Influence of Hop Polyphenols on Beer Flavor

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SUMMARY

There exists a substantial lack of information about the following properties of hop polyphenols:

- Quantity and composition of polyphenols besides the low-molecular components analysed by HPLC.
- Contribution of polyphenols to the formation of beer haze.
- Contribution of polyphenols to flavor stability of beer.

In this paper, hop polyphenols refers to the sum of all low-molecular components determined by HPLC.

The dosage of hop polyphenols is linked with interesting sensory effects. Beer flavor will be changed and, most of the time, improved. Hops for this purpose have to be carefully selected, treated, and dosed. Aroma varieties from temperate production zones are suitable. A high grade of freshness is very important, which can be achieved by using gentle procedures during harvesting, pellet production, and storage. Short boiling times for such aroma pellets (between 10 and 30 minutes) are preferred.

The food industry has a positive impression of polyphenols, a tendency that seems to be particularly justified for hops in beer. In most beers worldwide, the hop polyphenolic character is reduced or eliminated, thus ignoring a favourable trend. The high reputation of wine is also linked to "healthy polyphenols." It is a pity that the public image of beer is lower than that of wine. Apart from polyphenols, iso-α-acids and isoxanthohumol also show promising health benefits; for instance, they may reduce metabolic disorders. The trend toward lower bitterness in beer stands in contradiction to the potential health benefits of using more hops.

INTRODUCTION

Polyphenols in general are secondary metabolites of many plants. For the last 30 years, there has been a lot of research on the composition and effect of polyphenols. In vitro tests showed anti-oxidative properties of several polyphenols, and furthermore, some also exhibit anti-carcinogenic, estrogenic, and anti-inflammatory effects. Interpretation of these results in the media lead to statements that some polyphenols could provide a positive effect on human health. Examples are soy, wine, tea, and coffee. Polyphenolic extracts are added to some food to create food supplements or functional food.

While research, publications, and articles on positive plant polyphenols increased greatly in the last 30 years, the dosage of hop polyphenols in beer decreased significantly. Regarding the existing positive and promising reputation of polyphenols, there should be one question: are hop polyphenols really unimportant? This paper will deal with the positive influence of hop polyphenols on beer flavor and shall encourage making use of these substances.

HOP POLYPHENOLS IN GENERAL

Analysis of hop polyphenols

There exist two types of methods to analyse the polyphenols in hops. The first one is a non-selective, non-specific colouring method, using a test solution for calibration. Results show values between 2 and 7% (w/w). HPLC is the alternative to this method and provides the separation, identification, and calibration of more than 100 individual low-molecular components. Values vary between 0.5 and 3% (w/w) as a sum of all HPLC components. Besides the problem that the values of total polyphenols are doubtful, it is unclear what represents the difference between total polyphenols and HPLC polyphenols. Quantity, structure, and activity of about two-thirds of hop polyphenols are at least a black box. Regarding the uncertainty of the existence of "total polyphenols," the author defines hop polyphenols in this paper as a sum of all low-molecular components determined by HPLC.

Composition of hop polyphenols

Only a limited overlook of the polyphenol composition of hops is cited (Forster et al., 2002/2003). Table 1 shows the most important groups of polyphenols and the typical variation of contents in different hop varieties. Besides the prenyl flavonoids, all components are detectable in the cone leaves and are soluble in water, preferably in hot water.

Xanthohumol is the most important representative of the prenyl flavonoids and is a unique polyphenol which is only found in hops. It can be detected in the lupulin glands together with the hop resins and oils. The biogenesis seems to be linked more to these components than to all other polyphenols. Xanthohumol is soluble in solvents like ethanol, but it is not soluble in water. It is considered to be the polyphenol with the greatest potential for human health benefits. In vitro tests show clear anti-inflammatory, anti-oxidative, and anti-carcinogenic effects. In vivo research is considered to be very promising.

Table 2 gives an example for the influence of a crop year on the polyphenol content of

Table 1. Polyphenol groups in hops

Substance	Contents (mg/100 g)
Hydroxy benzoic acids	1 – 10
Hydroxy cinnamic acids	100 – 450
Proanthocyanidins	100 – 600
Flavanols	30 – 200
Flavonols	1 – 10
Quercetin flavonoids	50 – 250
Camphorol flavonoids	50 – 300
Prenyl flavonoids	1,000 – 10,000

Table 2. Comparison of the polyphenols in two crops (Hallertau) (% [w/w])

	Saazer	Hallertauer	Magnum
1997	2.0	1.5	0.7
1998	1.6	1.2	0.5

three varieties. In general, the deviation of polyphenols within different crop years is less pronounced in comparison to the α-acids.

