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This article is no longer up to date.

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 gallocatechin

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 Polyvinylpolypyrrolidone

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.

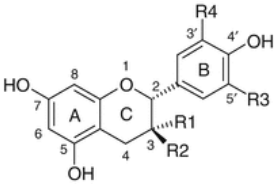
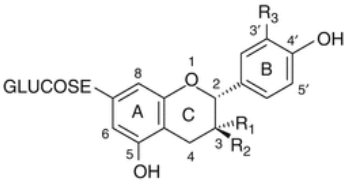
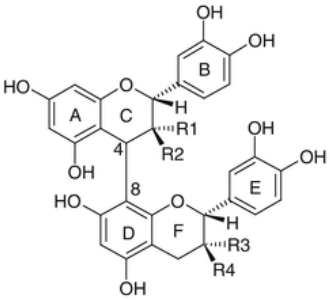
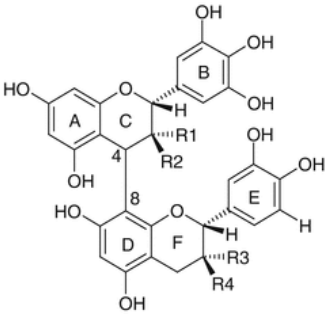
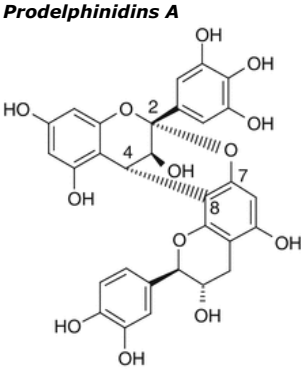
Catechins and Proanthocyanidins

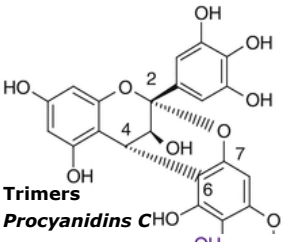
Even compared to grapes, hop emerges as an exceptional source of catechins and proanthocyanidins. Therefore, although added in 100 times lesser quantity than malt, it can account for 30% of total beer polyphenols. Among hop cultivars, the lower the bitterness, the higher the flavonoid level (up to 1% "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 (CO_2 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 2,821 and 1,483 ppm, respectively [*3–7*]. Malt contains only 10–100 ppm (+)-catechin (and no epicatechin at all) [*3*, *5*, *8–10*]. The main monomeric unit identified in beer is (+)-catechin (0.5 to 6.9 $mg.L^{-1}$), but (−)-epicatechin (0.8–1.9 $mg.L^{-1}$), (−)-catechin 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*]

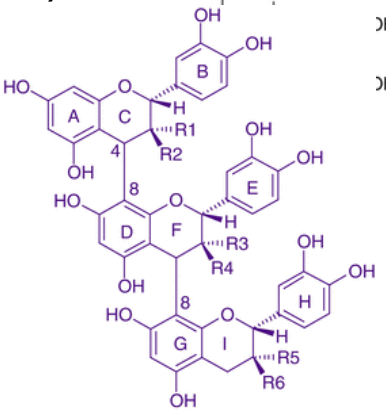
Concentrations

Structures	Compounds	R1	R2	R3	R4	in beer ($mg.L^{-1}$)
Monomers						
	(+)-Catechin	H	OH	H	OH	0.28–6.9
	(−)-Epicatechin	OH	H	H	OH	<0.10–1.9
	(−)-Catechin gallate	H	Gallate	H	OH	5–20
	(−)-Epicatechin gallate	Gallate	H	H	OH	5–20
	3-O-methylcatechin	H	OCH_3	H	OH	Detected
	Catechin-7-O-*β*-d-glucopyranoside	H	OH	OH		Detected
	Catechin-7-O-*β* (6″-O-nicotinoyl)-*β*-d-glucopyranoside	H	OH	OH		Detected
Dimers						
Procyanidins B						
	**B1**(−)-Epicatechin-(4*β*-8)-(+)-catechin	OH	H	H	OH	Detected
	B3(+)-Catechin-(4*α*-8)-(+)-catechin	H	OH	H	OH	Traces–3.1
	B4(+)-Catechin-(4*α*-8)-(−)-epicatechin	H	OH	OH	H	Detected
Prodelphinidins B						
	**B3**(−)-Gallocatechin-(4*α*-8)-(+)-catechin	H	OH	H	OH	Traces–3.3
	B9(−)-Epigallocatechin-(4*β*-8)-(+)-catechin	OH	H	H	OH	Detected
Prodelphinidins A						
	*ent*- (−)-Epigallocatechin -(4*α*-8, 2*α*-O-7)-(+)-catechin					Detected
	ent- (−)-Epigallocatechin -(4*α*-6, 2*α*-O-7)-(+)-catechin					Detected



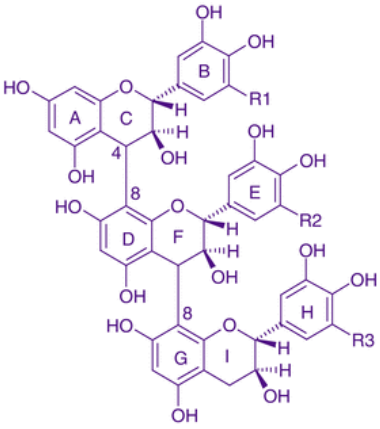
Trimers

Procyanidins C



C2(+)-Catechin-(4*a*-8)-(+)-catechin - (4*a*-8)-(+)-catechin	H	OH	and R5 = H	and R6 = OH	Detected

Prodelphinidins C



(−)-Gallocatechin-(4*a*-8)-(−)-gallocatechin-(4*a*-8)-(+)-catechin	OH	OH	H	/	Detected
(−)-Gallocatechin-(4*a*-8)-(+)-catechin-(4*a*-8)-(+)-catechin	OH	H	H	/	Detected

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) and hop (catechin, epicatechin, 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$_{\alpha}$-8-C-4$_{\alpha}$-8-C and two prodelphinidins, GC-4$_{\alpha}$-8-C-4$_{\alpha}$-8-C and GC-4$_{\alpha}$-8-GC-4$_{\alpha}$-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.

