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Differentiation of the World Hop Collection by Means of the Low Weight Molecular Polyphenols

Hop has three groups of variety-specific ingredients. These are the bitter substances, the essential oils and also some low molecular weight polyphenols like the flavonoids. In this work the focus was mainly on the composition of the quercetin- and kaempferol-glycosides, because these compounds are suitable to differentiate varieties. Initial work focused on the development of convenient methods for sample preparation and HPLC-analysis. Then nearly the whole available world hop collection (121 different varieties from 17 countries) was analysed. The data were evaluated by a principal component analysis to make differences and similarities visible. Some varieties are clearly distinguished from their polyphenol composition, while others, especially the old land races, were barely distinguishable from each other.

Descriptors: hop varieties, polyphenols, flavonoids, quercetin, kaempferol, principal component analysis

1 Introduction

The alpha acids have major importance, if the brewer want to add bitterness into the beer. In the case of late hopping the essential oils have priority. The polyphenols are usually of less interest. But especially the polyphenols have many additional beneficial effects to the health [1]. There are many publications about the antioxidative potential and radical scavenging capacity of the polyphenols [2]. It is proved that polyphenols can protect cells against oxidative processes and associated diseases like cancer and cardiovascular diseases e.g. atherosclerosis. Generally in the most publications the low molecular weight polyphenols are considered to promote health, therefore it is recommended to eat a lot of food with a high content of low molecular polyphenols. Fruits and vegetables have a high polyphenol content. The polyphenol content of hop ranges from 2 % to 8 % dry weight. The polyphenols are a heterogeneous group of chemical compounds, but they all have as a common structure element an aromatic ring with at least two hydroxyl-groups (Fig. 1). Polyphenols are occurring as bioactive substances in almost all plants [3]. They can easily be oxidized, therefore they are strong antioxidants themselves. Furthermore they have functions as dye and taste stuffs, they are able to protect plants against pests and fungi and in higher molecular form they act as tannins.

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Tables and figures see appendix

Former investigations in Hüll showed that the climate and the growing region have an influence on the total polyphenol content as well as on the polyphenol composition [4]. All polyphenols share elements of the same biosynthesis pathway (Fig. 2). A key step for the polyphenol biosynthesis is the transformation of the amino acid phenylalanine to cinnamic acid and from cinnamic acid all the other polyphenols are built up. Table 1 shows the distribution of the hop-polyphenols. Aromatic carboxyclic acids are present in hops only in lower concentrations. The main low molecular polyphenols are xanthohumol, the quercetin- and kaempferolglycosides as well as the catechins and their polymers, the oligomeric proanthocyanidins. A new group of hop ingredients are also the acylphloroglucinol derivatives e.g. multifidolglucoside. These compounds have anti-inflammatory properties and can make hops interesting for the pharmaceutical industry [5]. The larger part of hop polyphenols however are higher molecular weight substances like the tannins. The flavonoids are a subgroup of the polyphenols and were discovered by the Nobel prize winner Albert Szent-Györgyi Nagyropolt in the nineteen thirties and first called vitamin P, because they are able to influence the permeability of blood vessels. Later they got the name flavonoids, because they are derived from the structure flavone (Fig. 3). Especially the quercetin- and kaempferolglycosides are suitable for variety differentiation [6, 7, 8]. These compounds are occurring in hops only glycosidically bound. The basic work about flavonoids in hops was done by *I. McMurrough* and *C. F. van Sumere* in the nineteen eighties [9]. The sugars can be removed by hydrolysis and the aglycons quercetin and kaempferol were determined quantitatively by HPLC (Fig. 4). With this method nearly the whole available world hop collection had already been analysed five years ago [10]. But in this work also the glycosides should been taken into consideration. First convenient methods for sample preparation and HPLC analysis had to been worked out. Then the whole in Hüll available world hop collection was analysed and the data were evaluated by a principal component analysis to show differences and similarities.

2 Materials and methods

2.1 Sample preparation

For sample preparation 5 g dry weight ground hops are extracted with 50 ml acetone/water (3:1) in an ultrasonic bath for 15 minutes. Then the solution is filtered through a pleated filter. The filtrate was transferred into a separating funnel and shaken with 50 ml hexane. The non polar substances go into the hexane-phase and the flavanoids remain in the acetone/water phase. As an internal standard a solution of 1 ml flavone in acetone (250 mg flavone in 25 ml acetone) is added. Flavone is not a natural ingredient of hops and delimits the polar flavonoids from the non polar bitter substances, xanthohumol and the prenylated naringenins. All these substances elute after flavone.