The variety has an important influence on the amount and composition of polyphenols. As an example, the confidence intervals of Saazer, Hallertauer, and Magnum in the form of five groups of polyphenols are listed in Table 3. If the confidence interval, a statistical calculation, of a sample block (for example, variety A) does not overlap with the confidence interval of another sample block (for example, variety B), it is proven that a difference of quantities exists. Magnum shows significant lower contents of all polyphenols. Saazer and Hallertauer differ only in flavonoids and proanthocyanidins. There are also statistically proven differences of individual components in different varieties, such as the three examples in Table 4.

In general, the content of HPLC polyphenols varies across different classes of hops; for example, high α-hops (0.4–1.0% [w/w]), bitter hops (0.6–1.2 [w/w]), new types of aroma hops (0.8–1.4% [w/w]), and classical aroma hops (1.2–3.0% [w/w]).

The growing area also influences the composition of polyphenols (Forster et al., 2002). Two commercially grown varieties, Perle and Nugget, are compared in USA/Yakima and Germany/Hallertau. The mean values of all HPLC polyphenols of five lots in three crop years indicate higher levels in the Hallertau (Table 5). The three groups of hydroxy cinnamic acids, flavanols, and proanthocyanidins differ significantly (Table 6). Statistical differences of individual components could be confirmed 12 times by Perle and 14 times by Nugget.

While polyphenol contents in hop varieties normally correlate with α-acid content, xanthohumol behaves differently. Table 7 shows typical xanthohumol values in some hop

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Table 3. Confidence intervals of three varieties in crop year 1997 (mg/100 g as is)

	Saazer	Hallertauer	Magnum
Hydroxy cinnamic acids	184 – 264[a]	155 – 208[a]	65 – 72[b]
Flavonols	159 – 224[b]	83 – 116[b]	29 – 35[b]
Proanthycyanidins	505 – 691[b]	275 – 385[b]	100 – 116[b]
Quercetin flavonoids	202 – 249[a]	162 – 251[a]	64 – 73[b]
Camphorol flavonoids	264 – 343[a]	218 – 309[a]	66 – 76[b]

[a] Overlapping.
[b] Differentiation.

Table 4. Confidence intervals of three varieties in crop year 1998 (mg/100 g as is)

	Saazer	Hallertauer	Taurus
Procyanidine B1	90 – 131	41 – 72	7 – 16
Catechine	139 – 178	68 – 92	11 – 15
Camphorol malonyl hexocide	161 – 199	104 – 174	29 – 37

Table 5. Influence of growing area on polyphenols (% [w/w], average of 3 crop years)

	USA	Hallertau
Perle	0.81	0.97
Nugget	0.55	0.74

Table 6. Mean values of polyphenol subgroups (mg/100 g as is, average of 3 crop years)

	Perle		Nugget	
	USA	Hallertau	USA	Hallertau
Hydroxy cinnamic acids	242	304	139	213
Flavanols	85	112	78	103
Proanthocyanidins	142	197	107	163
Quercetin flavonoids	105	104	104	123
Camphorol flavonoids	101	87	49	56

Table 7. Xanthohumol in hops (% [w/w])

Saaz (CZ)	0.25
Hallertauer (HAL)	0.33
Perle (HAL)	0.60
Perle (USA)	0.45
Nugget (HAL)	0.69
Nugget (USA)	0.56
Magnum (HAL)	0.65
Taurus (HAL)	1.00

Table 8. Ratio of α-acids to polyphenols and xanthohumol

	α/polyphenols	α/xanthohumol
Saaz (CZ)	1	10
Hallertauer (HAL)	2	12
Perle (HAL)	4	10
Perle (USA)	6	14
Nugget (HAL)	7	14
Nugget (USA)	10	18
Magnum (HAL)	10	20
Taurus (HAL)	10	15

varieties (Forster et al., 1999). Here, Taurus has the highest level. The ratios of α-acids to polyphenols and xanthohumol are listed in Table 8. While the α-acid/polyphenol ratio varies in a wide range from 1 to 10, the ration for α-acid/xanthohumol varies only from 10 to 20.

