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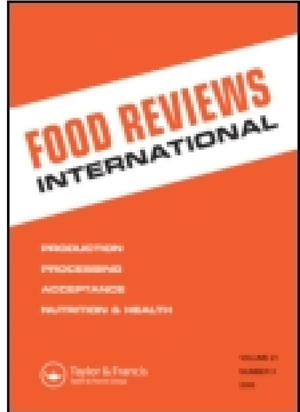

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Structure, Organoleptic Properties, Quantification Methods, and Stability of Phenolic Compounds in Beer—A Review

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Structure, Organoleptic Properties, Quantification Methods, and Stability of Phenolic Compounds in Beer—A Review

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Beer composition changes through storage, altering the quality of the product. In the past decade, many papers have been devoted to the compounds responsible for aged-beer off-flavors, mainly trans-2-nonenal, methional, and dimethyltrisulfide. Due to their huge antioxidant activity, polyphenols have often been described as key compounds for limiting beer staling. Yet phenolic structures also evolve through storage. Low-molecular-weight phenols like 4-vinylsyringol can impart off-flavors in aged beer, whilst flavonoids strongly influence astringency, haze, and color. The instability of stilbenes, prenylchalcones, and derived flavanones could also modify their health potential through storage. After reviewing the structures and properties of all phenolic compounds found in beer, this paper will try to assess the impact of their degradation through aging. Extraction procedures for their quantification and treatments for their removal are also described.

Keywords beer, storage, aging, flavor stability, polyphenols

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Introduction

Beer phenols issued from malt and hop can contribute directly to several characteristics of beer, mainly flavor, astringency, haze, body, and fullness.[1] Some phenolic structures can also impart very interesting health properties. Therefore, 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. It is currently very difficult to advise brewers as to which phenols should be kept in the final beer and at what levels.

The aim of the present paper is to review all phenolic structures that have been found in beer. Each family is discussed according to its properties and stability through storage. Extraction and analysis procedures and treatments for compound removal are also described.

Structure of Phenolic Compounds in Fresh Beer

This chapter describes all flavonoids, phenolic acids, and stilbenes detected in malt, hop, and beer (global definitions given in Scheme 1).

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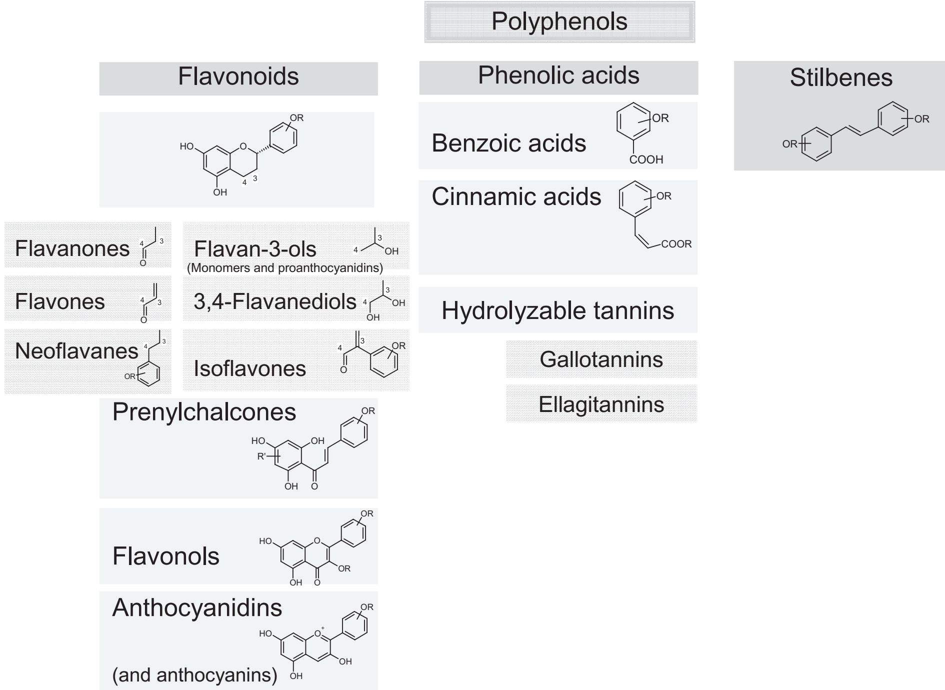

Scheme 1. Global definitions of all flavonoids, phenolic acids, and stilbenes detected in malt, hop, and beer.

