C. Schmidt and M. Biendl

Quantitative analysis of a large spectrum of hop phenolic compounds by LC-MS/MS

Besides of bitter acids, hop polyphenols can influence the sensory properties of beer. While the concentration of bitter acids is tightly monitored by the hop industry, comprehensive datasets concerning the polyphenols do not exist yet. To quantitate the hop phenolic compounds, LC-MS/MS analysis was applied to various hop varieties from different growing regions for the harvest year 2021 (> 400 samples). The list of compounds tested includes multifidol glucosides, flavonol glycosides and their aglycones, flavan-3-ols, procyanidins, and phenolic acids. The multifidol glucosides (co- and ad-multifidol glucoside) are typical to hops as intermediates in the biosynthesis of bitter acids. The detected concentrations for co-multifidol glucoside are between 8–200 mg/100 g depending on hop variety. Consistently low amounts (< 5 mg/100 g) were found for quercetin, kaempferol or myricetin as flavonol aglycones in comparison to their glycosides quercetin and kaempferol glucoside or quercetin and kaempferol rutinoside (up to 100 mg/100 g). Characteristics for single hop cultivars were observed for the procyanidins B1, B2 and B3, and for the flavan-3-ols catechin and epicatechin.

Descriptors: Hop polyphenols, multifidols, flavonols, flavan-3-ols, procyanidins.

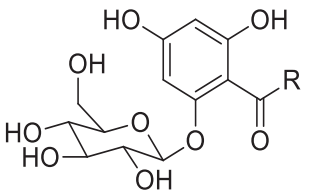
1 Introduction

The total polyphenol content of dried hop cones varies between 3 % and 8 %, depending on the hop variety [1]. Chemically, polyphenols are substances consisting of phenols or phenol-like units. Polyphenols are natural antioxidants [2] which can make a positive contribution to both bitterness [3] and body fullness of beer [4]. It is possible to differentiate between hop phenolic compounds specific to hops (or only few plants) like the multifidol glucopyranosides (short: multifidols), and polyphenols that are found in many plants like flavonol glycosides, flavan-3-ols or phenolic acids.

Multifidols as the first group of interest are acylpholoroglucinol derivatives, named after their discovery in *Jatropha multifida* L. [5]. They were isolated by Bohr et al. in 2005 from *Humulus lupulus* L. as three homologues of multifidols: co-multifidol glucoside (Co-M-glc), ad-multifidol glucoside (Ad-M-glc), and n-multifidol glucoside (N-M-glc) (Fig. 1) [6]. These substances are known intermediates in the biosynthesis of hop bitter acids from branched-chain amino acid precursors and therefore the acyl side chains of multifidol glucopyranosides are identical to those of alpha-acids (co-, ad-, and n-humulone) [7]. They are also showing anti-inflammatory activity [6]. Multifidols were identified as bitter molecules having a human recognition threshold concentration of 5 µmol/L (or 1.8 mg/L) for co-multifidol glucoside and 10 µmol/L (or 3.7 mg/L) for ad-multifidol glucoside [3]. In 2021, a study by Morcol et al. reports co-multifidol glucoside for the first time in wild hop cultivars *Humulus neomexicanus* [8].

Flavonol glycosides and their aglycones are a further group of chemical compounds found in hops but also in many other plants (Fig. 2). The amount varies within the different hop varieties but does not exceed 1 % [1]. The composition of quercetin and kaempferol glucosides in a world hop collection with 121 different varieties from 17 countries was investigated by Kammhuber in 2012 [9] and was described to be suitable for the differentiation of hop varieties. Regarding their contribution to beer bitterness, Dresel et al. described the lowest taste thresholds for kaempferol and quercetin glucoside [3].

Flavan-3-ols are a subgroup of flavonoids. They can also be found in many plants. The most important representatives of this group in hops are catechin with up to 0.2 %, the epimer epicatechin (Fig. 3) and gallocatechin [1]. Catechin is not only present in its free form but also bound to other (epi)catechin units. Chains with up to eight units (oligomers) are called proanthocyanidins. For polymers the



Co-M-glc $R = CH(CH_3)_2$

Ad-M-glc $R = CH(CH_3)CH_2CH_3$

Fig. 1 **Chemical structure of multifidol glucosides co-multifidol glucoside (Co-M-glc) and ad-multifidol glucoside (Ad-M-glc)**

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term tannin is used. The four procyanidins B1, B2, B3 and B4 are dimers of catechin and epicatechin. They are the principal compounds present in all hop varieties and represent up to 80 % of the total amount of procyanidins in hops [1].

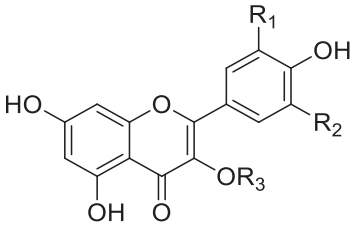
The flavan-3-ols in beer originate primarily from malt with up to 80 %. They are known to form complexes with proteins during wort boiling and are then mostly eliminated with hot break material. Thus, physical beer stability is improved although remaining polyphenols and proteins can still lead to turbidity or haze during beer storage [10–11].

Phenolic acids (Fig. 4) are also aromatic secondary plant metabolites. They are derivatives of benzoic and cinnamic acid. Phenolic acids are only minor components of hops. Ferulic acid was found in very low concentration of 0.01 % [1]. The amounts of further phenolic acids are even lower. Like flavan-3-ols, phenolic acids are transferred into the brewing process primarily through malt. Some phenolic acids are also discussed to be involved in colloidal changes during beer aging [11].

Recently the contents of various glycosidically bound polyphenols in a large range of hops and hop products from the crop years 2019 and 2020 were published [12]. The according glycosides are described to be relevant for beer taste in supplementation to the hop bitter acids [3].

To gain even more insights into the polyphenolic profile of different hop varieties, the list of hop phenolic compounds was enlarged to further flavonol glycosides, the aglycones quercetin (Q), kaempferol (K), and myricetin (Myr), the flavan-3-ols catechin and epicatechin, the procyanidins B1, B2 and B3, and phenolic acids (p-hydroxybenzoic acid, o-/m-/p-coumaric acid, ferulic acid) for the harvest year 2021. A total number of more than 400 raw hop and pellet samples were monitored using LC-MS/MS technique.

In the end the quantitative findings presented in this paper are not only useful for variety characterization but can also serve as base for the consideration of transfer rates from hops to beer, and finally the influence on beer quality.