2.2 UHPLC-method

UHPLC-Conditions: oven temperature: 40 °C

column: EC 125/2 NUCLEODURSphinx RP, 3 µm from Macherey & Nagel

eluent A: 100 ml methanol, 3 ml 85 % H_3PO_4 filled up to 1 l with water

eluent B: 700 ml methanol, 3 ml 85 % H_3PO_4 filled up to 1 l with water

eluent C: methanol

Linear gradient:	detection wavelength:	
0 min.: 100 % A	benzoic acid-derivates:	250 nm
5 min.: 100 % A	cinnamic acid-derivates:	280 nm
30 min.: 70 % A, 30 % B	catechins:	280 nm
55 min.: 10 % A, 90 % B	quercetin-,	
56 min.: 100 % C	kaempferolglycosides:	350 nm
60 min.: 100 % C	multifidolglucoside:	280 nm
61 min.: 100 % A		

3 Results and discussion

3.1. Identification of the chemical compounds

The hop varieties Opal, Hersbrucker Spät, Herkules and Zeus vary substantially in their flavonoid composition (Fig. 5). The quercetin- and kaempferolglycosides have an absorption maximum at a wavelength of 350 nm and the multifidolglycosides at 280 nm (Fig. 6), therefore it was decided to use the wavelengths 350 nm and 280 nm to get the best selectivity and sensitivity.

All main components could be identified with a masspectrometer [11] in cooperation with the TUM. The substances quercetin-3-galactoside, quercetin-3-glucoside and kaempferol-3-glucoside were also verified by reference substances. The chemical structures of these compounds are pictured in figure 7. Substance 1 is identified as 1-(2-methylpropanoyl)phloroglucinol-glucopyranoside a derivative of multifidolglucoside. The real multifidol-glucoside has the chemical designation 1-(2-methylbutyryl)phloroglucinol-glucopyranoside and its name is derived from the tropical plant jatropha multifida. Perle, Hall. Taurus and Herkules are varieties with high contents of substance 1. There was no correlation between alpha-acids content and the 1-(2-methylpropanoyl)phloroglucinol-glucopyranoside 1 content. Up to now a quantitative determination is not possible, because no standard is available. The structures of the compounds 5 and 7 were clarified by a masspectrometer after isolation by preparative HPLC. The determination of the absolute structures is not possible with this method. The substances 5 and 7 are identified as quercetin-3-(malonyl)hexoside and kaempferol-3-(malonyl)hexoside.

3.2 Differentiation of the world hop collection

In Hüll is a hop yard, in which nearly the whole hop world hop collection is grown (Table 2). These are 121 different varieties. All these varieties were analysed with the in point 2 described methods. The labelled eight peaks (Fig. 5) occurr in every variety, but with different levels in the different hop varieties. Especially peak 7 of the variety Herkules is not pure substance 7. It is possible that the peaks are slightly polluted with other chemical compounds. Samples of different growing locations show the same flavonoid compositions. This project was the first work, in which the flavonoid compositions of nearly the whole world hop collection were analysed. Some varieties are very good distinguishable, but others especially the old land races are very similar.

A principal component analysis PCA was then made with the eight in the chromatograms labelled peaks (Fig. 5) to make differences and similarities visible. The software SAS 9.1 was used for the calculation. Table 2 shows the first three principal components and figure 8 the graphic representation of the first two principal components. A three-dimensional figure is not lucid. The principal component analysis was carried out on the correlation matrix (Table 3). The eigenvalues, variances and cumulative variances are put together in table 4. The first two principal components describe 64,8 % and the first three principal components 81,2 % of the whole variance. Each point in the graphic presents a variety. Points, which are close together, are very similar. Points, which are wide apart, are very different. The drawn lines illustrate the contribution of the single peaks to the principal component analysis. A group formation based on the flavonoid composition is not observable nor according to countries. The most varieties are within the marked ellipse.