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Hydroxybenzoic Acids, Hydroxycinnamic Acids and Derived Compounds

As depicted in Table 1, malt and hop contain various hydroxybenzoic acids, which are retained at least partially up to the final beer. Malt is richer in gentisic acid, hop in vanillic and syringic acids.[2,3] Total hydroxybenzoic acids—mainly *p*-hydroxybenzoic, vanillic, and gallic acids—usually reach a few ppm in beer. They have also been found as glycosides or other bound forms.[4–13]

For some hydroxycinnamic acids, significantly higher concentrations can be found (Table 2). *p*-Coumaric, caffeic, ferulic, sinapic, and chlorogenic acids have been quantified at ppm levels in malt. Hop is an important source of *p*-coumaric, caffeic, and ferulic acids (more than 10 ppm in most cases).[10,14] Dimers of ferulic acid (6 isomers) have been detected in barley.[15] All these compounds are partially recovered in beer. Most of them are in combined forms in the raw materials, with quinic acid, glucose, or cell wall constituents.[4–13,16–18] In malt, *p*-coumaric and ferulic acids are esterified with arabinoxylans.[19] They can be both water-extracted and enzymatically solubilized by cinnamoyl esterases.[20] After mashing, an additional release of ferulic acid may occur during fermentation, due to yeast cinnamoyl esterases.[21]

Hydroxycinnamic acids have little impact by themselves on beer organoleptic properties but are precursors of very potent flavors (Table 2). Their decarboxylation can occur either by thermal degradation [22] during malt kilning and in the boiling kettle,[17,23] or during fermentation. In this last case, decarboxylation is catalyzed by the phenylacrylic acid decarboxylase found in *Saccharomyces cerevisiae* strains displaying the Pof^{+} phenotype

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Table 1
Hydroxybenzoic acids found in malt, hop, and beer

Structure	Compounds	R1	R2	R3	R4	Malt (Mg/Kg)	Hop (Mg/Kg)	Beer (Mg/L)
(R1, R2, R3, R4 substituted benzene ring with COOH)	*p*-Hydroxybenzoic acid	OH	H	H	H	1.0–1.7 (Wackerbauer *et al.*[3])	1.6–2.5 (Wackerbauer *et al.*[3])	0.4–3.09 (Wackerbauer and Kramer[34]) 0.3–1.8 (McMurrough *et al.*[10]) 0.017–0.068 (Montanari *et al.*[12]) 0.092 (Bartolomé *et al.*[5]) 16.84 (Floridi *et al.*[6])
	m-Hydroxybenzoic acid	H	H	OH	H	—	—	0.324 (Floridi *et al.*[6])
	Protocatechuic acid	OH	H	OH	H	0.1–0.5 (Wackerbauer *et al.*[3])	—	0.01–2.7 (Wackerbauer and Kramer[34]) 0.2–1.2 (McMurrough *et al.*[10]) 0.007–0.020 (Montanari *et al.*[12]) 0.840 (Floridi *et al.*[6]) <0.1 (Nardini and Ghiselli[13]) 0.66–5.1 (Garcia *et al.*[7])
	Gallic acid	OH	OH	OH	H	0.01–0.3 (Wackerbauer *et al.*[3])	0.5–1 (Wackerbauer *et al.*[3])	0.01–1.79 (Wackerbauer and Kramer[34]) 0.3–3.5 (McMurrough *et al.*[10]) 0.015–0.034 (Montanari *et al.*[12]) 2.9 (Gorinstein *et al.*[8]) 0.593 (Floridi *et al.*[6])
	Vanillic acid	OH	H	OCH_3	H	0.7–2.3 (Wackerbauer *et al.*[3]) 7 (McMurrough *et al.*[10])	59 (McMurrough *et al.*[10])	0.01–3.15 (Wackerbauer and Kramer[34]) 1.5–12.7 (McMurrough *et al.*[10]) 1.42–1.79 (Hayes *et al.*[9])1–10 (Moll[11]) 3.6 (Achilli *et al.*[4]) 0.062–0.097 (Montanari *et al.*[12]) 0.477 (Bartolomé *et al.*[5]) 0.737 (Floridi *et al.*[6]) 0.59–0.85 and 0.37–1.25* (Nardini and Ghiselli[13])
	Syringic acid	OH	OCH_3	OCH_3	H	0.02–0.5 (Wackerbauer *et al.*[3]) 3 (McMurrough *et al.*[10])	1.3–1.8 (Wackerbauer *et al.*[3]) 30 (McMurrough *et al.*[10])	0.35–1.3 (Wackerbauer and Kramer[34]) 0.7–2.2 (McMurrough *et al.*[10]) 0.68–1.16 (Hayes *et al.*[9]) 0.5 (Achilli *et al.*[4]) 0.017–0.027 (Montanari *et al.*[12]) 0.237 (Floridi *et al.*[6]) <0.1–0.23 and <0.1–0.26* (Nardini and Ghiselli[13])
	Gentisic acid	H	OH	H	OH	26–312 (Wackerbauer *et al.*[3])	—	0–1.5 (Moll[11]) 0.376 (Floridi *et al.*[6])