Kaempferol (K)	$R_1 = H$	$R_2 = H$	$R_3 = H$
Kaempferol-glc (K-glc)	$R_1 = H$	$R_2 = H$	R_3 = Glucose
Kaempferol-rut (K-rut)	$R_1 = H$	$R_2 = H$	R_3 = Rutinose
Quercetin (Q)	$R_1 = OH$	$R_2 = H$	$R_3 = H$
Quercetin-glc (Q-glc)	$R_1 = OH$	$R_2 = H$	R_3 = Glucose
Quercetin-rut (Q-rut)	$R_1 = OH$	$R_2 = H$	R_3 = Rutinose
Myricetin (Myr)	$R_1 = OH$	$R_2 = OH$	$R_3 = H$
Myricetin-glc (Myr-glc)	$R_1 = OH$	$R_2 = OH$	R_3 = Glucose

Fig. 2 Chemical structure of flavonol glycosides and aglycones

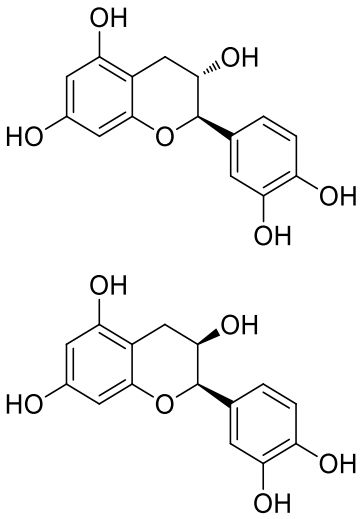
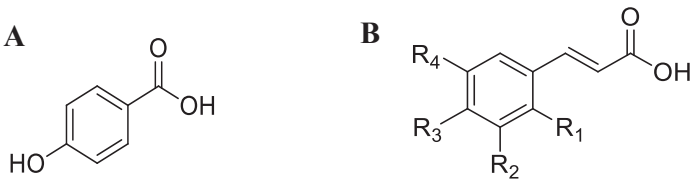


Fig. 3 Chemical structure of (+)-Catechin (top) and (–)-Epicatechin (bottom)



o-Coumaric acid	$R_1 = OH$	$R_2 = H$	$R_3 = H$	$R_4 = H$
m-Coumaric acid	$R_1 = H$	$R_2 = OH$	$R_3 = H$	$R_4 = H$
p-Coumaric acid	$R_1 = H$	$R_2 = H$	$R_3 = OH$	$R_4 = H$
Ferulic acid	$R_1 = H$	$R_2 = H$	$R_3 = OH$	$R_4 = OCH_3$

Fig. 4 Chemical structure of p-hydroxybenzoic acid (A) and further phenolic acids (B)

2 Materials and methods

2.1 Reagents

Following chemicals were obtained from commercial sources: water and methanol for LC-MS (Chemsolute®, Th. Geyer GmbH & Co. KG, Renningen, Germany);

Table 1 **Variety code, origin, variety name, and content of alpha-acids, beta-acids and xanthohumol for investigated hop cultivars**

Variety code [15]	Origin	Variety name	% Alpha-acids EBC 7.7 [16]	% Beta-Acids EBC 7.7 [16]	% Xanthohumol EBC 7.15 [16]
DE HKS	Germany	Herkules	13.0–17.0	4.0–5.5	0.6–0.8
DE PER	Germany	Perle	4.0–9.0	2.5–4.5	0.4–0.6
DE HTR	Germany	Hallertauer Tradition	4.0–7.0	3.0–6.0	0.3–0.5
DE HMG	Germany	Hallertauer Magnum	11.0–16.0	5.0–7.0	0.4–0.5
DE HEB	Germany	Hersbrucker Spät	1.5–4.0	2.5–6.0	0.2–0.3
DE SIR	Germany	Saphir	2.0–4.5	4.0–7.0	0.3–0.4
CZ SAZ	Czech Rep.	Saazer	2.8–3.5	3.0–5.0	0.2–0.4
DE TET	Germany	Tettnanger	2.5–5.5	3.0–5.0	0.3–0.4
DE SSE	Germany	Spalter Select	3.0–6.5	2.5–5.0	0.3–0.5
SI SSA	Slovenia	Super Styrian Aurora	7.5–8.8	3.3–5.0	0.3–0.6
DE HAL	Germany	Hallertauer Mittelfrüher	3.0–5.5	3.0–5.0	0.2–0.3
SI SGC	Slovenia	Styrian Golding Celeia	4.0–7.0	2.5–4.5	0.1–0.3
DE MBA	Germany	Mandarina Bavaria	7.0–10.0	4.0–7.0	0.4–0.8
SI SGB	Slovenia	Styrian Golding Bobek	3.0–7.0	4.0–7.0	0.3–0.4
DE CAL	Germany	Callista	2.0–5.0	5.0–10.0	0.3–0.6
DE PLA	Germany	Polaris	19.0–23.0	4.0–6.0	0.7–1.0
DE AKO	Germany	Akoya	8.0–10.0	4.0–5.0	0.9–1.0
DE SOL	Germany	Solero	9.0–10.0	5.0–6.0	0.7–0.8
PL LUB	Poland	Lublin	3.0–4.5	2.5–3.5	0.2–0.3
DE NBR	Germany	Northern Brewer	6.0–10.0	3.0–5.0	0.5–0.7
DE HTU	Germany	Hallertauer Taurus	12.0–17.0	4.0–6.0	0.9–1.0
DE SPA	Germany	Spalter	2.5–5.5	3.0–5.0	0.2–0.3
DE HBC	Germany	Hallertau Blanc	9.0–12.0	4.0–6.0	0.2–0.4
US CAS	USA	Cascade	4.5–7.0	4.5–7.0	0.1–0.4
US SUL	USA	Sultana	13.0–15.0	4.0–5.0	0.5–0.7
US EUE	USA	Eureka!	17.0–19.9	4.6–6.0	0.5–0.6
US BRO	USA	Bravo	14.0–17.0	3.0–5.0	0.8–1.0
US CEN	USA	Centennial	9.5–11.5	3.5–4.5	0.3–0.5
US LOT	USA	Lotus	13.0–17.0	5.5–6.0	0.8–1.0
US CPO	USA	Calypso	12.0–14.0	5.0–6.0	0.5–0.7
US LDP	USA	Lemondrop	5.0–7.0	4.0–6.0	0.2–0.3
AU GXY	Australia	Galaxy	11.0–16.0	5.0–9.0	0.7–0.8

