Polyphenols and Beer Quality

Abstract
Beer phenols issued from malt and hop can contribute directly to several characteristics of beer, mainly flavor, astringency, haze, body, and fullness. Some phenolic structures can also impart very interesting health properties. Yet phenolic structures also evolve through storage. Low-molecular-weight phenols like 4-vinylsyringol can impart off-flavors in aged beer, while oxidized flavonoids strongly influence astringency, haze, and color. The instability of stilbenes, prenylchalcones, and derived flavanones could also modify their health potential.

Keywords
Aging, beer, brewing process, colloidal stability, flavor, polyphenols

Abbreviations
- AEDA Aroma extract dilution analysis
- APCI Atmospheric-pressure chemical ionization
- C Catechin
- E Epicatechin
- EGC and GC Epigallocatechin and gallate
- ESI Electrospray ionization
- FD Dilution factor
- HPLC or LC High-performance liquid chromatography
- MS Mass spectroscopy
- MS/MS Tandem mass spectroscopy
- NP Normal phase
- P1 to P10 Procyanidins from monomers to decamers
- PVP Polyvinylpyrrolidone
- PVPP Polyvinylpyrrolidone
- RP Reversed phase
- UV Ultraviolet

Introduction
Beer phenols issued from malt and hop can contribute directly to several characteristics of beer, mainly color, flavor, astringency, and haze. As antioxidants, they can also protect raw materials from oxidative degradation throughout the process, minimizing therefore off-flavors such as trans-2-nonenal.

In this chapter, all phenolic structures that have been found in beer will be described.

Catechins and Proanthocyanidins
Even compared to grapes, hop emerges as an exceptional source of catechins and proanthocyanidins. Therefore, although added in 100 times lesser quantity than malt, it can account for 30% of total beer polyphenols. Among hop cultivars, the lower the bitterness, the higher the flavonoid level (up to 1% "total flavanoids" in Saaz pellets, as expressed in terminal unit weight [1]). During mashing, malt flavonoids are progressively dissolved in the wort (monomers dissolve much faster than oligomers). From mash filtration to boiling, a great proportion of them will be lost through oxidation, adsorption to spent grains, linkage to coagulated proteins, etc. According to the type of hop conditioning used (CO2 extracts are much poorer in polyphenols than pellets and cones) and the stage of addition, more or less flavonoids will be brought into the wort in the boiling kettle [2].

In dried hop cones or pellets, (+)-catechin and (−)-epicatechin monomers can reach up to 7 mg.L−1, but (−)-epicatechin (0.8–1.9 mg.L−1) and gallocatechin gallate, (−)-epicatechin gallate, and two glycosides have also been detected (Table 1) [8, 11–21].

Table 1 Flavan-3-ol monomers, polymers, and range of concentrations in beer [8, 11, 13, 14, 16, 19–21, 31, 62, 70, 124–126]
Structures

Monomers

- (+)-Catechin
- (-)-Epicatechin
- (-)-Catechin gallate
- (-)-Epicatechin gallate
- 3-O-methylcatechin
- Catechin-7-O-β-d-glucopyranoside
- Catechin-7-O-β (6'-O-nicotinoyl)-β-d-glucopyranoside

Dimers

Procyanidins B

- B1(-)-Epicatechin-(4β-8)-(+) catechin
- B3(+) - Catechin-H (4α-8)-(+) catechin
- B4(+) - Catechin-H (4α-8)-(−) epicatechin

Prodelphinidins B

- B3(-)-Gallocatechin-(4α-8)-(+) catechin
- B9(-)-Epigallocatechin-(4β-8)-(+) catechin

Prodelphinidins A

- ent-(-)-Epigallocatechin-(4α-6, 2α-O-7)-(+) catechin
- ent-(−)-Epigallocatechin-(4α-6, 2α-O-7)-(+) catechin