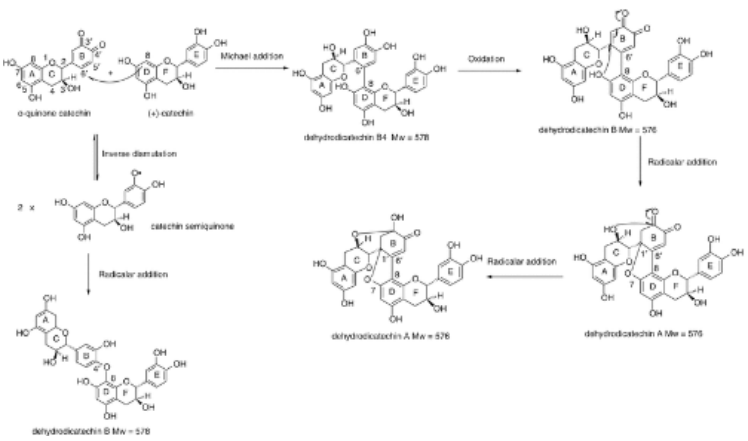


Fig. 1 Proposed degradation schemes of (+)-catechin to form colorless compounds with Mw = 578 and yellow compounds with Mw = 576 [*15*, *46*]

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 lipoxygenase 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*].

Prenylchalcones and Derived Flavanones

More than twenty prenylchalcones 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*].

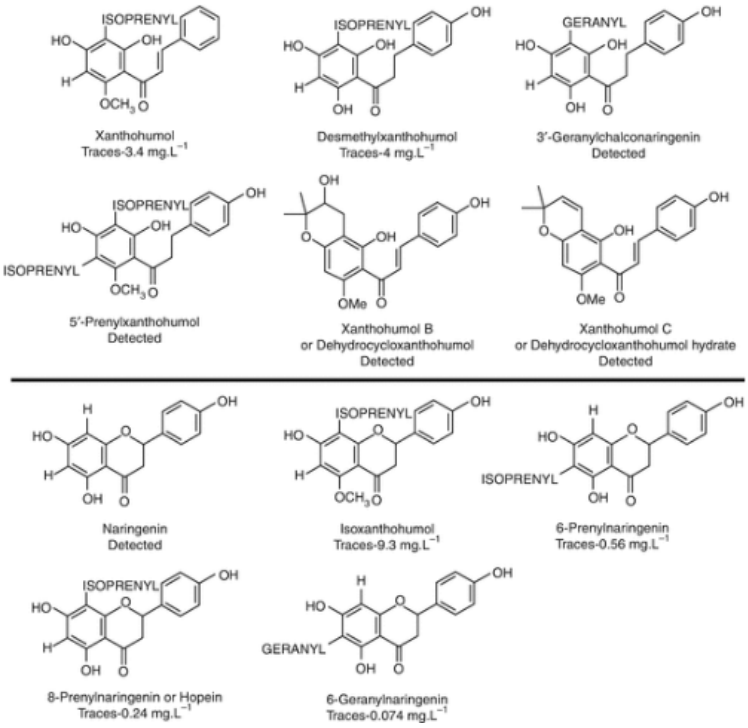


Fig. 2 Prenylchalcones, derived flavanones, and range of concentrations in beer (Adapted from [*31*])

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-CO_2 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 phytoestrogen. 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. Prenylchalcones 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*].

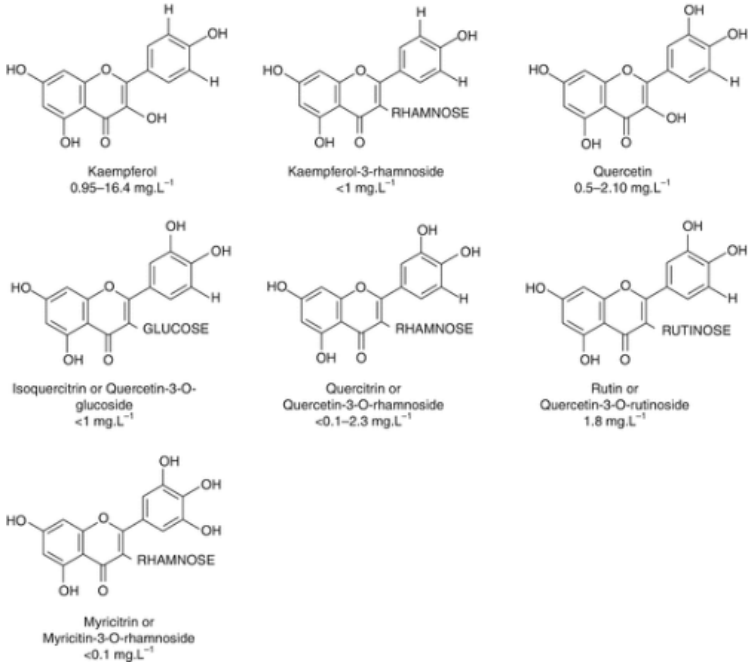


Fig. 3 Flavonols and range of concentrations in beer (Adapted from [*31*])

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*].