Hop polyphenols in beer

From the EBC manual *Hops and Hop Products*, the following statements on polyphenols exist:

> "It is generally agreed that the role of hop polyphenols with respect to beer taste, stability, and health-protective effects on consumers is of prime importance. The current state of knowledge, however, is very limited, unlike for other drinks, e.g., wine and tea. The well-known health-protective properties of tea and wine have repeatedly been shown to be caused by characteristic polyphenols. Studies on these and other drinks are ongoing.
>
> Although hops are reputed as a rich source of polyphenols, the potential for different applications has not been unlocked yet. In fact, the only efforts reported in the literature were focussed on the influence of flavanols and proanthocyanidins on the colloidal stability of beer in order to

prevent haze formation. Positive contributions of hop polyphenols have not been investigated in any depth. From a technological viewpoint, the role of polyphenolic anti-oxidants in stabilising the beer taste on storage has not been evaluated." (EBC-Manual, 1997).

This statement of 1997 represents more or less the common knowledge of today, aside from the intensive research on xanthohumol.

Hop polyphenols and beer haze

Polyphenols are recognized to provoke beer haze, but up to now no polyphenols have been found in beer haze apart of xanthohumol (Loch-Ahring and Almeida, 2005). Two observations are doubtless: First, a higher dosage of pellets increases haze formation in comparison to CO_2 extracts. Second, PVPP treatment reduces haze formation. PVPP adsorbs polyphenols in different ratios, which depend on the individual beer matrix and the dosed quantity of PVPP. As an example, the following figures for a relative adsorption rate are given:

- Flavanols, proanthocyanidnis: 60%
- Xanthohumol: 50%
- Flavonoids, hydroxy cinnamic acids: 25%

But only in the case of xanthohumol did a test confirm that the addition to beer correlated to a higher tendency of haze formation (Forster et al., 2002). Other information is still missing. In particular, little is known about the role of polyphenols with a molecular size greater than what is typical for standard HPLC analyses (that is, with a greater degree of polymerization than dimers or trimers of flavanols).

Hop polyphenols as anti-oxidants

There are many tests to prove anti-oxidative effects of biological materials. Through selection of a suitable test, one can gauge the influence on the result as the following example shows (Forster et al., 2001). Two different types of tests were carried out to prove the anti-oxidative potential of hop substances and two contradictory results were obtained.

- The Rancimat-test, similar to the Swift-test, showed an anti-oxidative potential for a hop solution with water as a solvent (polyphenols), but simulated a prooxidative potential for less polar extracts of bitter-acid character.
- A Fluoro-Scan-test, also called a Trolox-test, demonstrated a strong anti-oxidative potential for α-acids and extracts of bitter-acid character, but not for iso-α-acids and polyphenolic hop extracts.

Up to now there is no clear evidence if and how hop polyphenols can improve flavor stability of beers. Aeration of beers didn't show chemical changes of polyphenols. Nothing has been reported on electrochemical phenomena or on formation of complexes.

To summarize this chapter, the following statements could be given:

- There exists reasonable information on HPLC polyphenols in hops and hop varieties.
- Quantity and composition of the total polyphenols are unknown.
- What represents the fraction of total polyphenols (by spectrophotometry) minus HPLC polyphenols?
- Chemical structure and activity of approximately 50–70% of hop polyphenols are unknown.
- Which polyphenols, if any, contribute to the formation of beer haze? How?
- Can hop polyphenols improve the flavor stability of beer? How?

Table 9. Sensory scores of beers brewed with bitter hops

	Fresh beers			
	Pellets		Extracts	
	Beer quality	Bitterness quality	Beer quality	Bitterness quality
Fresh	4.1	3.8	3.9	3.7
Aged	3.3	2.9	3.7	3.3
	Aged beers			
	Beer quality	Bitterness quality	Beer quality	Bitterness quality
Fresh	3.7	3.4	3.4	3.3
Aged	2.7	2.2	3.0	2.8

Table 10. Sensory scores of beers brewed with aroma hops

	Fresh beers			
	Pellets		Extracts	
	Beer quality	Bitterness quality	Beer quality	Bitterness quality
Fresh	4.3	4.0	3.9	3.8
Aged	3.3	3.0	3.5	3.2
	Aged beers			
	Beer quality	Bitterness quality	Beer quality	Bitterness quality
Fresh	3.9	3.7	3.4	3.3
Aged	2.9	2.6	3.0	2.9

EVIDENCE FROM BREWING TRIALS

Brewing trial #1

Using a 200-L pilot brewery, simple tests where carried out by dosing pellets (with polyphenols) and CO_2 extracts (polyphenol-free). The goal was to achieve a bitter level of 20 IBU, so hop products were added at the beginning of the wort boiling. Three bitter and four aroma varieties were selected for the test program. Beer tasting took place in a panel (n = 16) based on school marks with 1 (insufficient) to 5 (very good). The beers were aged for 6 months at 22°C.