Compounds

<table>
<thead>
<tr>
<th>Compounds</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>R4</th>
<th>in beer (mg.L$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(+)-Catechin</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>0.28–6.9</td>
</tr>
<tr>
<td>(-)-Epicatechin</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>&lt;0.10–1.9</td>
<td></td>
</tr>
<tr>
<td>(-)-Catechin gallate</td>
<td>H</td>
<td>Gallate</td>
<td>H</td>
<td>OH</td>
<td>5–20</td>
</tr>
<tr>
<td>(-)-Epicatechin gallate</td>
<td>Gallate</td>
<td>H</td>
<td>OH</td>
<td>5–20</td>
<td></td>
</tr>
<tr>
<td>3-O-methylcatechin</td>
<td>H</td>
<td>OCH$_3$</td>
<td>H</td>
<td>Detected</td>
<td></td>
</tr>
<tr>
<td>Catechin-7-O-β-d-glucopyranoside</td>
<td>OH</td>
<td>OH</td>
<td>Detected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Catechin-7-O-β (6'-O-nicotinoyl)-β-d-glucopyranoside</td>
<td>OH</td>
<td>OH</td>
<td>Detected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B1(-)-Epicatechin-(4β-8)-(+) catechin</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>Detected</td>
<td></td>
</tr>
<tr>
<td>B3(+) - Catechin-H (4α-8)-(+) catechin</td>
<td>OH</td>
<td>OH</td>
<td>Traces–3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B4(+) - Catechin-H (4α-8)-(−) epicatechin</td>
<td>OH</td>
<td>OH</td>
<td>Detected</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B3(-)-Gallocatechin-(4α-8)-(+) catechin</td>
<td>H</td>
<td>OH</td>
<td>H</td>
<td>OH</td>
<td>Traces–3.3</td>
</tr>
<tr>
<td>B9(-)-Epigallocatechin-(4β-8)-(+) catechin</td>
<td>OH</td>
<td>H</td>
<td>H</td>
<td>Detected</td>
<td></td>
</tr>
</tbody>
</table>

Detected compounds:
- (-)-Epigallocatechin-(4α-8, 2α-O-7)-(+) catechin
- (-)-Epigallocatechin-(4α-6, 2α-O-7)-(+) catechin
- (-)-Epigallocatechin-(4α-8, 2α-O-7)-(+) catechin
- (-)-Epigallocatechin-(4α-6, 2α-O-7)-(+) catechin
Hop is also an excellent source of flavonoid oligomers (proanthocyanidins, known as anthocyanogens in the brewing field). For instance, B3 and B4 procyanidin dimers have been detected at levels up to 0.1% \([3, 5–7]\). Malt contains two B3 dimers (prodelphinidin and procyanidin) at lower levels than in hop, but with higher amounts of gallocatechin units \([3, 5, 10, 22]\). Many trimers have also been detected in malt (catechin and gallocatechin units, but always a catechin unit at the terminal position). Thiolysis hyphenated to RP-HPLC-ESI(-)-MS/MS was recently optimized by our group to investigate beer polyphenolic oligomers \([17]\). Thiolysis indicated that most beer dimers are procyanidins B3 (two catechin units), while most trimers are prodelphinidins (catechin in terminal units and gallocatechins or catechins in extension units). Despite the absence of chromatographic peaks corresponding to oligomers above trimers, an apparent degree of polymerization (mDP) close to 6 was calculated in a total LH20 extract. Detailed structures were determined by RP-HPLC-ESI(-)-MS/MS \([16]\). Four dimers were identified: three procyanidins (B1, B3, and B4) and one prodelphinidin (B3) (Table 1). Previously detected in hop or malt, three trimers (the procyanidin C-4∝-8-C-4∝-8-C and two prodelphinidins, GC-4∝-8-C-4∝-8-C and GC-4∝-8-GC-4∝-8-C), were distinguished for the first time in beer. As expected, according to previous thioacidolysis data, most beer proanthocyanidins carry a catechin as terminal unit.