4 Conclusion – summary

The composition of the quercetin- und kaempferolglycosides of hops is genetically determined and therefore depending on the variety. Some varieties are very well distinguishable, however other varieties like the old land races are very similar. The analytics of the flavonoids is a useful additional tool to differentiate varieties. It is not possible to make groups based on the flavonoid composition.

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Appendix

Table 1 The distribution of the hop polyphenols

substances and groups of substances	concentrations
aromatic carboxylic acids	
1) benzoic acid-derivates	< 0,01 %
2) cinammic acid-derivates	0,01 – 0,03 %
flavonoids	
3) xanthohumol	0,20 – 1,70 %
4) 8-, 6-prenylnaringenin	< 0,01 %
5) quercetin-glycosides	0,05 – 0,23 %
6) kaempferol-glycosides	0,02 – 0,24 %
7) catechins and epicatechins	0,03 – 0, 30 %
8) proanthocyanidins	0,20 – 1,30 %
9) acylphloroglucinol-derivates (multifidols)	0,05 – 0,50 %
higher molecular substances	
10) tannins	2,00 – 7,00 %

Table 2 World hop collection and the first three PCA-values (harvest 2009, 2010)

Sorte	PCA 1	PCA 2	PCA 3	Sorte	PCA 1	PCA 2	PCA 3
Admiral	1,1682	–0,5349	1,0614	Hall. Magnum	2,8912	1,0239	0,4025
Agnus	2,3061	–0,5189	0,9039	Hall. Merkur	1,3086	0,7450	0,3580
Ahil	2,2231	–0,2418	–1,2286	Hall. Taurus	2,2465	0,8289	–0,9431
Alliance	–1,9321	–1,4778	1,3508	Hall. Tradition	1,0473	–1,1677	–1,5281
Alpharoma	–2,2172	–0,7703	1,3347	Hallertauer Mfr.	–0,5632	–2,0694	–0,1420
Apolon	0,6569	0,3291	–0,9330	Harmony	1,3107	–0,3659	–0,0423
Aquila	1,5885	3,0754	1,5011	Herald	0,3043	–0,1575	–1,2075
Aromat	0,0367	–1,5485	–0,5999	Herkules	1,5148	1,5725	–1,8072
Atlas	–0,6711	2,1336	–0,1297	Hersbrucker Pure	–0,2882	–1,1079	0,3737
Aurora	–0,2010	–1,6635	0,6439	Hersbrucker Spät	–3,0965	0,7484	1,0776
Backa	1,2217	1,1048	0,0661	Horizon	–0,4303	–0,7081	0,8742
Belgischer Spalter	0,0865	–0,1480	–0,7276	Hüller Anfang	–0,6423	–2,1060	–0,3398
Blisk	0,9699	1,1906	–0,5516	Hüller Aroma	–0,5397	–1,6429	–0,2945
Boadicea	–1,1622	0,6009	–0,7538	Hüller Bitter	–0,6432	0,4300	0,2369