formic acid and ammonium formate (Merck, Darmstadt, Germany). The substances quercetin-3-O-glucoside, quercetin-3-O-rutinoside, kaempferol-3-O-glucoside were obtained from Merck (Darmstadt, Germany), kaempferol-3-O-rutinoside, myricetin-3-O-glucoside, procyanidin B1, B2 and B3 were obtained from Extrasynthese, (Lyon, France), (+)-catechin, (–)-epicatechin, p-hydroxybenzoic acid, o-/m-/p-coumaric acid, ferulic acid, hesperetin-7-O-rutinosid, kaempferol, quercetin and myricetin were obtained from PhytoLab (Vestenbergsgreuth, Germany). The internal standard dicamba was obtained from LGC Standards GmbH (Wesel, Germany). The purified standards for co-multifidol glucoside and ad-multifidol glucoside were provided by Dr. Philip Wietstock from the Technical University of Berlin after isolation according to Kunz et al. [13].

2.2 Sampling

The investigated samples from Europe were raw hops (dried cones) and pellets taken during process control in a large-scale pellet plant throughout the whole campaign 2021/2022.

Each sample was representative of variety specific batches with sizes up to 30 tons of hops. The more batches of a variety had been processed the more samples were available for our study. In total more than 400 single samples from 23 different varieties of the main European growing areas were analysed. Most of them came from Germany, the others from Czech Republic, Slovenia, and Poland.

Moreover, a few pellet samples outside Europe were supplied by Hopsteiner, USA (S. S. Steiner, Inc., Yakima). They only represented one or two samples per variety. For one variety 3 samples were investigated. In total 14 single samples from 8 different US varieties and one from Australia were analysed.

Table 1 shows all hop varieties investigated in this study and gives their characteristics regarding the main hop bitter compounds (alpha-acids, beta-acids), and the main hop prenylflavonoid (xanthohumol), all well-known from literature [1]. These data are taken from the Hopsteiner webpage [14].

2.3 Sample preparation

1 g of milled hop cones or pellets was weighed into a 100 mL screw cap bottle and 20 mL of HPLC solvent mixture (solvent A + solvent B, 50/50, v/v, see more details to HPLC solvents in chapter 2.4) was added. The sample was extracted for 30 min with the help of a shaking device (225 rpm). An aliquot of this sample was then centrifuged for 15 min at 13500 rpm and 1 mL of the supernatant was analysed using LC-MS/MS in the negative mode after addition of dicamba as internal standard (c = 200 ng/mL).

2.4 Sample analysis with liquid chromatography – tandem mass spectrometry (LC-MS/MS)

The ExionLCTM system, consisting of a binary pump, a degas-

ser, an auto-sampler and a thermostatted column oven with capacity for 2 analytical columns (SCIEX, Darmstadt, Germany), was coupled with a 5500+ Q-TRAP mass spectrometer (SCIEX, Darmstadt, Germany) equipped with an electrospray ionization (ESI) source running in the negative ion mode. Samples were introduced by HPLC at a solvent flow of 500 µl/min, which required the use of turbo gas at a temperature of 350 °C. The ion spray voltage was set to −4500 V, the declustering potential and the MS/MS parameters were optimized for each substance to induce fragmentation of the pseudo molecular ion $[M-H]^-$ to the corresponding target product ions after collision-induced dissociation. The collision energy (CE), the declustering potential (DP) as well as the cell entrance potential (CEP) were set as given in table 2. Nitrogen was used as the collision gas. The quantitation was done using the scheduled multiple reaction monitoring (MRM) mode of the instrument with the fragmentation parameters optimized prior to analysis and the retention times of the corresponding reference compounds. Data processing was performed by using Analyst software version 1.7.1 and data integration was done by SCIEX OS software version 1.7 (SCIEX, Darmstadt, Germany). For chromatography, an analytical 50 x 2.0 mm Synergi 4µ Fusion-RP 80A column (Phenomenex, Aschaffenburg, Germany) equipped with a guard column of the same type (Phenomenex, Aschaffenburg, Germany) served as the stationary phase. 5 mM ammonium formate containing 0.1 % formic acid in water was used as solvent A and methanol with 5 mM ammonium formate and 0.1 % formic acid as solvent B. The temperature of the column oven was set at 40 °C. The injection volume was 2 µl. Chromatography was performed by increasing solvent B from 20 to 100 % within 8 min and holding for 2 min. Quantitation was done by external calibration in a range between 100 and 10000 ng/ml with dicamba as internal standard.

Table 2 Specific mass transitions and optimized parameters for the LC-MS/MS for all phenolic compounds and the internal standard (Dicamba)

Compound	Mass transitions m/z Q1→Q3	DP[a] [V]	CE[b] [V]	CXP[c] [V]
Co-M-glc	356.9 → 194.9[d]	−85	−26	−2
	356.9 → 151.0	−85	−46	−2
Ad-M-glc	371.0 → 209.1[d]	−80	−22	−4
	371.0 → 165.1	−80	−48	−4
Kaempferol (K)	284.9 → 117.0[d]	−130	−54	−11
	284.9 → 185.0	−130	−38	−15
K-glc	446.8 → 284.1[d]	−115	−34	−2
	446.8 → 226.9	−115	−64	0
K-rut	593.1 → 285.0[d]	−210	−44	−17
	593.1 → 255.0	−210	−74	−17
Quercetin (Q)	301.0 → 151.0[d]	−150	−30	−13
	301.0 → 121.0	−150	−38	−11
Q-glc	462.8 → 300.1[d]	−110	−40	−2
	462.8 → 270.8	−110	−60	−2
Q-rut	608.9 → 300.0[d]	−115	−48	−4
	608.9 → 271.1	−115	−74	−4
Myricetin (Myr)	316.9 → 151.0[d]	−150	−34	−11
	316.9 → 137.0	−150	−36	−11
Myr-glc	479.0 → 316.0[d]	−175	−40	−19
	479.0 → 271.1	−175	−60	−19
Hes-rut	609.1 → 301.0[d]	−205	−38	−17
	609.1 → 164.0	−205	−76	−13
Catechin/Epicatechin	289.0 → 245.1[d]	−115	−22	−17
	289.0 → 203.1	−115	−28	−15
p-/m-/o-Coumaric acid	162.9 → 119.1[d]	−70	−22	−19
	162.9 → 93.1	−70	−42	−9
Ferulic acid	193.0 → 178.0[d]	−80	−18	−13
	193.0 → 149.1	−80	−16	−11
p-Hydroxybenzoic acid	136.9 → 93.1[d]	−5	−22	−13
	136.9 → 65.1	−5	−40	−9
Dicamba (IntStd)	218.9 → 174.9[d]	−55	−8	−4
	218.9 → 35.1	−55	−26	−4
Procyanidin B1	576.9 → 125.0[d]	−170	−56	−11
	576.9 → 289.0	−170	−36	−17
Procyanidin B2	577.0 → 125.0[d]	−150	−56	−11
	577.0 → 289.1	−150	−36	−21
Procyanidin B3	577.1 → 125.0[d]	−140	−56	−11
	577.1 → 289.1	−140	−36	−17