Colloidal Instability

Colloidal instability due to interactions between polyphenols and proteins limits the shelf life of beer. A lag phase is usually observed in lager beers before chill-haze development \([23–25]\). The time needed to form critical amounts of tanning polyphenols leading to visible chill-haze particles corresponds to the lag phase. As described by Leemans et al. \([25]\) for different batches, the longer the lag phase, the better the colloidal stability. Chill haze (or reversible haze), defined by non-covalent bonds between polyphenols and active proteins, can eventually turn into permanent haze that no longer dissolves as the beer warms. Catechin does not rapidly induce strong haze. Upon storage, however, it does. Likewise, colloidal instability caused by dimers and trimers is enhanced after oxidation (not true for tetramers and pentamers) \([26–28]\). Free radicals are known to enhance haze \([29]\). Tannoids have been defined by Chapon \([30]\) as intermediates in the oxidation of simple flavanoids to tannins, forming complexes with proteins. On the other hand, according to O’Rourke et al. \([28]\), oxidized flavanols cause chill haze, but only subsequent polymerization leads to tannoids and permanent haze \([28, 31]\).
Leemans et al. [25] have proposed a model in which aldehydes and oxygen play key roles in tanning polyphenol formation [25, 31]. Not only dissolved oxygen but also shaking, higher temperature, polyphenol-rich raw materials, light, and heavy metals will significantly increase colloidal instability [25, 31].

Beer contains less haze-active polyphenols than haze-active proteins. Derived from barley hordeins, haze-active proteins (10–30 kDa) are acidic hydrophilic polypeptides, rich in both proline and glutamic acid [26] and glycosylated [32]. Much more haze is produced near pH 4.0 than at pH 3.0 or above pH 4.2. At the beer pH, ethanol at low concentration causes a modest decline of haze, while strong haze is observed at higher concentrations [33].

To preserve beer colloidal stability, brewers usually remove haze-active materials [34]. To get rid of haze-active proteins, precipitation with tannic acid, hydrolysis with papain and adsorption to bentonite [35] or silica gel [36, 37] are very effective, but unfortunately in some cases, such procedures also remove foam proteins. To remove haze-active polyphenols, the most usual way is adsorption to polyvinylpolypyrrolidone-PVPP. Because of the structural analogy between these compounds and proline [38], pyrrolidone rings bind polymerized flavanoids through hydrogen and ionic bonds.

New combined absorbents are now proposed to brewers, such as PVPP mixed with silica xerogel, PVP bound onto silica, and tannin linked to silica [23, 39]. Another innovative way is the use of flavan-3-ol and proanthocyanidin-free malt which allows affording an excellent colloidal stability [40].

**Astringency**

In beer, flavanoids could be also responsible for astringency [41, 42]. Catechin and epicatechin thresholds lie between 1 and 20 ppm [41, 42], with higher values for the beer matrix (20 ppm). Astringency is intensified at low pH, especially near 4.0–4.2 [43], but a higher astringency has been measured by François et al. [44] in beers with a pH close to 5. In this case, it was suspected that the pH of the samples fell in the mouth before polyphenol/protein interactions occurred. Sensory analyses applied to top-fermented beers have shown that storage (20°C or 40°C with air in the headspace) decreases bitterness and post-bitterness but intensifies astringency [45]. On the other hand, no significant astringency-related deterioration was measured in lager beers aged for 5 days at 40°C (with or without oxygen) [44]. In both cases, an increase in DP (global assay) and a decrease in total flavanoids were mentioned, especially at higher temperature or pH, in the presence of air [44, 45].

**Color**

Beer color increases through storage, especially in the presence of oxygen and at higher temperature. At pH 3, colorless catechin-derived products are formed after enzymatic oxidation, whereas at pH 6 after chemical degradation, yellow products including dehydrodicatechin A dimers differing by their interflavan linkages can be detected in model media (Fig. 1) [46, 47]. Recently, Callemien and Collin [15] detected dehydrodicatechin A in a beer spiked with catechin after storage.