Bobek	0,7563	–1,3901	–0,3582	Hüller Fortschritt	–1,2859	–1,7462	0,3518
Bor	0,7966	–0,3426	–1,0197	Hüller Start	–0,9317	–2,2770	0,1523
Bramling Cross	–2,0159	2,6388	–0,6440	Japan C 730	–0,6456	0,0283	1,7829
Braustern	0,8084	–1,2310	–0,7422	Japan C 845	1,6744	–0,0063	–2,4914
Brewers Gold	2,3456	0,9341	0,0525	Kirin 1	–0,5663	4,3001	–0,6098
Brewers Stand	–0,8525	2,9484	0,1179	Kirin 2	–0,6803	4,5611	–0,4765
Buket	–0,4146	–1,4976	1,1649	Kitamidori	0,4046	0,2743	–1,8071
Bullion	0,5911	0,8128	–0,4729	Kumir	0,4719	–0,7643	0,2004
Cascade	0,7359	–0,0825	–0,5093	Lubelski	1,0551	–1,3945	–0,4113
Chang Bei 1	–1,5525	–0,8015	0,6504	Malling	–2,1140	1,0422	0,2514
Chang Bei 2	–1,5555	–0,4521	0,6733	Marynka	–0,9812	2,6990	0,1190
College Cluster	–2,9899	2,4738	0,6321	Mt. Hood	–0,2745	–0,8995	0,5223
Columbus	0,9282	3,0808	–1,2409	Neoplanta	–1,0720	–1,1345	1,0980
Comet	1,1808	0,5673	–0,1616	Neptun	4,6159	0,0358	5,2798
Crystal	–2,9592	1,1544	0,8979	New Zealand Hallertauer	–1,3090	1,0854	–0,0906
Density	–1,8294	2,5229	–0,8132	Northern Brewer	3,9825	–0,3649	1,4789
Diva	–0,8184	–0,9396	–0,7198	Nugget	–1,2975	–0,3105	0,8997
Early Choice	–1,0962	–0,9869	–0,7157	Olympic	–1,3420	–0,2178	0,7355
Eastern Gold	–0,7137	4,3263	–0,1320	Opal	–2,0242	–1,4161	0,5223
Eastwell Golding	–0,9016	–0,4953	–0,2306	Orion	1,2338	–0,4060	–1,7365
Emerald	1,9226	–0,4544	–2,5513	Pacific Gem	–2,2264	0,9394	1,5129
Eroica	0,5112	2,9135	–1,2670	PCU 280	0,8562	–0,9419	–0,6114
Estera	–1,4200	0,6819	–0,0872	Perle	2,3792	–0,4904	–3,0422
First Gold	–0,9611	–0,5190	–0,2654	Phoenix	–0,8352	–0,8661	0,8535
Fuggle	–0,4894	0,5915	0,4451	Pilgrim	–0,6419	–0,7377	–0,9443
Galena	2,0862	2,0949	–1,5645	Pilot	–2,0300	0,1094	–0,2576
Ging Dao Do Hua	–0,7069	4,1741	–0,3927	Pioneer	–1,7790	0,7577	0,3629
Glacier	–1,4959	–1,4693	–0,0567	Premiant	1,3224	–0,6696	–0,6105
Golden Star	–0,6494	4,3068	–0,4637	Pride of Kent	–1,6595	–1,9667	0,3066
Granit	–0,3470	1,0616	0,3112	Pride of Ringwood	–1,7599	1,7763	0,4951
Green Bullet	–1,7257	–0,5629	0,9473	Progress	–0,8397	2,9648	0,6149
Hall. Gold	0,1733	–1,1510	–0,9658	Rubin	–1,7520	–1,0825	0,5508