From each variety, four products resulted:

1. fresh pellets (hop storage index [HSI] = 0.22) – fresh
2. (from 1) aged pellets (HSI = 0.55) – aged
3. (from 1) CO_2 – extract from fresh pellets
4. (from 2) CO_2 – extract from aged pellets

In Tables 9 and 10, the results of the sensory evaluations are listed, divided in quality of beers and quality of bitterness.

The results can be summarised as follows:

- Pellet beers with aroma hops were slightly better than those with bitter hops.
- Fresh pellet beers were slightly better than fresh extract beers.

Table 11. Difference and preference tests on fresh beers, first run

	BB[a] vs. 600/90[b]	BB vs. 300/30[c]	BB vs. 600/30[d]
No. of tasters	12	11	14
No. correct	12	10	14
Preference for BB	4	2	2
Preference for treatment	8	8	12

[a] Comparison beer without hop polyphenols.
[b] 600 g/hL HPF hopped (90 min boiled).
[c] 300 g/hL HPF hopped (30 min boiled).
[d] 600 g/hL HPF hopped (30 min boiled).

- Aging of pellets decreased beer quality drastically, while CO2 extract of aged pellets didn't reduce beer quality at the same rate.
- Trends in aged beers were maintained.
- Flavor stability in fresh pellet beers was slightly better than in extract beers.

The results about the aging character of pellets are obvious. Pellets have to be fresh; only then do beers have a tendency to be better than extract-beers and provide a better flavor stability.

Brewing trial #2

The following brewing trials were carried out in a 120-L pilot brewery (Forster et al., 1995). Hop polyphenols in the form of a bracteole fraction, as produced by the mechanical concentration of hop powder type 45, were added to the brew hopped with pure resin extract free of polyphenols. The comparison or neutral brew did not contain any of this hop polyphenol fraction (HPF). In the first run the dosage, except in the case of the comparison brew, was 300 and 600 g of an HPF from Saaz hops per hectolitre, not only at the beginning of the boil (90 min boiling time), but also later (30 min boiling time). In the second run, amounts between 50 and 300 g/hL HPF were used, besides the comparison brew. The factors of hop variety and boiling time of the HPF in the wort were varied. The features of foam, colour, physical stability, polyphenol values, and particularly taste were observed.

The results of the first run can be summarized as follows:

- Colour and foam were not influenced by the dosage of an HPF.
- The physical stability suffered, especially when the HPF was boiled for a longer time.
- The beers were tested in a triangle test with the comparison beer and with 11–14 tasters.

In the first run, four beers were available:

- Comparison beer without hop polyphenols = BB
- 600 g/hL HPF hopped (90 min boiled) = 600/90
- 300 g/hL HPF hopped (30 min boiled) = 300/30
- 600 g/hL HPF hopped (30 min boiled) = 600/30

There were significant findings with regard to differentiation and preference (Table 11), by which the beers containing spent hops surprised the tasters with their pleasant, hoppy, slightly fruity aroma and taste. It is still not clear whether these impressions of taste can also be linked to the observations on glycosidic compounds in hops (Goldstein et al., 1999). Only the 600/90 beer had a somewhat broader bitterness.

The results were unchanged when the beer-tasting was repeated with the same samples. In the second run, the same tasters again preferred the neutral beer or the beers with HPF. According to this finding, definite sensory effects, which the majority of tasters judged positively, can be attained with the addition of an HPF.

Table 12. Polyphenols in five beers each in three test brews[a]

	Polyphenol Values (average of 3 varieties)				
Dosage of HPF (g/hL)	0	50	100	150	300
Dosage of polyphenols with HPF (mg/L)	-	10	20	30	60
Polyphenol content in beer (mg/L)	35	43	49	55	72
Polyphenol yield in beer (% rel. of dosed hop polyphenols)		80	65	67	53

[a] Second run. Base beer; 50/100/150/300 g of hop polyphenol fraction (HPF), boiling time 30 min; three hop varieties (Saazer, Hallertauer, Northern Brewer).

Table 13. Normal xanthohumol yields[a]

	Wort	Young Beer	Beer
Xanthohumol (mg/L)	0.4	0.2	0.04
Iso-xanthohumol (mg/L)	1.5	1.3	0.73
Total (mg/L)	1.9	1.5	0.77
Percentage of dosage	38	30	15

[a] Dosage 5 mg/L (which is 100–150 g of pellets per hectolitre).

This basic judgement did not change after four weeks of storage at 27°C. The beers were aged badly over 15 months at 22°C. The beer-tasting again produced clear results. The comparison beer without HPF was totally aged and undrinkable. This one was followed by the beer with 600 g of HPF, hopped at the beginning of the boil (600/90). The beers with 300 and 600 g of HPF and boiled for only 30 min had aged but were still drinkable. However, it must be considered that the freshly bottled beer from the pilot brewery had a total oxygen content of about 0.8 to 1.0 mg/L.