— = Not determined; and * = Total amount measured after hydrolysis (free + bound forms occuring as esters, glycosides or bound complexes).

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Table 2
Hydroxycinnamic acids and derived compounds found in malt, hop, and beer

Structure	Compounds	R1	R2	R3	R4	Malt (mg/kg)	Hop (mg/kg)	Beer (mg/l)
R1, R2, R3, R5, $COOR_4$	*p*-Coumaric acid	OH	H	H	and R5 = H	0.3–1.3 (Wackerbauer *et al.*[3]); 3 (McMurrough *et al.*[10])	2.2–2.8 (Wackerbauer *et al.*[3]); 13 (McMurrough *et al.*[10])	0.06–0.27 (Kenyhercz and Kissinger[16]); 0.21–1.45 (Wackerbauer and Kramer[34]); 0.6–4.6 (McMurrough *et al.*[10]); 0.57–0.92 (Hayes *et al.*[9]); 0.027–0.129 (Montanari *et al.*[12]); 2.1 (Gorinstein *et al.*[8]); 0.77 (Bartolomé *et al.*[5]); 1.36 (Floridi *et al.*[6]); 0.34–0.76 and 0.15–1.62* (Nardini and Ghiselli[13]); 0.11–0.73 (Garcia *et al.*[7]); 1.41 and 1.67* (Vanbeneden *et al.*[18])
	o-Coumaric acid	H	H	H	H and R5 = OH	—	—	0.15–0.18 (Montanari *et al.*[12]); 1.73 (Floridi *et al.*[6])
	m-Coumaric acid	H	H	OH	and R5 = H	—	—	0.07–0.33 (Montanari *et al.*[12]); 0.22 (Floridi *et al.*[6])
	Caffeic acid	OH	OH	H	and R5 = H	0.29–2.13 (Wackerbauer *et al.*[3]); 1 (McMurrough *et al.*[10])	1.7–3.6 (Wackerbauer *et al.*[3]); 38 (McMurrough *et al.*[10])	0.01–0.98** (Wackerbauer and Kramer[34]); Trace-1 (McMurrough *et al.*[10]); 0.13–0.29 (Hayes *et al.*[9]); 0.006-0.019 (Montanari *et al.*[12]); 0.074 (Bartolomé *et al.*[5])); 0.566 (Floridi *et al.*[6]); 0.15–0.20 and 0.94–1.01*(Nardini and Ghiselli[13]); 0.19–0.41 (Garcia *et al.*[7])