**Health Properties**

Flavan-3-ols induce cardioprotective effects, including antioxidant effects (protection against...
LDL oxidation) and inhibition of platelet activity and vasodilatation [48, 49]. Flavonoids might reduce the risk of cancer, although some procarcinogenic activities have also been reported [48, 50]. Flavonoids alter the synthesis of eicosanoids (mediators of inflammation). They decrease the leukotriene/prostacyclin ratio by modifying lipoygenase activity [51, 52]. Immune regulation has also been observed [53]. Hop proanthocyanidins can help prevent nitric oxide-related disorders such as Alzheimer's and Parkinson's diseases [7].

**Prenylchalones and Derived Flavanones**

More than twenty prenylchalones and derived flavanones, studied mainly for their biological effects, have been identified in hop [54]. Concentrations higher than 0.6%, with a predominance of xanthohumol and desmethylxanthohumol, are usually found (Fig. 2) [55]. Levels of 80 and 90 ppm have been reported for the corresponding flavanones, isoxanthohumol and hopein. The higher the α-acid content (bitter acids in hop), the higher the xanthohumol level [56].

Since hop is the only source of these compounds in beer, a relation can be established between their concentration and the rate of hopping. Xanthohumol isomerizes easily during the brewing process into isoxanthohumol [57]. Only 15–50% hop xanthohumol remains in the final beer [58, 59], leading to concentrations often below 1 mg.L⁻¹ [18, 55, 60, 61]. Stout- and Porter-style beers are characterized by slightly higher levels because dark malts contain compounds inhibiting xanthohumol isomerization [57]. The use of xanthohumol-enriched hop products (obtained by ethanol-CO₂ extraction) combined with late hopping makes it possible to increase significantly the xanthohumol and isoxanthohumol potential of beer (close to 10 mg.L⁻¹).

**Health Properties**

Xanthohumol is a "broad-spectrum" cancer chemopreventive agent acting on all three stages of carcinogenesis. Xanthohumol and isoxanthohumol are both active ROS scavengers, while only the former is active in superoxide scavenging assays. Isoxanthohumol, 8-prenylnaringenin, and xanthogalenol may also exert chemopreventive effects [18, 61–64].

Prenylflavanones have mainly been studied for their estrogenic activity. Hopein is a very potent phytostrogen. The authors recommend its application in prevention or treatment of (post)menopausal symptoms and osteoporosis [61, 63, 65]. Weak estrogenic activity has been observed for close analogs like 6-prenylnaringenin, 8-geranylnaringenin, 6,8-diprenylnaringenin, and isoxanthohumol. Prenylchalones like xanthohumol and
xanthogalenol also show low activity [66].

**Flavonols**

Sixteen flavonol glycosides (mainly mono-, di-, and triglycosides of *quercetin* and *kaempferol*) have been detected in hop [31]. Although boiling can extract 91% of the *kaempferol* and 88% of the *quercetin* glycosides, only a few ppms of flavonols are found in the final beer (Fig. 3) [8, 11, 13, 67, 68].

**Bitterness**

In beer, flavonols could be responsible for bitterness but do not participate in beer haze formation [31].

**Health Properties**

Flavonols induce cardioprotective effects, including antioxidant effects (protection against LDL oxidation) and inhibition of platelet activity and vasodilatation [48, 69], while very little information is available on their potential anticancer effects.

**Hydroxybenzoic Acids, Hydroxycinnamic Acids, and Derived Compounds**

Malt and hop contain various hydroxybenzoic acids, which are retained at least partially up to the final beer (Fig. 4). Total hydroxybenzoic acids – mainly *p*-hydroxybenzoic, vanillic, and gallic acids – usually reach a few ppms in beer. They have also been found as glycosides or other bound forms [8, 11–14, 70–74].
Hydroxycinnamic acids are partially recovered in beer (Fig. 4). Most of them are in combined forms in the raw materials, either with quinic acid, glucose, or cell-wall constituents [8, 11–14, 70–78]. In malt, p-coumaric and ferulic acids are esterified with arabinoxylans [79]. They can be both water extracted and enzymatically solubilized by cinnamoyl esterases [80]. After mashing, an additional release of ferulic acid may occur during fermentation due to yeast cinnamoyl esterases [81].