[a] Declustering potential. [b] Collision energy. [c] Cell exit potential. Entrance potential (EP) = 10 for all compounds. [d] Quantifier ion. IntStd: Internal standard

For the analysis of procyanidins a LiChrospher 5 µm, RP-18, 100A, 250 x 4.6 mm analytical column (Phenomenex, Aschaffenburg, Germany) equipped with a guard column of the same type (Phenomenex, Aschaffenburg, Germany) as well as water with 0.1 % formic acid (solvent C) and methanol with 0.1 % formic acid (solvent D) were used with a solvent flow of 300 µl/min for chromatographic separation. The temperature of the column oven was set at 40 °C. The injection volume was 2 µl. Chromatography was performed by increasing solvent D from 15 to 40 % within 15 min, increasing further to 100 % D within further 15 min and holding for 5 min. Quantitation was done by external calibration in a range between 100 and 10000 ng/ml with dicamba as internal standard. Specific mass transitions and optimized parameters for the LC-MS/MS analysis of procyanidins are also given in table 2.

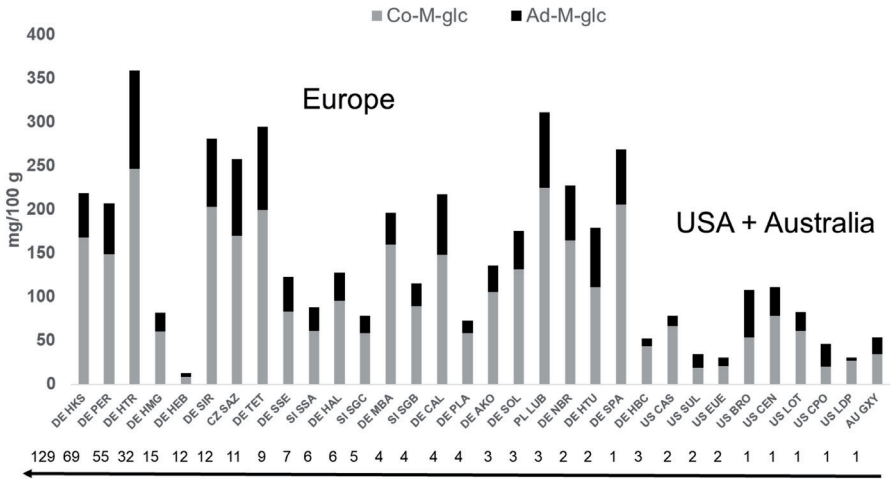


Fig. 5 **Amounts of multifidol glucosides co-multifidol glucoside (Co-M-glc) and ad-multifidol glucoside (Ad-M-glc) in mg/100 g in different hop varieties from Europe, USA, and Australia for the crop 2021. The rising numbers of samples are given along the arrow (from right to left)**

3 Results and discussion

The recently published method about glycosidically bound polyphenols in hops and hop products [12] has been enlarged. 16 further parameters namely kaempferol, quercetin, myricetin, kaempferol-rutinoside, myricetin-glucoside, hesperidin-rutinoside, catechin, epicatechin, p-, m- and o-coumaric acid, ferulic acid, p-hydroxybenzoic acid, and the procyanidins B1-B3 have been added. Samples from the crop year 2021 were evaluated, the results are summarized in groups and discussed in the following sections.

3.1 Multifidol glucosides

The concentrations for co-multifidol glucoside (Co-M-glc) and ad-multifidol glucoside (Ad-M-glc) are presented in figure 5 as mean values in mg/100 g. The highest number of samples (n = 129) was investigated for the hop variety Herkules (DE HKS) from Germany. The lowest sample numbers were tested from USA and Australia. The abbreviation of each hop cultivar is corresponding to the international list of hop varieties published by the International Hop Growers' Convention (IHGC) [15]. In total, 32 different varieties from Europe, USA, and Australia were investigated.

For the European hop cultivars, the amount of co-multifidol glucoside is always higher in comparison to the amount of ad-multifidol glucoside but the ratio (Co/Ad) is variety dependent and varies between 1.6 for German Hallertauer Taurus (DE HTU) and around 5 for the German hop cultivars Mandarina Bavaria (DE MBA) and Hallertau Blanc (DE HBC). The highest concentrations of the two homologues were found for the hop variety German Hallertauer Tradition (DE HTR) with 358.8 mg/100 g and the lowest for the cultivar German Hersbrucker Spät (DE HEB) with 12.5 mg/100 g. The sum of co-multifidol glucoside and ad-multifidol glucoside for further cultivars in this study varies between 52.3 mg/100 g (DE HBC) and 311.4 mg/100 g (PL LUB). The detected concentrations in crop 2021 are higher in comparison to the two previous crops described in [12]. The total amount of the multifidol glucosides for the variety Hallertauer Tradition (DE HTR) was e.g. 177.4 mg/100 g in 2020 in comparison to 358.8 mg /100 g in 2021.