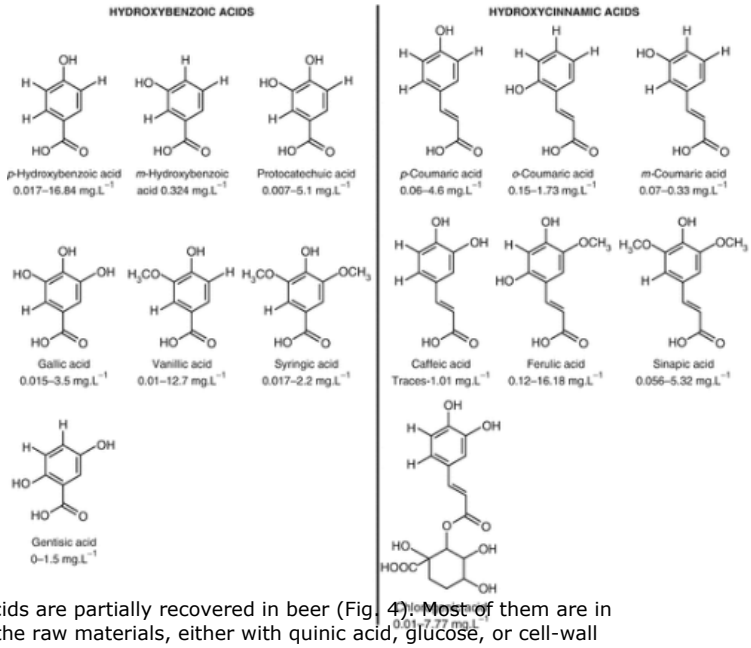


Fig. 4 Hydroxybenzoic acids and hydroxycinnamic acids and range of concentrations in beer (Adapted from [*31*])

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*].

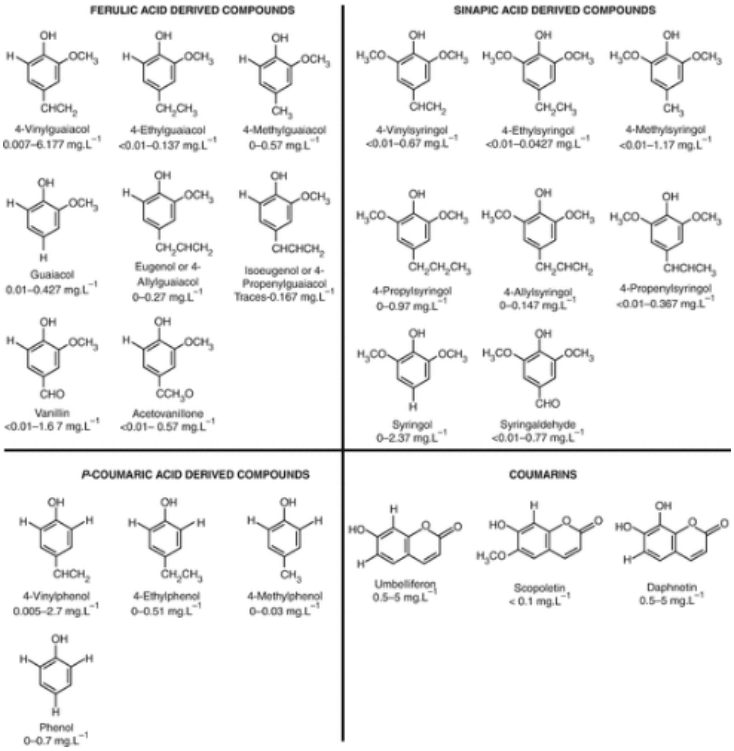


Fig. 5 Hydroxybenzoic- and hydroxycinnamic acid-derived compounds and range of concentrations in beer (Adapted from [*31*])

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^+ 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*, *90–97*]. 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*].

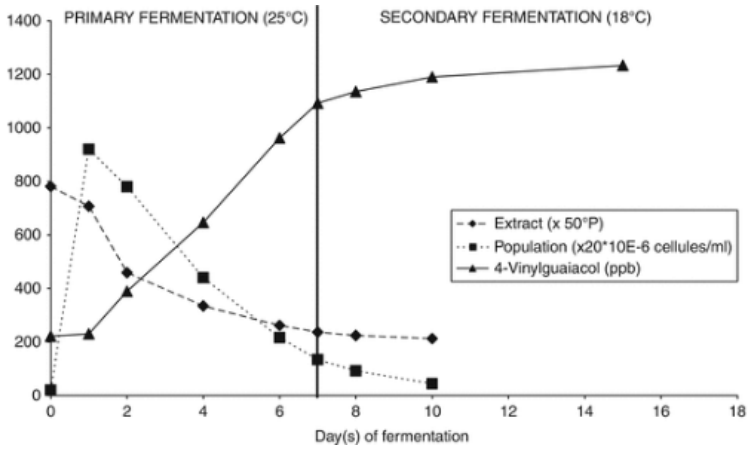


Fig. 6 Evolution of extract, cell population, and 4-vinylguaiacol concentration through fermentation of a Belgian white beer

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-vinylsyringol 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 pelletization induces strong degradation [*111*, *112*]. *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 $\mu g.L^{-1}$ *trans*-resveratrol was detected by our group in Belgian commercial beers [*114*].

