27] *Vandeginste B.G.M., Massart D.L., Buydens L. M. C., DeJong S., Lewi P.J., Smyers-Verebeke J.*: Handbook of Chemometrics and Qualimetrics: Part B, 1998, Elsevier: 57-86.
- [28] *Schuster B., Herrmann K.*: Formation of hydroxybenzoic acids from flavonoids by enzymic and alkaline hydrolyses. *Z Lebensm Unters Forsch* 1985; 181: 467-469.

Address of the authors:

Michael Nagl, Gregor Reich, Gerhard Sontag*
 Institute of Analytical Chemistry and Food Chemistry
 University of Vienna
 Waehringerstrasse 38, A-1090 Vienna, Austria
 t +43-1-4277-52303
 f +43-1-4277-952
 e-mail: gerhard.sontag@univie.ac.at

Reinhard Eder*, Silvia Wendelin
 Höhere Bundeslehranstalt und Bundesamt für Wein
 und Obstbau
 Wienerstrasse 74, A-3400 Klosterneuburg, Austria
 t +43 (0)2243-37910-223
 f +43(0) 2243-37910-411
 e-mail: reinhard.eder@hblawo.bmlfuw.gv.at

* corresponding authors

Eingelangt am: 8.2.06
 Akzeptiert am: 25.6.06



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