The beer-tasting results of the second run, with 50 up to 300 g/hL HPF, can be summarized as follows:

- The addition of an HPF can be seen with 50–100 g/hL and one-star significance, with 150 g/hL and two-star significance, and from 300 g/hL upwards with three-star significance.
- The significance of the classification decreased with a longer boiling time and with bitter hops.
- Having tasted the beer several times, a minority of tasters preferred the base beer, but a majority preferred the beers with HPF.
- This preference was clearer when the boiling times were short and when aroma hops were added.

The beers with HPF were designated "typically hoppy," "fruity," or "grassy." A so-called tannin bitterness was only found in beers from 300 g/hL HPF upwards that had been combined with a long boiling time. From this, the following conclusions for the dosage of hop polyphenols can be drawn:

- There was no negative influence on foam and colour of the beers.
- No harsh bitterness occurred with shorter boiling times, while the opposite tends to be more likely.
- Definite tastes were achieved.
- The stability of flavor improved.
- The tendency for beer to become turbid increased following longer boiling times.

Table 14. Development of hop dosage (lager beer)

	1960–1970	2005
Bitterness (IBU)	20–25	18
Isomerisation ratio (% rel.)	20–25	30
α-Acids in hops (% [w/w])	4	15
Polyphenols in hops (% [w/w])	2	1
Dosage of α-acids (mg/L)	100	60
Dosage of polyphenols (mg/L)	50	4[a]

[a] Value is 0 when extracts or isomerized products are used.

Table 12 shows the yield of polyphenols, dosed via the polyphenol fraction. It varied between 53 and 80%.

As far as the prenyl flavonoids are concerned (main representative: xanthohumol), the ratios were different (Table 13). In this trial, 5.0 mg of xanthohumol were added per litre of wort with a boiling time of 35 min. Xanthohumol converted mainly into isoxanthohumol. The high losses can be explained by a poor solubility and adsorption. Even with long boiling times and reduced discharge due to turbidity and filtration, only about 10–20% yield in the form of isoxanthohumol was observed in relation to the xanthohumol used.

CONCLUSION

Changes in hop dosage since 1960

Up to 1960, hopping was quite uniform. Hops had an α-acid content between 3 and 5% and two to four hop dosages took place during wort boiling in the form of leaf hops. In the last 45 years, the following changes took place:

- Hop varieties are now bred with up to 16% α-acids.
- Only one or two hop additions are normal.
- Wort boiling became much more efficient and improved the isomerisation rate.
- Hop products like different types of extracts and pellets replaced leaf hops.
- In most of the beers, the bitterness was reduced by about one-third.

As a consequence the dosage of α-acids was reduced from approximately 8 to 9 mg/L of beer to 5 mg/L.

Table 14 explains these tendencies on the example of a German lager beer. When hop dosage was reduced from 100 to 60 mg of α-acids per litre, the resulting bitterness dropped from 20–25 to 18 IBU. As a consequence of the use of high α-hops, the dosage of polyphenols dropped from 50 down to 4 mg/L. In many beers, a hop polyphenolic character is lost, in most cases more or less unintentionally.

As indicated at the beginning of this paper, the trend toward beers without clear hop polyphenolic character doesn't reflect newer tendencies for food with favourable polyphenols. The author believes there should be chances for special beers with hop polyphenolic character. The following example stands for several possible proposals to create a speciality:

- At least 100 g or more of fresh aroma pellets per hectolitre should be dosed in the middle of wort boiling.
- This represents 50 mg of α-acids and 20 mg of polyphenols per litre.

Fresh hops or pellets should preferably be used instead of aged ones, in order to maintain the low-molecular character of the polyphenols. The boiling time for the respective hopping should be limited. Pure resin extract can be added at the beginning of the boil, with pellets added only after the boiling time is halfway finished. In order to keep the doses of polyphenols constant over the years, the annual fluctuations of the α-acids can be balanced by

means of a flexible mechanical concentration (variable type). Therefore, the α-acid-to-polyphenol ratio remains constant. A lupulin concentration also makes sense, because the hop spindle, with a relatively low polyphenol content, is discharged and therefore the result is a more favourable ratio of polyphenols to nitrate in the pellet.

The term freshness of aroma pellets includes a clear quality chain from harvest onwards via drying, conditioning, storage of baled hops, and gentle pellet production with respect to air, temperature, packaging, and storage (Forster, 2003).

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