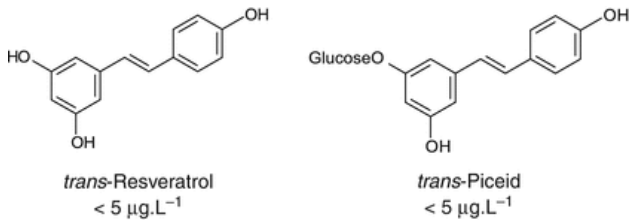


Fig. 7 Stilbenes and range of concentration in beer [*114*]

Taking into account a concentration of 1–10 ppm stilbenes in hop and hopping close to 200 $g.hL^{-1}$ in wort, a maximum of 2–20 $\mu g.L^{-1}$ stilbenes could be expected in beer. Moreover, massive degradation of *trans*-resveratrol is known to occur in the boiling kettle (60% degradation after 7 min of boiling – Fig. 8a). *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, *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 *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).

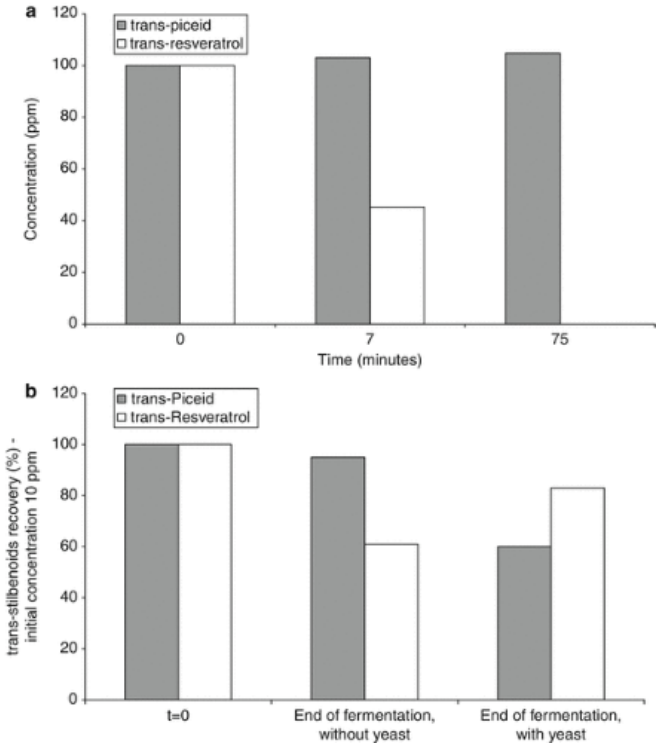


Fig. 8 Follow-up of the degradation of *trans*-resveratrol and *trans*-piceid (**a**) in an aqueous model medium

previously flushed by nitrogen-mimicking wort ebullition (**b**) through wort fermentation [*112*, *114*]

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 [*121*]. As flavonoids, *trans*-resveratrol alters the synthesis of eicosanoids (mediators of inflammation) and decreases the leukotriene/prostacyclin ratio by modifying lipoxygenase 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 advice 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

1. Lermusieau G, Liégeois C, Collin S (2001) Reducing power of hop cultivars and beer ageing. Food Chem 72:413–418

2. Moll M, Fonknechten G, Carnielo M, Flayeux R (1984) Changes in polyphenols from raw materials to finished beer. Tech Q Master Brew Assoc Am 21:79–87

3. McMurrough I (1981) High-performance liquid chromatography of flavonoids in barley and hops. J Chromatogr A 218:683–693

4. Callemien D, Jerkovic V, Rozenberg R, Collin S (2005) Hop as an interesting source of resveratrol for brewers: optimization of the extraction and quantitative study by liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry. J Agric Food Chem 53:424–429

5. Jerumanis J (1985) Quantitative analysis of flavanoids in barley, hops and beer by high-performance liquid chromatography (HPLC). J Inst Brew 91:250–252

6. Li HJ, Deinzer ML (2006) Structural identification and distribution of proanthocyanidins in 13 different hops. J Agric Food Chem 54:4048–4056

7. Stevens JF, Miranda CL, Wolthers KR, Schimerlik M, Deinzer ML, Buhler DR (2002) Identification and *in vitro* biological activities of hop proanthocyanidins: inhibition of nNOS activity and scavenging of reactive nitrogen species. J Agric Food Chem 50:3435–3443

8. Moll M (1991) Beers & coolers: definition, production, and composition. Collection sciences et techniques agro-alimentaires. Tec & Doc–Lavoisier et Apria, Paris

9. Friedrich W, Galensa R (2002) Identification of a new flavanol glucoside from barley (*Hordeum vulgare* L.) and malt. European Food Research and Technology 214:388–393

10. Zimmermann BF, Galensa R (2007) One for all- all for one: proof of authenticity and tracing of foods with flavonoids - Analysis of proanthocyanidins in barley and malt. European Food Research and Technology 224:385–393

11. Achilli G, Cellerino GP, Gamache PH, Deril GVM (1993) Identification and determination of phenolic constituents in natural beverages and plant-extracts by means of a coulometric electrode array system. J Chromatogr 632:111–117

12. Bartolome B, Pena-Neira A, Gomez-Cordoves C (2000) Phenolics and related substances in alcohol-free beers. Eur Food Res Technol 210:419–423

13. Gorinstein S, Caspi A, Zemser M, Trakhtenberg S (2000) Comparative contents of some phenolics in beer, red and white wines. Nutr Res 20:131–139

14. Hayes PJ, Smyth MR, McMurrough I (1987) Comparison of electrochemical and ultraviolet detection methods in high-performance liquid-chromatography for the determination of Phenolic-compounds commonly found in beers. 2. Analysis of beers. Analyst 112:1205–1207

15. Callemien D, Collin S (2007) Involvement of flavanoids in beer color instability during storage. J Agric Food Chem 55:9066–9073