Continued table 2

Continuation table 2

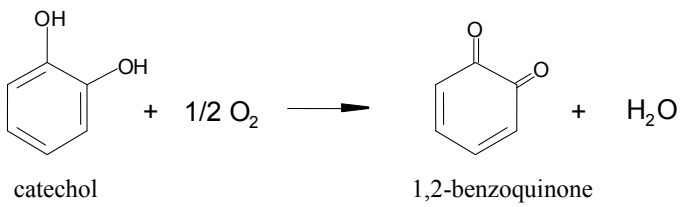
Sorte	PCA 1	PCA 2	PCA 3	Sorte	PCA 1	PCA 2	PCA 3
Saazer	–0,1950	–1,6743	0,5515	Urozani	–0,0948	–0,9667	1,1646
Saphir	–1,3506	–1,3976	–0,3865	USDA 21055	–1,5491	3,8642	–0,3581
Serebrianker	–0,7182	–1,9525	1,0076	Vojvodina	–0,8368	–1,7339	0,0174
Sirem	0,9910	–1,4713	–0,7268	WFG	0,9388	–1,4671	–0,6445
Sladek	0,8235	–0,7533	–1,0480	Williamette	–2,2056	1,2095	0,0951
Smaragd	–1,8402	–1,1527	0,3351	Wye Northdown	0,7422	–0,6856	–1,5281
Spalter	–0,0589	–1,8434	0,3222	Wye Target	0,8986	–0,6559	0,7886
Spalter Select	–0,8540	–1,8748	0,0160	Wye Viking	–0,0075	–0,6130	0,4524
Sterling	–1,6244	–0,0231	0,7788	Yeoman	–0,5796	–1,1030	0,1024
Sticklebrackt	–2,1959	2,0770	0,9805	Zatecki	–0,6515	0,8889	0,1565
Strisselspalter	–3,1147	1,3959	0,9958	Zenith	–1,0167	–1,5939	0,2939
Super Alpha	–1,5729	1,0136	0,9513	Zeus	0,1323	3,6007	–0,3090
Talisman	1,1639	–0,7823	–0,7213	Zitic	1,3728	–0,5409	–2,1116
Tettnanger	0,0549	–1,5640	0,1947	Zlatan	0,5318	–1,4985	–0,2262
Toyomidon	2,5675	0,1553	–0,3233				