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Ferulic acid	OH	OCH_3	H		and R5 = H	7.8–12.8 (Wackerbauer *et al.*[3]); 14 (McMurrough *et al.*[10])	13.2–14.1 (Wackerbauer *et al.*[3]); 24 (McMurrough *et al.*[10])	0.15–0.61 (Kenyhercz and Kissinger[16]); 5.6–13.1 (Wackerbauer and Kramer[34]); 1.1–10.8 (McMurrough *et al.*[10]); 1.07–1.90 (Hayes *et al.*[9]); 6.5 (Achilli *et al.*[4]); 0.7–6.6 (McMurrough *et al.*[17]); 0.116–0.274 (Montanari *et al.*[12]); 6.8 (Gorinstein *et al.*[8]);1.305 (Bartolomé *et al.*[5])); 2.41 (Floridi *et al.*[6]); 1.36–2.31 and 10.75–15.39* (Nardini and Ghiselli[13]); 0.66–2.4 (Garcia *et al.*[7]); 1.2–14.2 (Coghe *et al.*[21]); 2.22 and 16.18* (Vanbeneden *et al.*[18])
Sinapic acid	OH	OCH_3		OCH_3	and R5 = H	1.0–4.3 (Wackerbauer *et al.*[3])	4.1–5.1 (Wackerbauer *et al.*[3])	0.056–1.04 (Kenyhercz and Kissinger[16]); 2.4-4.5 (Wackerbauer and Kramer[34]); 0.2–3.0 (McMurrough *et al.*[10]); 0.15–0.24 (Hayes *et al.*[9]); 0.09 (Bartolomé *et al.*[5]); 0.15 (Floridi *et al.*[6]); 0.20–0.84 and 2.8–5.32* (Nardini and Ghiselli[13]); 0.24 and 2.74 *(Vanbeneden *et al.*[18])
Chlorogenic acid	OH	OH		H	[structure: * , COOH, OH, OH, OH] and R5 = H	0.3–6.6 (Wackerbauer *et al.*[3])	4–7 (Wackerbauer *et al.*[3])	0.01–7.77 (Wackerbauer and Kramer[34]); 0.028–0.089 (Montanari *et al.*[12]); 0.21–0.26 (Nardini and Ghiselli[13]); 0.901 (Floridi *et al.*[6])

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Table 2
(*Continued*)

Structure	Compounds	R1	R2	R3	R4	Malt (mg/kg)	Hop (mg/kg)	Beer (mg/l)
FERULIC ACID DERIVED COMPOUNDS								
R1, R2, R3, R4-substituted benzene ring	4-Vinylguaiacol	OH	OCH_3	H	$CHCH_2$	Detected (Fickert and Schieberle[36])	Detected (Steinhaus and Schieberle[28])	0.05–0.55 (Tressl *et al.*[23,32]); 0.007–0.074 (Wackerbauer *et al.*[14]); 0.19–4.3*** (Wackerbauer and Kramer[34]); 0.098*, 2.51***, 0.2**** (Wackerbauer *et al.*[3]); 0.49–6.17*** (Kieninger *et al.*[29]); 0.051–0.17 (Villareal *et al.*[33]); 0.05–0.55 (Moll[11]); 0.12–0.53*, 0.9*** (Schieberle[31]); 0.04–0.07*, 0.68*** (Madigan and McMurrough[30]); 0–0.09*, 0.57*** (McMurrough *et al.*[17]); 0–0.28*, 0.16–2.23**, 0.15–2.42*** (Coghe *et al.*[21]); 0.1390 (Vanbeneden *et al.*[18])
	4-Ethylguaiacol	OH	OCH_3	H	CH_2CH_3	0.40 (Kieninger and Boeck[37])	—	<0.01 (Tressl *et al.*[23,32]); 0–0.6*** (Wackerbauer and Kramer[34]); 0*and ***, 0.13*** (Wackerbauer *et al.*[3])
	4-Methylguaiacol	OH	OCH_3	H	CH_3	0.80 (Kieninger and Boeck[37])	—	0–0.15*** (Wackerbauer and Kramer[34]); 0*, 0.04***, 0.5**** (Wackerbauer *et al.*[3])
	Guaiacol	OH	OCH_3	H	H	0.12 (Kieninger and Boeck[37])	—	0.01–0.02 (Tressl *et al.*[23,32]); 0–0.3 0-0.3*** (Wackerbauer and Kramer[34]); 0.014*, 0.02***, 0.42**** (Wackerbauer *et al.*[3])