The concentrations for US varieties of crop 2021 are lower in comparison to the amounts of multifidol glucosides in European cultivars. The maximum concentrations were detected for the two US varieties Bravo (US BRO) and Centennial (US CEN) with 107.5 mg/100 g and 111.3 mg/100 g. An outstanding position is given for the hop variety US

Table 3 **Mean value ± standard deviation (σ), and min/max values of co-multifidol glucoside (Co-M-glc) and ad-multifidol glucoside (Ad-M-glc) in mg/100 g for hop varieties with sample numbers n > 10**

Variety code [15]	n		Co-M-glc	Ad-M-glc
DE HKS	129	Mean (± σ)	168.3 (± 35.9)	50.2 (± 10.5)
		Min-Max	105.4–271.8	31.3–80.8
DE PER	69	Mean (± σ)	148.6 (± 36.4)	58.8 (± 13.9)
		Min-Max	87.3–256.0	32.5–102.0
DE HTR	55	Mean (± σ)	247.2 (± 49.7)	111.6 (± 21.6)
		Min-Max	144.8–351.4	70.7–148.1
DE HMG	32	Mean (± σ)	60.9 (± 16.5)	21.2 (± 7.4)
		Min-Max	37.3–97.2	12.6–45.4
DE HEB	15	Mean (± σ)	8.7 (± 4.4)	3.7 (± 2.3)
		Min-Max	3.1–16.3	1.3–8.3
DE SIR	12	Mean (± σ)	203.3 (± 40.2)	77.9 (± 13.8)
		Min-Max	121.7–259.2	61.2–105.7
CZ SAZ	12	Mean (± σ)	170.3 (± 37.7)	87.5 (± 23.5)
		Min-Max	115.9–220.6	55.7–131.6
DE TET	11	Mean (± σ)	199.8 (± 36.5)	95.2 (± 19.5)
		Min-Max	128.6–250.0	70.6–125.9

n = number of samples tested per variety, ± standard deviation for n samples tested

Table 4A Mean value ± standard deviation (σ), and min/max values in mg/100 g for selected varieties with n > 10 of flavonol glycosides and aglycones

Variety code [15]		K	K-glc	K-rut	Q	Q-glc	Q-rut	Myr-glc
DE HKS	Mean ± σ	<1	13.4 (± 2.5)	8.3 (± 2.5)	<1	46.7 (± 10.2)	11.6 (± 2.8)	2.7 (± 0.7)
	Min-Max		8.1–20.9	3.3–16.0		23.1–80.6	6.8–18.7	1.0–6.1
DE PER	Mean ± σ	<1	11.7 (± 2.6)	7.3 (± 2.9)	<1	34.5 (± 9.0)	10.3 (± 2.7)	1.8 (± 0.5)
	Min-Max		7.0–19.5	1.7–13.7		17.0–61.3	6.1–17.9	1.0–3.5
DE HTR	Mean ± σ	<1	17.8 (± 4.1)	7.9 (± 2.5)	<1	49.0 (± 13.0)	13.5 (± 3.4)	2.1 (± 0.7)
	Min-Max		12.2–29.3	4.0–13.7		30.6–88.7	8.8–23.7	1.0–4.0
DE HMG	Mean ± σ	<1	4.5 (± 1.7)	18.9 (± 5.9)	1.1	17.3 (± 5.4)	29.6 (± 8.2)	<1
	Min-Max		2.3–8.8	5.7–36.7		10.9–32.1	12.1–52.6	
DE HEB	Mean ± σ	<1	27.8 (± 4.4)	16.3 (± 2.7)	<1	74.3 (± 13.6)	23.6 (± 3.7)	1.6 (± 0.3)
	Min-Max		22.7–36.2	12.6–23.0		59.8–98.1	19.3–31.0	1.3–2.5
DE SIR	Mean ± σ	<1	24.2 (± 3.6)	17.9 (± 4.8)	<1	56.1 (± 10.4)	25.3 (± 4.6)	<1
	Min-Max		17.6–29.3	12.3–27.8		36.7–75.5	18.6–34.0	
CZ SAZ	Mean ± σ	<1	19.0 (± 3.7)	13.2 (± 5.2)	<1	53.9 (± 12.5)	21.5 (± 4.8)	1.0
	Min-Max		14.4–26.7	5.4–23.1		36.9–85.2	16.8–34.7	
DE TET	Mean ± σ	<1	18.8 (± 3.6)	12.1 (± 3.8)	<1	55.7 (± 13.6)	21.2 (± 4.2)	<1
	Min-Max		14.4–25.6	5.5–19.0		33.8–81.0	16.1–29.9	

Mean values of various raw hop and pellet samples for the crop 2021, number of samples (n) tested per variety can be taken from table 3, ± standard deviation for n samples tested

Bravo (US BRO) which contains a ratio of 1:1 for the co- and ad-multifidol glucoside.

The multifidol glucosides are known as intermediates in the biosynthesis of bitter acids in hops but no correlation between the alpha-acids amounts and the determined contents of multifidol glucosides could be observed for the varieties presented here. Table 1 gives an overview of the bitter acid content. For example, the US hop cultivar Eureka! (US EUE) has the highest alpha-acid content of the US hop varieties investigated here but, together with the low-alpha US variety Lemondrop (US LDP), the lowest amount of multifidol glucosides.

For all cultivars inside and outside Europe the variety-dependent trends were similar to crop 2021 as compared to the two previous crop years [12].

In addition to mean values of all hop varieties given in figure 5, table 3 presents min and max values as well as mean values together with the standard deviation in mg/100 g for 8 hop varieties (DE HKS, DE PER, DE HTR, DE HMG, DE HEB, DE SIR, CZ SAZ, DE TET) with the highest sample numbers.

The results show large concentration ranges for the different hop varieties for both compounds and demonstrates the importance of a high sample number for variety differentiation. It is essential that mean values for one variety are only reliable and representative enough if at least 10 samples (of big commercial lots) per variety are investigated. If the sample number is between 3 and 9, the data represent a varietal trend and in case of one or two samples only, it illustrates not more than a snapshot that requires further verification by follow-up analyses.

3.2 Flavonol glycosides and aglycones

In addition to the three most relevant representatives of the glycosidically bound polyphenols kaempferol-3-O-glucoside (K-glc), quercetin-3-O-glucoside (Q-glc), and quercetin-3-O-rutinoside (Q-rut), further flavonol glycosides namely kaempferol-3-O-rutinoside (K-rut), myricetin-3-O-glucoside (Myr-glc), hesperetin-7-O-rutinosid (H-rut) as well as the aglycones kaempferol (K), quercetin (Q), and myricetin (Myr) were investigated in the hop samples. Hesperitin-7-O-rutinoside (H-rut) and the aglycone myricetin (Myr) were not detected at all.

Table 4A and 4B summarise the achieved results for flavonol glycosides and the aglycones. Only low concentrations (< 5 mg/100 g) were found for myricetin-3-O-glucoside (Myr-glc) and no differences between European and US varieties were noticed. Very low concentrations were determined for the aglycones kaempferol (K) and quercetin (Q). The highest amounts of the aglycone quercetin (Q) were measured in the US hop varieties Bravo (US BRO) and Lotus (US LOT) with 7.0 and 10.3 mg/100 g. The aglycone kaempferol (K) was only detectable in one German (DE SOL) and two US varieties (US BRO and US LOT).