Table 3 Correlation matrix

	Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	Peak 6	Peak 7	Peak 8
Peak 1	1,00	–0,57	–0,20	–0,39	–0,58	–0,32	0,19	0,13
Peak 2	–0,57	1,00	0,37	–0,30	0,61	–0,42	–0,32	–0,23
Peak 3	–0,20	0,37	1,00	–0,50	–0,07	–0,52	–0,05	0,11
Peak 4	–0,39	–0,30	–0,50	1,00	–0,11	0,69	–0,07	–0,19
Peak 5	–0,58	0,61	–0,07	–0,11	1,00	0,13	–0,38	–0,30
Peak 6	–0,32	–0,42	–0,52	0,69	0,13	1,00	–0,22	–0,27
Peak 7	0,19	–0,32	–0,05	–0,07	–0,38	–0,22	1,00	0,81
Peak 8	0,13	–0,23	0,11	–0,19	–0,30	–0,27	0,81	1,00

Table 4 Eigenvalues and variances

PCA	eigenvalue %	variance %	cumulative variance %
1	2,72	34,0	34,0
2	2,47	30,8	64,8
3	1,31	16,4	81,2
4	0,79	9,9	91,1
5	0,44	5,5	96,6



catechol 1,2-benzoquinone

Fig. 1 Oxidation of catechol to 1,2-benzoquinone

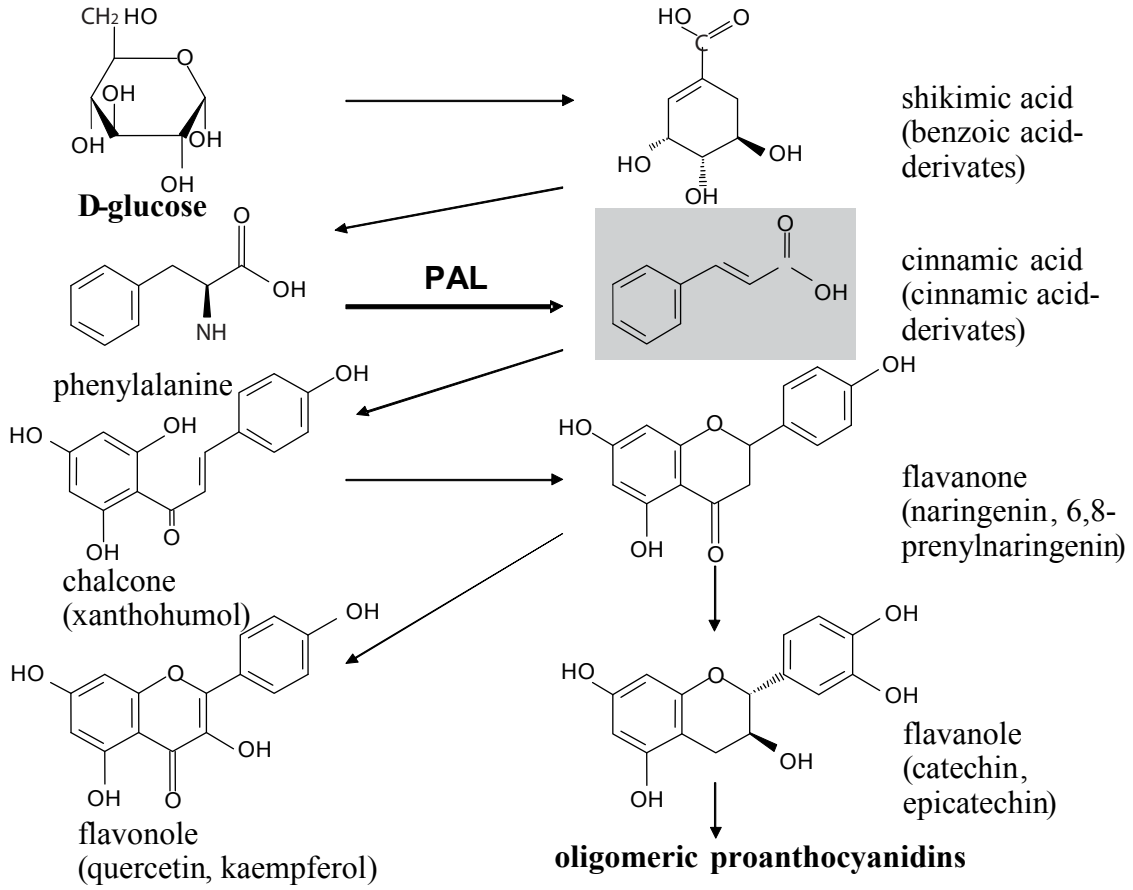


Fig. 2 Biosynthesis pathway of the polyphenols

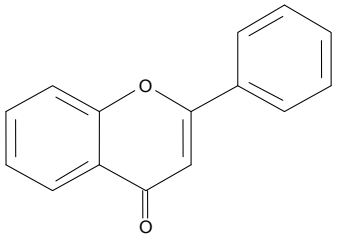


Fig. 3 Flavone

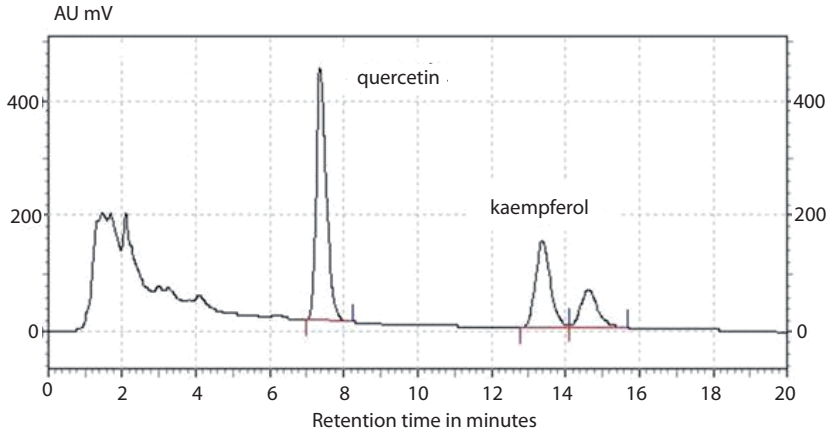


Fig. 4 HPLC chromatogram of querecetin and kaempferol (365 nm), eluent H_2O-methanol-85 % H_3PO_4 (49,75: 49,75: 0,5, v/v)