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	Eugenol or 4-Allylguaiacol	OH	OCH_3	H	CH_2CHCH_2	—	—	0–0.2*** (Wackerbauer and Kramer[34]); 0.01*, 0***, 0.2**** (Wackerbauer *et al.*[3])
	Isoeugenol or 4-Propenylguaiacol	OH	OCH_3	H	$CHCHCH_3$	3.30 (Kieninger and Boeck[37])	—	<0.01 (Tressl *et al.*[23,32]); 0–0.16*** (Wackerbauer and Kramer[34]); 0*, 0.04***, 0**** (Wackerbauer *et al.*[3])
	Vanillin	OH	OCH_3	H	CHO	—	—	<0.01 (Tressl *et al.*[23,32]); 0.030*, < 0.005*** and **** (Wackerbauer *et al.*[3]); 1.6 (Achilli *et al.*[4]); 0.028 (Bartolomé *et al.*[5])
	Acetovanillone	OH	OCH_3	H	$COCH_3$	—	—	<0.01 (Tressl *et al.*[23,32]); 0–0.5*** (Wackerbauer and Kramer[34]); 0.01*, 0.01***, 0.35**** (Wackerbauer *et al.*[3]); 0.01–0.3 (Moll[11])
***p*-Coumaric acid derived compounds**								
	4-Vinylphenol	OH	H	H	$CHCH_2$	—	—	0.04–0.15 (Tressl *et al.*[23,32]); 0.005–0.172 (Wackerbauer *et al.*[14]); 0.2–2.7*** (Wackerbauer and Kramer[34]); 0.01*, 1.25***, 0.2**** (Wackerbauer *et al.*[3]); 0.3–3.17*** (Kieninger *et al.*[29]); 0.0453 (Vanbeneden *et al.*[18])
	4-Ethylphenol	OH	H	H	CH_2CH_3	4.41 (Kieninger and Boeck[37])	—	0–0.1*** (Wackerbauer and Kramer[34]); 0 *and ***, 0.51**** (Wackerbauer *et al.*[3])
	4-Methylphenol	OH	H	H	CH_3	—	—	0–0.03*** (Wackerbauer and Kramer[34])
	Phenol	OH	H	H	H	0.16 (Kieninger and Boeck[37])	—	0–0.03*** (Wackerbauer and Kramer[34]); 0.03*, 0.7*** (Wackerbauer *et al.*[3])

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Table 2
(*Continued*)

Structure	Compounds	R1	R2	R3	R4	Malt (mg/kg)	Hop (mg/kg)	Beer (mg/l)
SINAPIC ACID DERIVED COMPOUNDS								
	4-Vinylsyringol	OH	OCH_3	OCH_3	$CHCH_2$	—	—	<0.01 (Tressl *et al.*[23,32]) 0.9–0.6*** (Wackerbauer and Kramer[34]); 0.04*, 0.2***, 0.14**** (Wackerbauer *et al.*[3])
	4-Ethylsyringol	OH	OCH_3	OCH_3	CH_2CH_3	—	—	<0.01 (Tressl *et al.*[23,32]); 0*, 0***, 0.042**** (Wackerbauer *et al.*[3])
	4-Methylsyringol	OH	OCH_3	OCH_3	CH_3	—	—	<0.01 (Tressl *et al.*[23,32]); 0*, 0***, 1.1**** (Wackerbauer *et al.*[3])
	4-Propylsyringol	OH	OCH_3	OCH_3	$CH_2CH_2CH_3$	—	—	0* and ***, 0.9**** (Wackerbauer *et al.*[3])
	4-Allylsyringol	OH	OCH3	OCH_3	CH_2CHCH_2	—	—	0* and ***, 0.14**** (Wackerbauer *et al.*[3])
	4-Propenyl-syringol	OH	OCH_3	OCH_3	$CHCHCH_3$	—	—	<0.01 (Tressl *et al.*[23,32]); 0* and ***, 0.36**** (Wackerbauer *et al.*[3])
	Syringol	OH	OCH_3	OCH_3	H	—	—	0* and ***, 2.3**** (Wackerbauer *et al.*[3])
	Syringaldehyde	OH	OCH_3	OCH_3	CHO	—	—	<0.01 (Tressl *et al.*[23,32]) 0.7 (Achilli *et al.*[4])
COUMARINS								
	Umbelliferon	OH	H	H	/	—	—	0.5–5 (Moll *(11)*)
	Scopoletin	OH	H	OCH_3	/	—	—	< 0.1 (Moll *(11)*)
	Daphnetin	OH	OH	H	/	—	—	0.5–5 (Moll *(11)*)

— = Not determined; and * = Total amount measured after hydrolysis (free + bound forms occuring as esters, glycosides or bound complexes).

(Phenolic-Off-Flavor),[24,25] and in some contaminating microorganisms like *Brettanomyces/Dekkera* spp.[26] or *Enterobacteriacea*.[27] In this way, 4-vinylguaiacol is issued from ferulic acid while 4-vinylphenol derives from *p*-coumaric acid. 4-Vinyguaiacol has been found in hop.[28] In Belgian white beer production, enzymatic decarboxylation of ferulic acid occurs linearly through fermentation at a rate close to 140 ppb/day. 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) (Collin, unpublished data). Concentrations up to 6.2 ppm in 4-vinylguaiacol and up to 3.2 ppm in 4-vinylphenol have been reported in wheat beers (Table 2).[3,11,14,17–19,21,29–34] These vinyl compounds can be further oxidized or reduced into smaller molecules like vanillin, 4-ethylguaiacol, guaiacol, and 4-ethylphenol through chemical reactions [35] or through the activity of wild yeasts like *Brettanomyces/Dekkera* spp.[26]

Three coumarins issued from orthohydroxycinnamic acid cyclization have also been found in beer (Table 2).[10,11] Very few data concerning their occurrence in malt and hop are available in the literature.