16. Callemien D, Collin S (2008) Use of RP-HPLC-ESI(−)-MS/MS to differentiate various

proanthocyanidin isomers in lager beer extracts. J Am Soc Brew Chem 66:109–115

17. Callemien D, Guyot S, Collin S (2008) Use of thiolysis hyphenated to RP-HPLC-ESI(−)-MS/MS for the analysis of flavanoids of fresh lager beers. Food Chem 110:1012–1018

18. Gerhauser C, Alt A, Heiss E, Gamal-Eldeen A, Klimo K, Knauft J, Neumann I, Scherf HR, Frank N, Bartsch H, Becker H (2002) Cancer chemopreventive activity of Xanthohumol, a natural product derived from hop. Mol Cancer Ther 1:959–969

19. Kirby W, Wheeler RE (1980) The extraction of beer polyphenols and their assay by H.P.L.C. J Inst Brew 86:15–17

20. Madigan D, McMurrough I, Smyth MR (1994) Determination of proanthocyanidins and catechins in beer and barley by high-performance liquid chromatography with dual-electrode electrochemical detection. Analyst 119:863–868

21. McMurrough I, Madigan D, Smyth MR (1996) Semipreparative chromatographic procedure for the isolation of dimeric and trimeric proanthocyanidins from barley. J Agric Food Chem 44:1731–1735

22. Mulkay P, Touillaux R, Jerumanis J (1981) Proanthocyanidins of barley: separation and identification. J Chromatogr 208:419–423

23. McMurrough I, Kelly R, Byrne J, O'Brien M (1992) Effect of the removal of sensitive proteins and proanthocyanidins on the colloidal stability of lager beer. J Am Soc Brew Chem 50:67–76

24. Gardner RJ, McGuinness JD (1977) Complex phenols in brewing – a critical survey. Tech Q Master Brew Assoc Am 14:250–261

25. Leemans C, Pellaud J, Melotte L, Dupire S (2003) Opportunities for lag phase prediction: a new tool to assess beer colloidal stability. In: Proceedings of the 29th European Brewery Convention (Dublin), pp 88/1–88/11, Fachverlag Hans Carl, Nürnberg, Germany

26. Asano K, Shinagawa K, Hashimoto N (1982) Characterization of haze-forming proteins of beer and their roles in chill haze formation. J Am Soc Brew Chem 40:147–154

27. Eastmond R, Gardner RJ (1974) Effect of various polyphenols on the rate of haze formation in beer. J Inst Brew 80:192–200

28. O'Rourke T, Ianniello R, McMurrough I, Springle A (1998) The role of tannoids in the colloidal stabilization of beer. In: Proceedings of the 25th Convention – Institute of Brewing, Asia Pacific Section (Adelaide), pp 143–146, Commercial Printing Company

29. Kaneda H, Kano Y, Osawa T, Kawakishi S, Kamimura M (1990) Effect of free-radicals on haze formation in beer. J Agric Food Chem 38:1909–1912

30. Chapon L (1994) The mechanics of beer stabilisation. Brewers' Guardian 123:50

31. Callemien D, Collin S (2010) Structure, organoleptic properties, quantification methods, and stability of phenolic compounds in beer – a review. Food Rev Int 26:1–84

32. Leiper KA, Stewart GG, McKeown IP (2003) Beer polypeptides and silica gel - Part I. Polypeptides involved in haze formation. J Inst Brew 109:57–72

33. Siebert KJ, Carrasco A, Lynn PY (1996) Formation of protein-polyphenol haze in beverages. J Agric Food Chem 44:1997–2005

34. Siebert KJ (1999) Effects of protein-polyphenol interactions on beverage haze. Stabilization and analysis. J Agric Food Chem 47:353–362

35. De Clerck J (1969) The use of proteolytic enzymes for the stabilization of beer. Tech Q Master Brew Assoc Am 6:136–140

36. Siebert KJ, Lynn PY (1997) Mechanisms of adsorbent action in beverage stabilization. J Agric Food Chem 45:4275–4280

37. Siebert KJ, Lynn PY (1997) Mechanisms of beer colloidal stabilization. J Am Soc Brew Chem 55:73–78

38. Siebert KJ, Lynn PY (1998) Comparison of polyphenol interactions with polyvinylpolypyrrolidone and haze-active protein. J Am Soc Brew Chem 56:24–31

39. Leiper KA, Stewart GG, McKeown IP, Nock T, Thompson MJ (2005) Optimising beer stabilisation by the selective removal of tannoids and sensitive proteins. J Inst Brew 111:118–127

40. Erdal K, Outtrup H, Ahrenst-Larsen B (1985) Barley and hop proanthocyanidins and the colloidal stability of beer. In: Proceedings of the 20th European Brewery Convention (Helsinki). IRL Press, Oxford, pp 459–466

41. Counet C (2004) Détermination des structures chimiques des polyphénols du chocolat et étude de leurs principales propriétés fonctionnelles. Thesis, UCL, Belgium

42. Dadic M, Belleau G (1973) Polyphenols and beer flavors. Proc Am Soc Brew Chem 4:107–114

43. Siebert KJ (2004) pH and colloidal stability. In: Proceedings of XIth De Clerck Chair. Louvain-la-Neuve, Belgium

44. Francois N, Guyot-Declerck C, Hug B, Callemien D, Govaerts B, Collin S (2006) Beer astringency assessed by time-intensity and quantitative descriptive analysis: influence of pH and accelerated aging. Food Qual Prefer 17:445–452

45. Vanderhaegen B, Neven H, Coghe S, Verstrepen KJ, Verachter H, Derdelinckx G (2003) Evolution of chemical and sensory properties during aging of top-fermented beer. J Agric Food Chem 51:6782–6790