Three coumarins issued from orthohydroxycinnamic acid cyclization have also been found in beer (Fig. 5) [8, 73].
**Flavor**

Hydroxybenzoic and hydroxycinnamic acids are characterized by relatively high flavor thresholds (> ppm, mainly bitter taste and astringency) [82]. On the other hand, their decarboxylated derivatives (Fig. 5) can impart very strong phenolic/clove/smoked flavors to beer because of their low threshold values (ppb order).

Decarboxylation can occur either by thermal degradation [83] during malt kilning and in the boiling kettle [77, 84], or during fermentation. In this last case, decarboxylation is catalyzed by the phenylacrylic acid decarboxylase found in *Saccharomyces cerevisiae* strains displaying the Pof<sup>+</sup> phenotype (phenolic off-flavor) [85, 86] and in some contaminating microorganisms like *Brettanomyces/Dekkera* spp. [87] or *Enterobacteriaceae* [88]. In this way, 4-vinylguaiacol is issued from ferulic acid, while 4-vinylphenol derives from *p*-coumaric acid. 4-Vinylguaiacol has been also found in hop [89].

In Belgian white beer production, enzymatic decarboxylation of ferulic acid occurs linearly through fermentation at a rate close to 140 ppb/day (Fig. 6). The rate decreases strongly during secondary fermentation, down to 20 ppb/day. Compared to *p*-coumaric acid, ferulic acid is preferentially degraded by yeast (*p*-coumaric acid remains unmodified until the ferulic acid concentration reaches 2 ppm) [31]. Concentrations up to 6.2 ppm in 4-vinylguaiacol and up to 3.2 ppm in 4-vinylphenol have been reported in wheat beers [8, 77–79, 81–97]. For instance, 4-vinylguaiacol contributes to the specificity of Belgian white beers (made with unmalted wheat) and German rauch and weizen beers (made with malted wheat) [92, 94, 97, 98]. According to its concentration, 4-vinylguaiacol can lead either to strong pharmaceutical off-flavor defects [91] or to pleasant clove flavors [79], while 4-vinylphenol is always considered to be an off-flavor [79]. These vinyl compounds can be further oxidized or reduced into smaller molecules like vanillin, 4-ethylguaiacol, guaiacol, and 4-ethylphenol through chemical reactions [99] or through the activity of wild yeasts like *Brettanomyces/Dekkera* spp. [87].

![Fig. 6 Evolution of extract, cell population, and 4-vinylguaiacol concentration through fermentation of a Belgian white beer](image)

Degradation of 4-vinylguaiacol through natural aging (25% after 20 days) or at 40°C (50% after 20 days) has been reported [77, 94, 100]. This compound could be partially transformed to 4-ethylguaiacol, vanillin, and guaiacol [79, 101].

By using the AEDA methodology on aged lager beers, 4-vinilsyringol was identified as a strong old-beer-like phenolic odorant (FD value as high as that of *trans*-2-nonenal, responsible for the cardboard off-flavor in aged beer) [102, 103]. Its release through aging should be due to acidic hydrolysis of a glycoside, since sinapic acid decarboxylation occurs much earlier in the process, either in the boiling kettle or during fermentation.

**Color**

Phenolic acids do not participate in beer haze formation [27, 104], but vinylphenol and cinnamic acid have been described as potential pigments [105–108].