In addition to mean values, table 4A presents min and max values in mg/100 g for 8 hop varieties (DE HKS, DE PER, DE HTR, DE HMG, DE HEB, DE SIR, CZ SAZ, DE TET) with the highest sample numbers to reveal information about the variability of the results.

For all varieties, the concentration of quercetin glucoside (Q-glc) is higher than the kaempferol glucoside (K-glc) amount. The ratio between these two compounds (Q-glc/K-glc) varies depending on the cultivar and ranges between 1.3 (DE PLA) and 7.2 (US BRO). The most European cultivars are showing a ratio of Q-glc/K-glc be-

Table 4B Mean values of flavonol glycosides and aglycones in hop varieties from Europe, USA, and Australia in mg/100 g for the harvest year 2021

Variety code [15]	n	K	K-glc	K-rut	Q	Q-glc	Q-rut	Myr-glc
DE SSE	9	<1	21.9	14.7	<1	58.6	20.5	<1
SI SSA	7	<1	26.3	12.2	<1	62.8	19.2	1.9
DE HAL	6	<1	19.5	10.5	<1	61.2	17.7	1.3
SI SGC	6	<1	32.9	13.7	<1	84.7	17.5	2.0
DE MBA	5	<1	20.9	9.5	<1	44.9	14.4	1.4
SI SGB	4	<1	22.3	14.5	<1	53.2	20.7	1.2
DE CAL	4	<1	34.0	17.3	<1	61.0	20.6	1.3
DE PLA	4	<1	18.5	15.0	<1	24.6	9.0	3.7
DE AKO	4	<1	22.1	12.0	1.0	75.7	28.5	2.0
DE SOL	3	1.3	35.1	95.8	4.1	86.2	119.7	2.8
PL LUB	3	<1	18.7	14.7	<1	48.6	21.1	1.2
DE NBR	3	<1	10.5	7.0	<1	27.6	11.1	1.7
DE HTU	2	<1	7.2	7.2	<1	32.5	14.4	1.8
DE SPA	2	<1	16.6	13.7	<1	47.6	20.7	<1
DE HBC	1	<1	38.8	30.5	1.6	82.2	33.4	2.1
US CAS	3	<1	16.8	49.0	1.7	34.1	41.4	<1
US SUL	2	<1	10.2	7.2	4.2	64.2	22.2	2.9
US EUE	2	<1	11.1	6.5	2.2	42.4	11.9	1.5
US BRO	2	1.2	12.4	8.8	7.0	89.3	25.2	2.6
US CEN	1	<1	16.0	35.3	1.6	34.3	34.9	3.7
US LOT	1	2.8	8.8	34.2	10.3	34.8	43.7	1.0
US CPO	1	<1	5.1	4.2	5.1	30.1	12.1	<1
US LDP	1	<1	21.0	6.5	<1	34.9	6.0	<1
AU GXY	1	<1	19.4	32.0	2.1	33.8	35.5	1.8

Mean values of various raw hop and pellet samples, n = number of samples tested per variety

tween 2 and 4. The hop variety German Solero (DE SOL) shows the highest concentrations for this compound group within all European hop cultivars presented here. Only for the kaempferol glucoside (K-glc) the variety German Hallertau Blanc (DE HBC) shows the highest amount with 38.8 mg/100 g. The lowest concentration for kaempferol glucoside (K-glc) was detected for German Hallertauer Magnum (DE HMG) with 4.5 mg/100 g.

Besides the highest amount for quercetin glucoside (Q-glc) with 86.2 mg/100 g, Solero (DE SOL) has the highest content for quercetin rutinoside (Q-rut) with 119.7 mg/100 g and for kaempferol rutinoside (K-rut) with 95.8 mg/100 g. All other cultivars show concentrations below 44 mg/100 g for quercetin rutinoside (Q-rut) and below 50 mg/100 g for kaempferol rutinoside (K-rut).

The lowest amount was detected for the US hop variety Lemondrop (US LDP) with only 6 mg/100 g for quercetin rutinoside (Q-rut) and for the US cultivar Calypso (US CPO) with 4.2 mg/100 g for kaempferol rutinoside (K-rut).

Special observation could be done for the hop varieties German Polaris (DE PLA) and the US variety Cascade (US CAS). Only for these varieties the amount of kaempferol rutinoside (K-rut) is higher than the amount of quercetin rutinoside (Q-rut). Varieties with a 1:1 ratio for quercetin and kaempferol rutinoside are US Centennial (US CEN) and Australian GXY (AU GXY). All other cultivars have higher amounts of quercetin rutinoside (Q-rut) in comparison to kaempferol rutinoside (K-rut).

The quantitative data of kaempferol glucoside (K-glc) and kaempferol rutinoside (K-rut) show a higher amount of kaempferol glucoside (K-glc) compared to kaempferol rutinoside (K-rut) with exception of the varieties German Hallertauer Magnum (DE HMG), German Solero (DE SOL), US Cascade (US CAS), US Centennial (US CEN), US Lotus (US LOT) and Australian Galaxy (AU GXY). As described for the kaempferol glycosides, the quercetin glucoside (Q-glc) concentration is higher than the quercetin rutinoside (Q-rut) concentration with exception of the same hop varieties mentioned above.

As reported by Kammhuber in 2012 [9], the composition of quercetin and kaempferol glucosides was described to be suitable to differentiate hop varieties. The additional data for kaempferol and quercetin rutinoside offer a further option for varietal differentiation. But to valid these observations, a higher sample number with at least 10 samples for each hop variety is essential especially if considering the large varietal ranges.