46. Guyot S, Vercauteren J, Cheynier V (1996) Structural determination of colourless and yellow dimers resulting from (+)-catechin coupling catalysed by grape polyphenoloxidase. Phytochemistry 42:1279–1288

47. Young DA, Young E, Roux DG, Brandt EV, Ferreira D (1987) Synthesis of condensed tannins.19. Phenol oxidative coupling of (+)-catechin and (+)-mesquitol - conformation of bis-(+)-catechins. J Chem Soc Perkin Trans 1:2345–2351

48. Santos-Buelga C, Scalbert A (2000) Proanthocyanidins and tannin-like compounds – nature, occurrence, dietary intake and effects on nutrition and health. J Sci Food Agric 80:1094–1117

49. Oszmianski J, Cheynier V, Moutounet M (1996) Iron-catalyzed oxidation of (+)-catechin in model systems. J Agric Food Chem 44:1712–1715

50. Prior RL, Gu LW (2005) Occurrence and biological significance of proanthocyanidins in the American diet. Phytochemistry 66:2264–2280

51. Schewe T, Kuhn H, Sies H (2002) Flavonoids of cocoa inhibit recombinant human 5-lipoxygenase. J Nutr 132:1825–1829

52. Schramm DD, Wang JF, Holt RR, Ensunsa JL, Gonsalves JL, Lazarus SA, Schmitz HH, German JB, Keen CL (2001) Chocolate procyanidins decrease the leukotriene-prostacyclin ratio in humans and human aortic endothelial cells. Am J Clin Nutr 73:36–40

53. Sanbongi C, Suzuki N, Sakane T (1997) Polyphenols in chocolate, which have antioxidant activity, modulate immune functions in humans *in vitro*. Cell Immunol 177:129–136

54. Stevens JF, Taylor AW, Nickerson GB, Ivancic M, Henning J, Haunold A, Deinzer ML (2000) Prenylflavonoid variation in *humulus lupulus*: distribution and taxonomic significance of xanthogalenol and 4'-O-methylxanthohumol. Phytochemistry 53:759–775

55. Stevens JF, Taylor AW, Deinzer ML (1999) Quantitative analysis of xanthohumol and related prenylflavonoids in hops and beer by liquid chromatography-tandem mass spectrometry. J Chromatogr A 832:97–107

56. De Keukeleire J, Ooms G, Heyerick A, Roldan-Ruiz I, Van Bockstaele E, De Keukeleire D (2003) Formation and accumulation of alpha-acids, beta-acids, desmethylxanthohumol, and xanthohumol during flowering of hops (*Humulus lupulus* L.). J. Agric. Food Chem 51:4436–4441

57. Walker CJ, Lence CF, Biendl M (2003) Studies on xanthohumol levels in stout/porter beer. Brauwelt 143:1709–1712

58. Biendl M, Mitter W, Peters U, Methner FJ (2002) Use of a xanthohumol-rich hop product in beer production. Brauwelt Int I:39–42

59. Biendl M, Methner FJ, Stettner G, Walker C (2004) Brewing trials with a xanthohumol-enriched hop product. Brauwelt Int III:182–184

60. Stevens JF, Taylor AW, Clawson JE, Deinzer ML (1999) Fate of xanthohumol and related prenylflavonoids from hops to beer. J Agric Food Chem 47:2421–2428

61. Stevens JF, Page JE (2004) Xanthohumol and related prenylflavonoids from hops and beer: to your good health! Phytochemistry 65:1317–1330

62. Gerhauser C, Alt A, Klimo K, Knauft J, Frank N, Becker H (2002) Isolation and potential cancer chemopreventive activities of phenolic compounds of beer. Phytochem Rev 1:369–377

63. De Keukeleire D, Milligan SR, Kalita J, Pocock V, De Cooman L, Heyerick A, Rong H, Roelens F (2001) Prenylated hop flavonoids are key agents in relation to health-beneficial properties of beer. In: Proceedings of 28th European Brewery Convention (Budapest), Fachverlag Hans Carl, Nürnberg, Germany

64. Gerhauser C (2005) Beer constituents as potential cancer chemopreventive agents. Eur J Cancer 41:1941–1954

65. Milligan SR, Kalita JC, Heyerick A, Rong H, De Cooman L, De Keukeleire D (1999) Identification of a potent phytoestrogen in hops (*Humulus lupulus* L.) and beer. J Clin Endocrinol Metab 84:2249–2252

66. Milligan SR, Kalita JC, Pocock V, Van de Kauter V, Stevens JF, Deinzer ML, Rong H, De Keukeleire D (2000) The endocrine activities of 8-prenylnaringenin and related hop (*Humulus lupulus* L.) flavonoids. J Clin Endocrinol Metab 85:4912–4915

67. McMurrough I, Henningan GP, Loughrey ML (1982) Quantitative analysis of hop flavonols using high-performance liquid chromatography. J Agric Food Chem 30:1102–1106

68. Vancraenenbroeck R, De Brackeleire C, Willems Y, Devreux A (1983) Quantitative determination of flavonols in beer. Cerevisia (1976–1990) 8:13–19

69. Prior RL, Lazarus SA, Cao G, Muccitelli H, Hammerstone JF (2001) Identification of procyanidins and anthocyanins in blueberries and cranberries (*vaccinium spp*.) using high-performance liquid chromatography/mass spectrometry. J Agric Food Chem 49:1270–1276