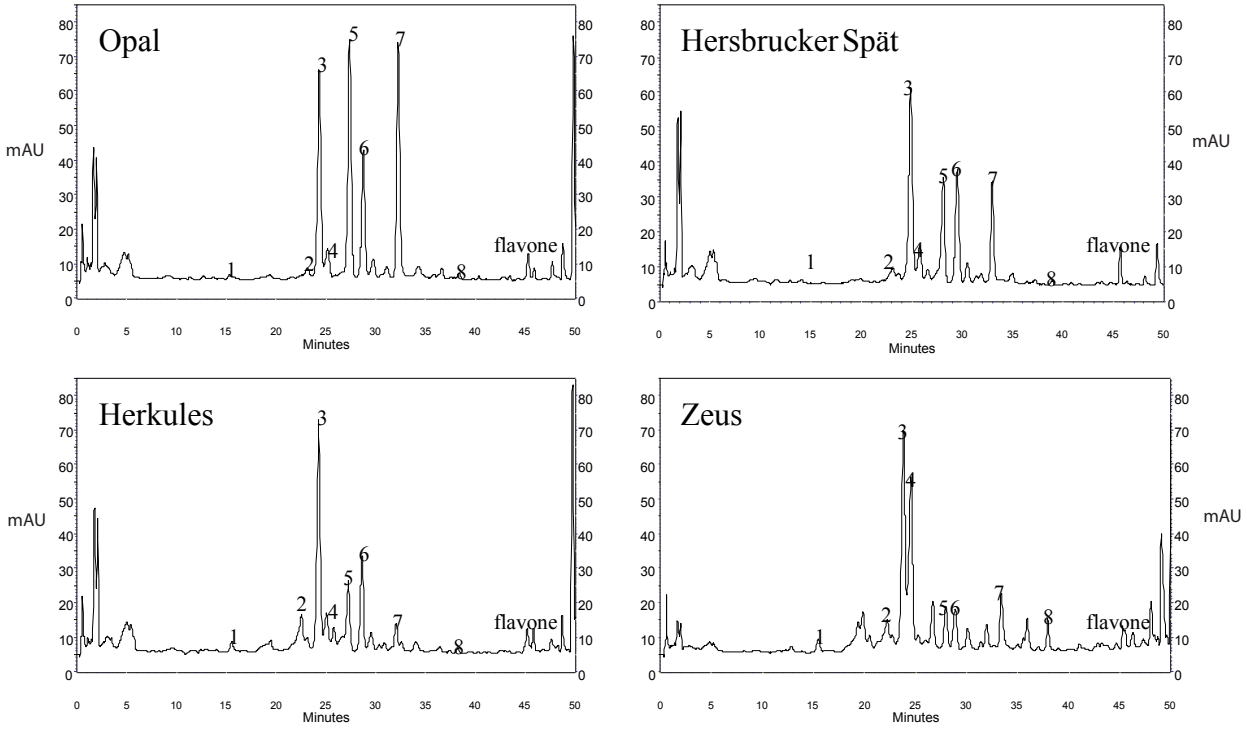


Fig. 5 HPLC chromatogram of the different varietíes (350 nm)

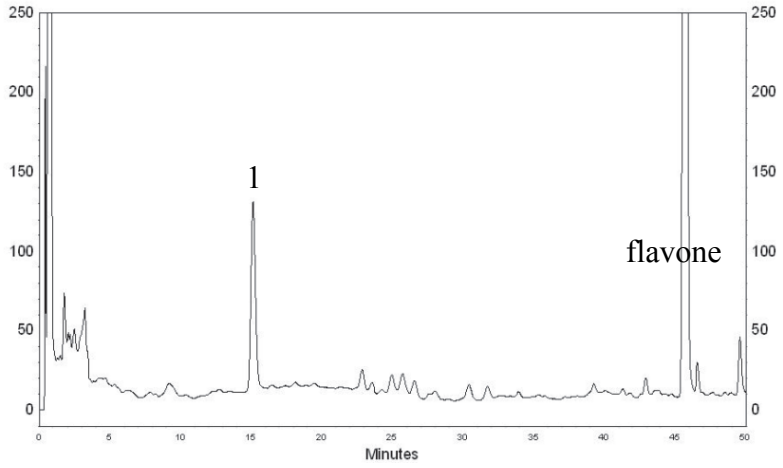


Fig. 6 HPLC chromatogram of the flvonoids (280 nm), this wavelength supplies the optimal selectivity and sensitivity for the multi-fidolglucosides