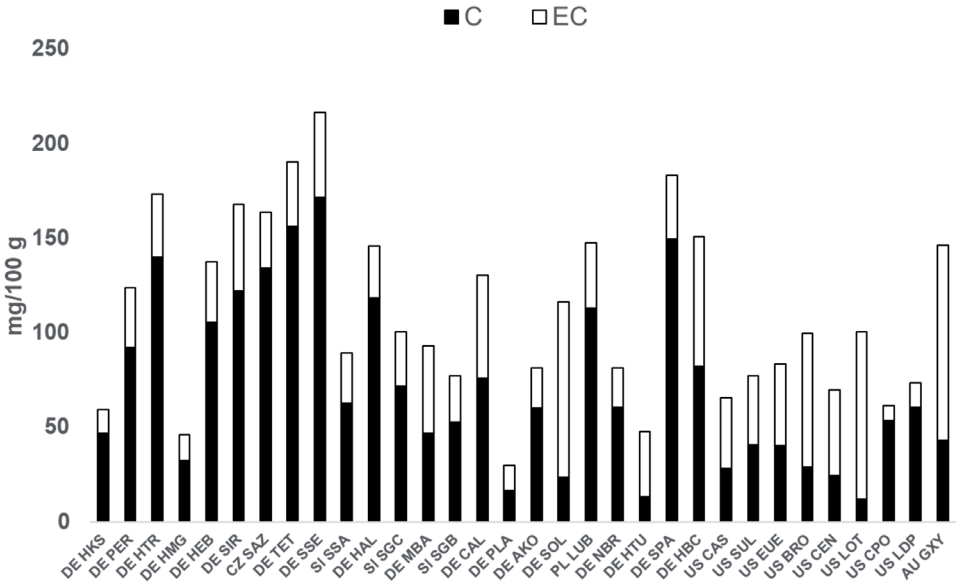


Fig. 6 Amounts of flavan-3-ols catechin (C) and epicatechin (EC) in mg/100 g in different hop varieties from Europe, USA, and Australia for the crop 2021. The numbers of samples can be taken from figure 1

3.3 Flavan-3-ols and procyanidins

For the flavan-3-ols, catechin and epicatechin as the most important representatives of this group were screened using the LC-MS/MS technique. The results are shown as mean values in mg/100 g in figure 6. The highest sum of these two flavan-3-ols was detected for the hop variety German Spalter Select (DE SSE) with 224.4 mg/100 g and the lowest for the hop variety German Polaris (DE PLA) with 29.7 mg/100 g. Catechin is the dominant compound for most hop cultivars. A ratio of catechin and epicatechin of 1:1 was detected for the hop varieties German Mandarina Bavaria (DE MBA), German Hallertau Blanc (DE HBC), and German Polaris (DE PLA) as well as for the US hop cultivars Cascade (US CAS), Sultana (US SUL), and Eureka! (US EUE). For the US varieties Bravo (US BRO), Centennial (US CEN), and Lotus (US LOT) as well as for the Australian hop variety Galaxy (AU GXY) higher concentrations of epicatechin were observed in comparison to catechin. Epicatechin is also the predominant flavan-3-ol for the two European cultivars German Solero (DE SOL) and German Hallertauer Taurus (DE HTU).

Table 6 reveals information about the variability of the results including mean values and standard deviation for the samples investigated in this study. In addition, the concentration ranges with min and max values demonstrate also for the compounds catechin and epicatechin a large fluctuation.

The determined concentrations of procyanidins B1, B2 and B3 from different hop cultivars are given in table 7A and 7B. The min/max values as well as the mean value together with the standard deviation are also presented for the hop varieties with samples number n > 10. It is important to know that procyanidin B1 is consisting of (–)-epicatechin and (+)-catechin units bonded between position 4 and 8' in β-configuration, procyanidin B2 has two molecules of (–)-epicatechin bonded between position 4 and 8' in β-configuration whereas procyanidin B3 has two molecules of (+)-catechin bonded between position 4 and 8' in α-configuration. As the procyanidin B1, B2 and B3 are dimers from catechin and epicatechin, the highest contents for these compounds were also detected for the hop variety German Spalter Select (DE SSE) with a total amount of 177.4 mg/100 g and the lowest for the cultivar German Polaris (DE PLA) with only 30.2 mg/100 g in total.

Besides the fact that procyanidin B3, a dimer with catechin only, is the predominant component in all varieties, it is possible to group tentatively the cultivars tested as follows.

First group consists of hop varieties with the highest amount of procyanidin B3, followed by procyanidin B2 and followed by procyanidin B1, or with comparable content of procyanidin B2 and B1 (B3>B2≥B1). In this group we can find the German varieties Hallertauer Magnum (DE HMG), Hallertauer Taurus (DE HTU), Mandarina Bavaria (DE MBA), Polaris (DE PLA), Hallertau Blanc (DE HBC), and Solero (DE SOL) for the European varieties and all US varieties with exception of Calypso (US CPO), Lemondrop

Table 6 Mean value ± standard deviation (σ), and min/max values of catechin (C) and epicatechin (EC) in mg/100 g for hop varieties with sample numbers n > 10

Variety code [15]	n		Catechin (C)	Epicatechin (EC)
DE HKS	129	Mean (± σ)	46.7 (± 10.2)	12.6 (± 2.1)
		Min-Max	23.8–80.7	7.1–21.1
DE PER	69	Mean (± σ)	92.4 (± 21.1)	31.3 (± 13.9)
		Min-Max	51.9–126.3	19.1–58.5
DE HTR	55	Mean (± σ)	139.9 (± 32.0)	33.5 (± 6.8)
		Min-Max	68.3–210.0	19.3–54.0
DE HMG	32	Mean (± σ)	32.2 (± 6.6)	14.0 (± 3.0)
		Min-Max	15.2–49.3	5.9–22.6
DE HEB	15	Mean (± σ)	105.6 (± 11.9)	31.8 (± 3.8)
		Min-Max	83.0–120.2	26.1–41.9
DE SIR	12	Mean (± σ)	122.1 (± 21.9)	45.9 (± 6.9)
		Min-Max	75.4–155.7	35.4–59.1
CZ SAZ	12	Mean (± σ)	134.1 (± 32.3)	29.4 (± 7.0)
		Min-Max	74.5–188.8	17.4–42.0
DE TET	11	Mean (± σ)	156.4 (± 33.1)	33.8 (± 6.5)
		Min-Max	85.6–194.4	20.1–42.0

n = number of samples tested per variety, ± standard deviation for n samples tested

Table 7A Mean value ± standard deviation (σ), and min/max values of procyanidins in mg/100 g for selected cultivars with samples n > 10