70. Garcia AA, Grande BC, Gandara JS (2004) Development of a rapid method based on solid-phase extraction and liquid chromatography with ultraviolet absorbance detection for the determination of polyphenols in alcohol-free beers. J Chromatogr A 1054:175–180

71. Floridi S, Montanari L, Marconi O, Fantozzi P (2003) Determination of free phenolic acids in wort and beer by coulometric array detection. J Agric Food Chem 51:1548–1554

72. McMurrough I, Roche GP, Cleary KC (1984) Phenolics acids in beers and worts. J Inst Brew 90:181–187

73. Montanari L, Perretti G, Natella F, Guidi A, Fantozzi P (1999) Organic and phenolic acids in beer. LWT - Food Sci Technol 32:535–539

74. Nardini M, Ghiselli A (2004) Determination of free and bound phenolic acids in beer. Food Chem 84:137–143

75. Stewart D, Robertson GW, Morrison IH (1994) Phenolic-acids dimers in the cell-walls of barley. Biol Mass Spectrom 23:71–74

76. Kenyhercz TM, Kissinger PT (1977) A new approach to the phenolic components in beer. Application to the determination of sinapic, ferulic, and p-coumaric acids. J Agric Food Chem 25:959–961

77. McMurrough I, Madigan D, Donnelly D, Hurley J, Doyle AM, Hennigan G, McNulty N, Smyth MR (1996) Control of ferulic acid and 4-vinyl guaiacol in brewing. J Inst Brew 102:327–332

78. Vanbeneden N, Delvaux F, Delvaux FR (2006) Determination of hydroxycinnamic acids and volatile phenols in wort and beer by isocratic high-performance liquid chromatography using electrochemical detection. J Chromatogr A 1136:237–242

79. Tressl R, Renner R (1975) Gas chromatographic-mass spectrometric study of some lactones in beer. Monatsschrift fuer Brauerei 28:195–197

80. Vanbeneden N, Delvaux F, Delvaux FR (2006) Release of phenolic flavour precursors during mashing. In: Proceedings of XIIth De Clerck Chair, Leuven

81. Coghe S, Benoot K, Delvaux F, Vanderhaegen B, Delvaux FR (2004) Ferulic acid release and 4-vinylguaiacol formation during brewing and fermentation: indications for feruloyl esterase activity in *Saccharomyces cerevisiae*. J Agric Food Chem 52:602–608

82. Meilgaard MC (1975) Flavor chemistry of beer. II: Flavor and threshold of 239 aroma volatiles. Tech Q Master Brew Assoc Am 12:5–13

83. Fiddler W, Parker WE, Wasserman AE, Doerr RC (1967) Thermal decomposition of ferulic acid. J Agric Food Chem 15:757–761

84. Tressl R, Kossa T, Renner R (1975) Gas-chromatographic-mass spectrometric studies of volatile substances of hops, wort, and beer and their genesis. II. Formation of phenols and oxygen-containing heterocyclic compounds in beer. In: Proceedings of the 15th European Brewery Convention (Nice). Elsevier, Scientific Pub, pp 737–756

85. Clausen M, Lamb CJ, Megnet R, Doerner PW (1994) PAD1 encodes phenylacrylic acid decarboxylase which confers resistance to cinnamic acid in *Saccharomyces cerevisiae*. Gene 142:107–112

86. Meaden PG, Taylor NR (1991) Cloning of a yeast gene which causes phenolic off-flavors in beer. J Inst Brew 97:353–357

87. Chatonnet P, Dubourdieu D, Boidron JN, Pons M (1992) The origin of ethylphenols in wines. J Sci Food Agric 60:165–178

88. Lindsay RF, Priest FG (1975) Decarboxylation of substituted cinnamic acids by enterobacteria: the influence on beer flavour. J Appl Bacteriol 39:181–187

89. Steinhaus M, Schieberle P (2000) Comparison of the most odor-active compounds in fresh and dried hop cones (*Humulus lupulus* L. variety spalter select) based on GC-olfactometry and odor dilution techniques. J Agric Food Chem 48:1776–1783

90. Wackerbauer K, Kramer P, Siepert J (1982) Phenolic aromatic substances in beer. Phenolic carboxylic acid and phenols in the raw material and beer preparation. Brauwelt 122:618–20, 622, 624, 626.

91. Wackerbauer K, Kossa T, Tressl R (1978) Phenol formation by yeasts. Monatsschrift fuer Brauerei 31:52–55

92. Kieninger H, Narziss L, Miedaner H, Hecht S (1984) Changes of quality-determining compounds in the preparation of Bavarian wheat beers. Monatsschrift fuer Brauwissenschaft 37(9–18):55

93. Madigan D, McMurrough I (1994) Rapid determination of 4-vinyl guaiacol and ferulic acid in beers and worts by high-performance liquid chromatography. J Am Soc Brew Chem 52:152–155

94. Schieberle P (1991) Primary odorants of pale lager beer – differences to other beers and changes during storage. Zeitschrift fur Lebensmittel-Untersuchung und-Forschung 193:558–565

95. Tressl R, Renner R, Apetz M (1976) Volatile phenolic components in beer, smoked beer and sherry. Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung 162:115–122

96. Villarreal R, Sierra JA, Cardenas L (1986) A rapid and sensitive method for the determination of 4-vinyl guaiacol in beer by electron-capture gas–liquid chromatography. J Am Soc Brew Chem 44:114–117

97. Wackerbauer K, Kramer P (1982) Bavarian wheat beer – an alternative. Production and composition. Brauwelt 122:758–60–762

98. Narziss L, Miedaner H, Nitzsche F (1990) The formation of 4-vinylguaiacol during the manufacture of Bavarian wheat beers (Weizenbier). Monatsschrift fuer Brauwissenschaft 43:96–100