Flavonols

Sixteen flavonol glycosides (mainly mono, di, and triglycosides of quercetin and kaempferol) have been detected in hop (Table 3).[2,38–42] Myricetin is found only in trace amounts.[2,43] Although boiling can extract 91% of the kaempferol and 88% of the quercetin glycosides, only a few ppm of flavonols are found in the final beer.[2,4,8,11,44]

Catechins and Proanthocyanidins

Even compared to grapes, hop emerges as an exceptional source of catechins and proanthocyanidins. Therefore, although added in one-hundred-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 [45]). 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.[46]

In hop dried cones or pellets, (+)-catechin and (-)-epicatechin monomers can reach up to 2821 and 1483 mg/kg, respectively (Table 4).[38,43,47–49] Malt contains only 10-100 mg/kg (+)-catechin (and no epicatechin at all).[11,38,47,50,51] The main monomeric unit identified in beer is (+)-catechin (0.5 to 6.9 mg/L), but (-)-epicatechin (0.8-1.9 mg/L), gallocatechin, galloepicatechin, (-)-catechin gallate, (-)-epicatechin gallate, and two glycosides have also been detected (Table 4).[4,5,8,9,11,52–58]

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% (Table 5).[38,47–49] Malt contains two B3 dimers (prodelphinidin and procyanidin), at lower levels than in hop, but with higher amounts of gallocatechin units.[38,47,51,59] As depicted in Table 6, 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). Two years ago, very few

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Table 3
Flavonols found in malt, hop, and beer

Structure	Compounds	R1	R2	R3	R4	Malt (mg/kg)	Hop (mg/kg)	Beer (mg/l)
KAEMPFEROL DERIVED COMPOUNDS								
HO, OH, O, O, 3, R4, R2, R1, R3	Kaempferol	OH	H	H	OH	—	820–1630** (McMurrough *et al.*[2]); 16 (Callemien *et al.*[43])	0.95 (McMurrough *et al.*[2]); 1.15–2.0** (Vancraenenbroeck *et al.*[44]); 0.6–2 (Moll[11]); 16.4 (Achilli *et al.*[4]))
	Astragalin or Kaempferol-3-O-glucoside	OH	H	H	Glucose	—	Detected (Vancraenenbroeck *et al.*[40]); 310–620 (McMurrough[38])	—
	Kaempferol-3-rhamnoside	OH	H	H	Rhamnose	—	Detected (Vancraenenbroeck *et al.*[40]; McMurrough *et al.*[2])	<1 (Moll[11])
	Kaempferol-3-O-rutinoside	OH	H	H	Rutinose	—	Detected (Vancraenenbroeck *et al.*[41,42]); 600–1160 (McMurrough *et al.*[2])	—
	Kaempferol-3-O-neohesperidoside	OH	H	H	Neohesperidose	—	Detected (Vancraenenbroeck *et al.*[41,42]; McMurrough *et al.*[2])	—

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	Kaempferol-(3,1)-β-glucose-(6,1)-α-rhamnose-(2,1)-α-rhamnose	OH	H	H	Triglycoside	—	Detected (Vancraenenbroeck *et al.*[41,42]; McMurrough *et al.*[2])	—
	Kaempferol-(3,1)-β-glucose-[(6,1)-α-rhamnose-(2,1)-α-rhamnose]	OH	H	H	Triglycoside	—	Detected (Vancraenenbroeck *et al.*[41,42]; McMurrough *et al.*[2])	—
QUERCETIN DERIVED COMPOUNDS								
	Quercetin	OH	OH	H	OH	—	320–1440** (McMurrough *et al.*[2]); 132 (Callemien *et al.*[43])	1.35–2.10** 0.5 (McMurrough *et al.*[2]); (Vancraenenbroeck *et al.*[44]); 0.95 (Gorinstein *et al.*[8])
	Isoquercitrin or Quercetin-3-O-glucoside	OH	OH	H	Glucose	—	Detected (Vancraen enbroeck *et al.*[40]); 80-1040 (McMurrough[38])	<1 (Moll[11])
	Quercitrin or Quercetin-3-O-rhamnoside	OH	OH	H	Rhamnose	—	Detected (Vancraenenbroeck *et al.*[40]; McMurrough *et al.*[2])	<0.1–2.3 (Moll[11])
	Rutin or Quercetin-3-O-rutinoside	OH	OH	H	Rutinose	—	130–910 (McMurrough[38]); 1931 (Callemien *et al.*[43])	1.8 (Achilli *et al.*[4])
	Quercetin-3-O-neohesperidoside	OH	OH	H	Neohesperidose	—	Detected (Vancraenenbroeck *et al.*[41,42]; McMurrough *et al.*[2])	—