**Stilbenes**

Our group recently discovered three stilbenes in hop: *trans*-resveratrol, *trans*-piceid, and *cis*-piceid [4, 109]. Concentrations ranging from 0.7 to 11 ppm *trans*-piceid and from 0.03 to 2.3 ppm *trans*-resveratrol have been reported in hop cones [110]. A strong influence of geographic origin and harvest year has been shown [110], but American aromatic cultivars...
like Willamette and Cascade emerge in all cases as the best sources of stilbenes. Resveratrol is very sensitive to heat and light \[^{110}\]. Even during hop storage, a significant loss occurs, especially in highly oxygen-sensitive varieties, leading to new analogs like cis-resveratrol and dimers \[^{111}\]. Likewise, hop \textit{pelletization} induces strong degradation \[^{111, 112}\]. \textit{trans}-Resveratrol and glycosides are absent from malt \[^{113}\], so one should not be surprised to find only traces of stilbenes in beer (Fig. 7). Up to 5 µg.L\(^{-1}\) \textit{trans}-resveratrol was detected by our group in Belgian commercial beers \[^{114}\].

![Stilbenes and range of concentration in beer](http://www.springerreference.com/docs/html/chapterdbid/363148.html)

Taking into account a concentration of 1–10 ppm stilbenes in hop and hopping close to 200 g.hl\(^{-1}\) in \textit{wort}, a maximum of 2–20 µg.L\(^{-1}\) stilbenes could be expected in beer. Moreover, massive degradation of \textit{trans}-resveratrol is known to occur in the boiling \textit{kettle} (50% degradation after 7 min of boiling – Fig. 8a). \textit{trans}-Piceid is much more stable during heat treatment, and it can be converted to free resveratrol through wort fermentation (only 60% recovered from a beer prepared by spiking an industrial wort at 10 mg.L\(^{-1}\) before fermentation – Fig. 8b). When the wort was not pitched, \textit{trans}-piceid remained stable, suggesting that yeast enzymes catalyze this hydrolysis to free resveratrol, as previously described for wine \[^{114}\]. On the other hand, the \textit{trans}-resveratrol concentration decreased even in the absence of yeast (40% degradation), most likely because of reactions with wort components (apparent degradation of 17% in the presence of yeast due to the equilibrium with piceid).

![Follow-up of the degradation of \textit{trans}-resveratrol and \textit{trans}-piceid](http://www.springerreference.com/docs/html/chapterdbid/363148.html)
In order to increase the stilbene level, stilbene-enriched hop products and brewery process modifications are needed (e.g., adding a stilbene-enriched ethanolic hop extract after fermentation significantly increases the beer stilbene potential).

**Health Properties**

*trans*-Resveratrol shows an impact on platelet aggregation and vasodilatation, and through its effect on the antioxidant status, regulates gene expression and decreases the total lipid concentration (cholesterol and triglycerides) [115]. Although less potent, *cis*-resveratrol, *trans*-, and *cis*-piceid also improve the antioxidant activity [116, 117].

Piceid absorption is enhanced by the presence of its sugar [118].

*trans*-Resveratrol inhibits the initiation and growth of tumors. It inhibits cyclooxygenase, ornithine decarboxylase, and angiogenesis [119, 120]. *trans*-Piceid is a weaker inhibitor of ROS production [122]. As flavonoids, *trans*-resveratrol alters the synthesis of eicosanoids (mediators of inflammation) and decreases the leukotriene/prostacyclin ratio by modifying lipooxygenase activity [120–122]. Estrogenic activity has recently been reported for some stilbenes, especially *trans*-resveratrol. *cis*-Resveratrol appears less potent [120, 123].

**Conclusion**

The aim of the present chapter was to review all phenolic structures that have been found in beer. Each family was discussed according to its properties and stability through storage. However, at the end of this chapter, it is very difficult to advise brewers as to which phenols should be kept in the final beer and at what levels.

Beer phenols issued from malt and hop can contribute directly to several characteristics of beer, mainly flavor, astringency, haze, body, and fullness. Some phenolic structures also impart very interesting health properties. However, degradation of such compounds will inevitably lead to alteration of fresh beer. On the other hand, as antioxidants, these compounds can considerably protect raw materials from oxidative degradation throughout the process.

**References**