Variety code [15]		PB1	PB2	PB3	ΣPB1-PB3	Group
DE HKS	Mean (± σ)	11.6 (± 2.9)	7.6 (± 1.4)	28.2 (± 5.3)	47.4	2
	Min-Max	5.5–21.2	4.4–11.7	13.1–43.4		
DE PER	Mean (± σ)	17.6 (± 5.3)	13.1 (± 2.9)	65.7 (± 13.2)	96.4	2
	Min-Max	5.2–29.7	6.3–18.9	24.5–92.4		
DE HTR	Mean (± σ)	23.9 (± 6.4)	14.2 (± 3.1)	89.6 (±18.5)	127.7	2
	Min-Max	12.3–43.2	9.0–23.0	47.2–125.4		
DE HMG	Mean (± σ)	5.5 (± 2.2)	8.1 (± 2.0)	26.5 (± 6.1)	40.1	1
	Min-Max	2.9–6.9	5.2–13.9	17.8–41.7		
DE HEB	Mean (± σ)	30.3 (± 4.4)	13.3 (± 1.2)	78.5 (± 5.5)	122.1	2
	Min-Max	23.7–36.6	11.3–14.9	69.7–85.1		
DE SIR	Mean (± σ)	27.1 (± 8.2)	19.3 (± 4.4)	90.8 (± 19.7)	137.2	2
	Min-Max	14.9–44.7	12.1–27.4	51.5–122.5		
CZ SAZ	Mean (± σ)	28.9 (± 7.0)	15.3 (± 2.3)	92.5 (±14.2)	136.7	2
	Min-Max	17.8–42.0	12.5–20.3	73.0–121.6		
DE TET	Mean (± σ)	30.1 (± 7.3)	17.7 (± 3.2)	109.0 (± 19.3)	156.8	2
	Min-Max	20.5–38.6	13.0–22.3	69.4–136.1		

Mean values of various raw hop and pellet samples for the crop 2021, number of samples (n) tested per variety can be taken from table 3, ± standard deviation for n samples tested. Group 1: PB3>PB2≥PB1; Group 2: PB3>PB1>PB2; Group 3: PB3=PB2>PB1

(US LDP), and Lotus (US LOT).

The second group consists of varieties with the highest amount of procyanidin B3, followed by procyanidin B1 and followed by procyanidin B2 (B3>B1>B2). In this group, we can summarize all remaining hop cultivars from the European region and two US hop varieties Calypso (US CPO) and Lemondrop (US LDP).

The last group number 3 with procyanidin B3 and B2 as equal amount followed by procyanidin B1 (B3=B2>B1) contains the hop variety Lotus from USA (US LOT) only.

As already discussed for previous substances, the varietal ranges observed for procyanidins (see Table 7A) show again the need of high sample numbers and make a comparison with literature data, which often represent one sample only, difficult.

3.5 Phenolic acids

Very low concentrations were observed for the phenolic acids. Only hop varieties with amounts ≥ 1 mg/100 g are given in table 8. None of the investigated hop cultivars contains o- and m-coumaric acid. The hop variety German Perle (DE PER) was the only one with concentra-

Table 7B Concentrations of procyanidins in hop varieties from Europe, USA, and Australia in mg/100 for the harvest 2021

Variety code [15]	n	PB1	PB2	PB3	ΣPB1-PB3	Group
DE SSE	9	38.9	16.4	122.1	177.4	2
SI SSA	7	12.5	8.3	41.1	61.9	2
DE HAL	6	25.2	16.7	88.2	130.1	2
SI SGC	6	23.7	12.2	72.7	108.6	2
DE MBA	5	15.3	15.7	54.0	85.0	1
SI SGB	4	13.7	10.6	44.1	68.4	2
DE CAL	4	18.5	17.7	71.5	107.7	2
DE PLA	4	6.5	6.5	17.2	30.2	1
DE AKO	4	11.5	8.7	48.8	69.0	2
DE SOL	3	19.0	52.3	58.1	129.4	1
PL LUB	3	24.5	16.2	74.7	115.4	2
DE NBR	3	11.7	10.5	47.1	69.3	2
DE HTU	2	5.2	16.2	19.3	40.7	1
DE SPA	2	34.8	19.3	107.4	161.5	2
DE HBC	1	20.0	21.4	69.1	110.5	1
US CAS	3	12.8	15.3	37.2	65.3	1
US SUL	2	8.8	19.4	23.5	51.7	1
US EUE	2	9.7	16.2	39.2	65.1	1
US BRO	2	14.1	23.3	39.9	77.3	1
US CEN	1	13.2	18.5	37.6	69.3	1
US LOT	1	12.2	34.1	34.1	80.4	3
US CPO	1	13.9	7.1	39.2	60.2	2
US LDP	1	13.2	4.8	41.4	59.4	2
AU GXY	1	30.2	52.2	84.8	167.2	1

All concentrations are mean values of various raw hop and pellet samples, n = number of samples tested per variety. Group 1: PB3>PB2≥PB1; Group 2: PB3>PB1>PB2; Group 3: PB3=PB2>PB1

Table 8 Concentrations of phenolic acids in hop varieties from Europe, USA, and Australia in mg/100 g for the harvest 2021

Variety code [15]	n	Ferulic acid	p-Hydroxy-benzoic acid	p-Coumar-ic acid
DE HKS	129	<1	<1	1.2
DE PER	69	1.2	1.0	1.1
DE HTR	55	1.0	<1	<1
DE SIR	12	1.0	<1	1.1
DE SSE	9	1.1	<1	1.3
SI SGB	4	<1	1.1	1.3
DE CAL	4	<1	<1	1.1
DE PLA	4	1.7	<1	2.1
DE SOL	3	1.3	<1	1.2
PL LUB	3	<1	<1	1.1
DE NBR	3	1.4	<1	1.3
DE HTU	2	<1	<1	1.2
DE HBC	1	<1	1.3	<1
US SUL	2	<1	<1	1.0
US CEN	1	<1	<1	1.2
US CPO	1	<1	<1	1.3
AU GXY	1	1.2	<1	<1

All concentrations are mean values of various raw hop and pellet samples (n = number of samples tested per variety)

tions ≥ 1 mg/100 g for ferulic acid, p-hydroxybenzoic acid, and p-coumaric acid. The variety German Polaris (DE PLA) has the highest amount for ferulic acid and p-coumaric acid.

But even this amount is lower than described in literature with 0.01 % for ferulic acid [1]. No differences between the European hop cultivars and cultivars from USA and Australia could be observed.

4 Conclusion

LC-MS/MS technique offers a sophisticated quantitative analysis of a broad range of various phenolic compounds in hops. It enables the characterization of hop varieties from different growing regions worldwide regarding their phenolic profile. But for reliable variety specific values, many samples of each hop cultivar and observations during several crop years are required. The quantitative findings are useful for the determination of their transfer from hops to beer, and, combined with taste thresholds, for evaluation of their contribution to overall beer taste.

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