(*Continued*)

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Table 3
(*Continued*)

Structure	Compounds	R1	R2	R3	R4	Malt (mg/kg)	Hop (mg/kg)	Beer (mg/l)
	Quercetin-(3,1)-β-glucose-(6,1)-α-rhamnose-(2,1)-α-rhamnose	OH	OH	H	Triglycoside	—	Detected (Vancraenenbroeck *et al.*[41,42]; McMurrough *et al.*[2])	—
	Quercetin-(3,1)-β-glucose-[(6,1)-α-rhamnose-(2,1)-α-rhamnose]	OH	OH	H	Triglycoside	—	Detected (Vancraenenbroeck *et al.*[41,42]; McMurrough *et al.*[2])	—
Myricetin								
	Myricetin	OH	OH	OH	OH	—	Detected (McMurrough *et al.*[2]); 1 (Callemien *et al.*[43])	—
	Myricitrin or Myricitin-3-O-rhamnoside	OH	OH	OH	Rhamnose	—	Detected (McMurrough *et al.*[2])	<0.1 (Moll[11])
with Glucose:			Rutinose: β-Glucose (6,1)-α-Rhamnose		Rhamnose:		Neohesperidose: Glucose (2,1)-α-Rhamnose	

— = Not determined; * = unstabilized beer; and ** = measured after hydrolysis.

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Table 4
Flavan-3-ol monomers found in malt, hop, and beer

Structures	Compounds	R1	R2	R3	R4	Malt (mg/kg)	Hop (mg/kg)	Beer (mg/l)
	(+)-Catechin	H	OH	H	OH	30–95 (McMurrough[38]) 13–20 (Moll[11]) 29–55 (Jerumanis[47]) 6.1–28.3 (Friedrich and Galensa[50]) 6.5–33.4 (Zimmermann and Galensa[51])	260 (McMurrough[38]) 817–2821 (Jerumanis[47]) Detected (Stevens *et al.*[49]; Li and Deinzer[48]) 238 (Callemien *et al.*[43])	3.4–6.9 (Kirby and Wheeler[56]); 0.28–0.82 (Hayes *et al.*[9]) 5 .4 (Achilli *et al.*[4]) 1.6 and 2.8* (McMurrough and Baert[62]); 2.7 and 4.2*(Madigan *et al.*[57]); 1.7 and 4.3* (McMurrough *et al.*[58]) 0.463 (Bartolomé *et al.*[5]) 0.42–4.5 (Garcia *et al.*[7])
	(-)-Epicatechin	OH	H	H	OH	—	194 (McMurrough[38]) Detected (Stevens *et al.*[49]; Li and Deinzer[48]) 1483 (Callemien *et al.*[43])	0.8–1.9 (Kirby and Wheeler[56]); <0.10–0.25 (Hayes et al.[9]); 1.0 and 1.0* (McMurrough and Baert[62]); 0.6 and 1.1* (Madigan *et al.*[57]); 0.9 and 1.3* (McMurrough *et al.*[58]) <1 (Gorinstein *et al.*[8]) 0.18-0.21 (Garcia *et al.*[7])
	(-)-Gallocatechin	H	OH	OH	OH	—	Detected (Li and Deinzer[48])	Detected (Callemien and Collin [53])
	(-)-Galloepicatechin	OH	H	OH	OH	—	—	Detected (Callemien and Collin [53])
	(-)-Catechin gallate	H	Gallate	H	OH	—	—	5–20 (Moll[11])
	(-)-Epicatechin gallate	Gallate	H	H	OH	—	—	5–20 (Moll[11])
	3-O-methylcatechin	H	OCH_3	H	OH	—	—	Detected* (Gerhauser *et al.*[79])
	Catechin-7-O-β-D glucopyranoside	H	OH	OH	Glucose	26.1–67.5 (Friedrich and Galensa[50])	—	Detected* (Gerhauser *et al.*[79])
	Catechin-7-O- β–(6′′-O-nicotinoyl)-β-D-glucopyranoside	H	OH	OH	Glucose and nicotinoyl	—	—	Detected* (Gerhauser *et al.*[79])
with Gallate:					Glucose:		Glucose and nicotinoyl:	