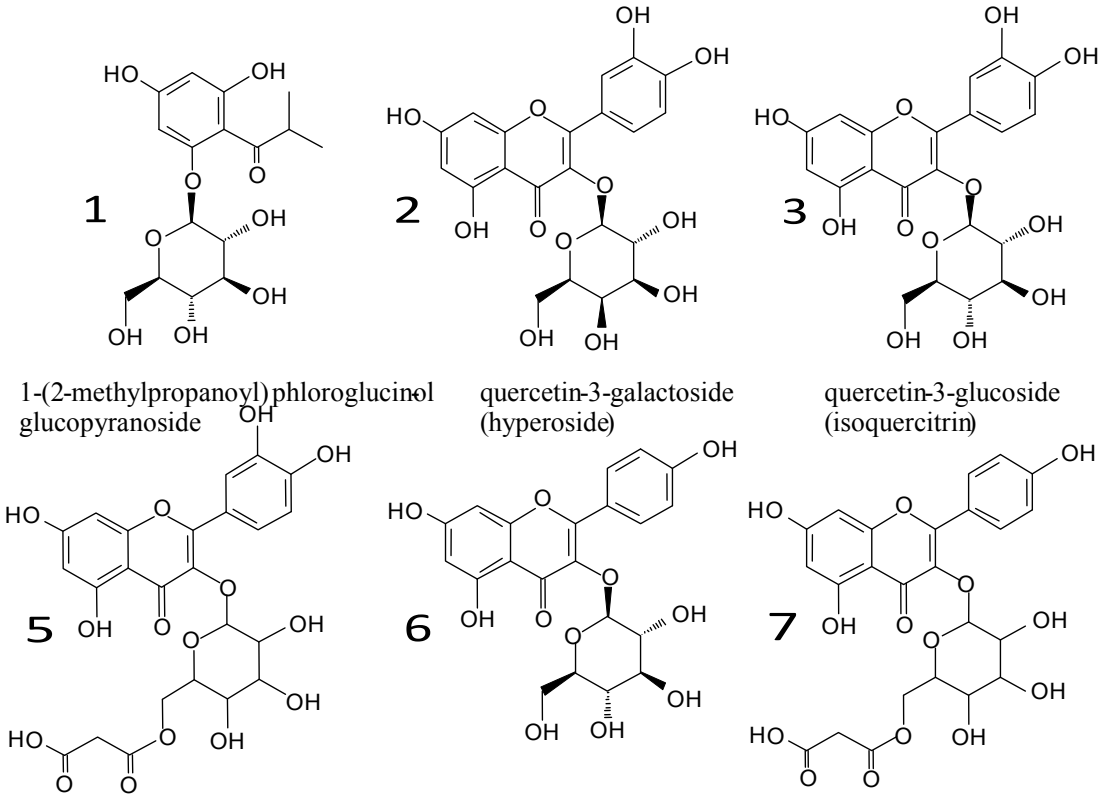


Fig. 7 Identified flvonoids

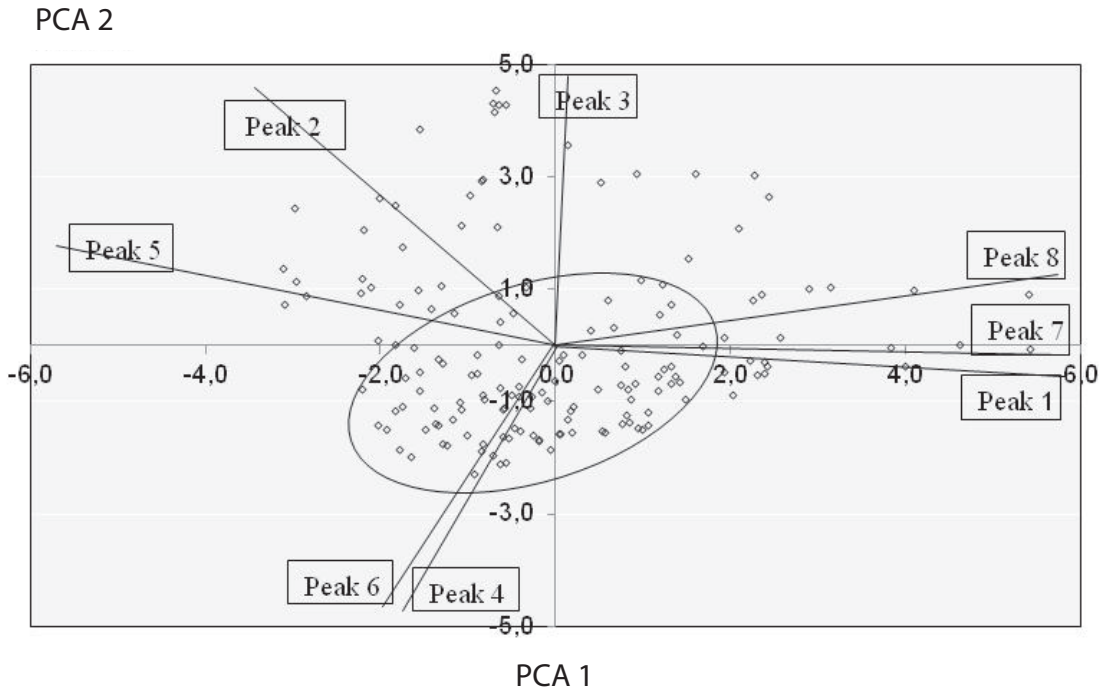


Fig. 8 Principal component analysis of the world hop collection