99. Tressl R, Kossa T, Renner R (1974) Formation of aroma compounds in malt, wort, and beer. Monatsschrift fuer Brauerei 27:98–102

100. Schieberle P, Komarek D (2003) Changes in key aroma compounds during natural beer aging. In: Freshness and shelf life of foods, ACS symposium series. ACS, Washington DC, pp 70–79

101. Moir M (1992) The 1990 Laurence Bishop silver medal lecture – The desideratum for flavor control. J Inst Brew 98:215–220

102. Callemien D, Dasnoy S, Collin S (2006) Identification of a stale-beer-like odorant in extracts of naturally aged beer. J Agric Food Chem 54:1409–1413

103. Gijs L, Chevance F, Jerkovic V, Collin S (2002) How low pH can intensify beta-damascenone and dimethyl trisulfide production through beer aging. J Agric Food Chem 50:5612–5616

104. Asano K, Ohtsu K, Shinagawa K, Hashimoto N (1984) Turbidity formed in beer at low

temperatures. Affinity of proanthocyanidins and their oxidation products for haze-forming proteins of beer and the formation of chill haze. Agric Biol Chem 48:1139–1146

105. Es-Safi NE, Le Guerneve C, Fulcrand H, Cheynier V, Moutounet M (1999) New polyphenolic compounds with xanthylium skeletons formed through reaction between (+)-catechin and glyoxylic acid. J Agric Food Chem 47:5211–5217

106. Es-Safi NE, Fulcrand H, Cheynier V, Moutounet M (1999) Studies on the acetaldehyde-induced condensation of (−)-epicatechin and malvidin 3-O-glucoside in a model solution system. J Agric Food Chem 47:2096–2102

107. Es-Safi NE, Cheynier V, Moutounet M (2000) Study of the reactions between (+)-catechin and furfural derivatives in the presence or absence of anthocyanins and their implication in food color change. J Agric Food Chem 48:5946–5954

108. Es-Safi NE, Le Guerneve C, Fulcrand H, Cheynier V, Moutounet M (2000) Xanthylium salts formation involved in wine colour changes. Int J Food Sci Technol 35:63–74

109. Callemien D, Counet C, Cawet Q, Collin S (2003) Hop as a determinant nutrition key for health? In: Proceedings of the 29th European Brewery Convention (Dublin). Fachverlag Hans Carl, Nürnberg, p 137

110. Jerkovic V, Collin S (2007) Occurrence of resveratrol and piceid in American and European hop cones. J Agric Food Chem 55:8754–8758

111. Jerkovic V, Collin S (2008) Fate of resveratrol and piceid through different hop processings and storage times. J Agric Food Chem 56:584–590

112. Jerkovic V, Callemien D, Collin S (2005) Determination of stilbenes in hop pellets from different cultivars. J Agric Food Chem 53:4202–4206

113. Jerkovic V, Collin S (2007) Resveratrol analogues in hop, malt, and adjuncts. In: Proceedings of the 31st European Brewery Convention, 76:1–6, Fachverlag Hans Carl, Nürnberg, Germany

114. Jerkovic V, Nguyen F, Timmermans A, Collin S (2008) Comparison of procedures for resveratrol analysis in beer. Assessment of stilbene stability through wort fermentation and beer aging. J Inst Brew 114:143–149

115. Delmas D, Jannin B, Latruffe N (2005) Resveratrol: preventing properties against vascular alterations and ageing. Mol Nutr Food Res 49:377–395

116. Fauconneau B, WaffoTeguo P, Huguet F, Barrier L, Decendit A, Merillon JM (1997) Comparative study of radical scavenger and antioxidant properties of phenolic compounds from *Vitis vinifera* cell cultures using *in vitro* tests. Life Sci 61:2103–2110

117. Orallo F (2006) Comparative studies of the antioxidant effects of *cis*- and *trans*-resveratrol. Curr Med Chem 13:87–98

118. Hollman PCH (1997) Bioavailability of flavonoids. Eur J Clin Nutr 51:S66–S69

119. Aggarwal BB, Bhardwaj A, Aggarwal RS, Seeram NP, Shishodia S, Takada Y (2004) Role of resveratrol in prevention and therapy of cancer: preclinical and clinical studies. Anticancer Research 24(5A):2783–2840

120. Baur JA, Sinclair DA (2006) Therapeutic potential of resveratrol: the *in vivo* evidence. Nat Rev Drug Discov 5:493–506

121. Jang DS, Kang BS, Ryu SY, Chang IM, Min KR, Kim Y (1999) Inhibitory effects of resveratrol analogs on unopsonized zymosan-induced oxygen radical production. Biochem Pharmacol 57:705–712

122. De la Lastra CA, Villegas I (2005) Resveratrol as an anti-inflammatory and anti-aging agent: mechanisms and clinical implications. Mol Nutr Food Res 49:405–430

123. Basly JP, Marre-Fournier F, Le Bail JC, Habrioux G, Chulia AJ (2000) Estrogenic/antiestrogenic and scavenging properties of (E)- and (Z)-resveratrol. Life Sci 66:769–777

124. McMurrough I, Baert T (1994) Identification of proanthocyanidins in beer and their direct measurement with a dual electrode electrochemical detector. J Inst Brew 100:409–416

125. Delcour JA, Tuytens GM (1984) Structure elucidation of three dimeric proanthocyanidins isolated from a commercial Belgian pilsner beer. J Inst Brew 90:153–161

126. Eastmond R (1974) Separation and identification of a dimer of catechin occurring in beer. J Inst Brew 80:188–192