— = Not determined; and * = unstabilized beer.

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Table 5
Dimers of flavan-3-ols found in malt, hop, and beer

Structures	Compounds	R1	R2	R3	R4	Malt (mg/kg)	Hop (mg/kg)	Beer (mg/l)
PROCYANIDINS B								
	B1 (-)-Epicatechin-(4β–8)-(+)-catechin	OH	H	H	OH	—	Detected (McMurrough[38]; Stevens *et al.*[49]; Li and Deinzer[48])	Detected (Callemien and Collin [53])
	B2 (-)-Epicatechin-(4β–8)-(-)-epicatechin	OH	H	OH	H	—	Detected (McMurrough[38]; Stevens *et al.*[49]; Li and Deinzer[48])	—
	B3 (+)-Catechin-(4α–8)-(+)-catechin	H	OH	H	OH	Detected (Mulkay *et al.*[59]); 65–350 (McMurrough[38]); 136–276 (Jerumanis[47]); 79.5–213.3 (Zimmermann and Galensa[51])	428–1472 (Jerumanis[47]); Detected (McMurrough[38]; Stevens *et al.*[49]; Li and Deinzer[48])	Detected (Eastmond[61]; Delcour and Tuytens[60]); 0.7 and 2.1* (McMurrough and Baert[62]); 0.3 and 3.1* (Madigan *et al.*[57]); 0.7 and 1.7* (McMurrough *et al.*[58]); Detected (Callemien and Collin [53])
	B4 (+)-Catechin-(4α–8)-(-)-epicatechin	H	OH	OH	H	—	1058 (Moll[11]); Detected (McMurrough[38]; Stevens *et al.*[49]; Li and Deinzer[48])	
PRODELPHINIDINS B								
	B3 (-)-Gallocatechin-(4α-8)-(+)-catechin	H	OH	H	OH	Detected (Mulkay *et al.*[59]); 105–450 (McMurrough[38]); 272–362 (Jerumanis[47]); 76.7–194.1 (Zimmermann and Galensa[51])	Detected (Li and Deinzer[48])	Detected (Delcour and Tuytens[60]); 0.5 and 2.7* (McMurrough and Baert[62]); 0.5 and 3.3* (Madigan *et al.*[57]); 1.1 and 2.5* (McMurrough *et al.*[58]) Detected (Callemien and Collin [53])
	B9 (-)-Galloepicatechin-(4β-8)-(+)-catechin	OH	H	H	OH	—	—	Detected (Delcour and Tuytens[60])

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Structure	Compound	R1	R2	R3	R4			
	(+)-Catechin-(4α-8)-(-)-gallocatechin	H	OH	H	OH	—	Detected (Li and Deinzer[48])	—
	(+)-Catechin-(4α-6)-(-)-gallocatechin	H	OH	H	OH	—	Detected (Li and Deinzer[48])	—
	(-)-Gallocatechin-(4α-6)-(+)-catechin	H	OH	OH	H	—	Detected (Li and Deinzer[48])	—
Prodelphinidins A								
	ent- (-)-Epigallocatechin -(4α-8, 2α-O-7)-(+)-catechin					—	—	Detected* (Gerhauser *et al.*[79])
	ent- (-)-Epigallocatechin - (4α-6, 2α-O-7)-(+)-catechin					—	—	Detected* (Gerhauser *et al.*[79])
Propelargonidin B								
	(+)-Afzelechin-(4α–8)-(+)-catechin					—	Detected (Li and Deinzer[48])	—
Other Dimers								
	2,3-*cis*-3,4-*trans*- 2-[2,3-*trans*-3,3′,4′,5,7- pentahydroxyflavon-8-yl]-4-(3,4-dihydroxyphenyl)-3,5,7-trihydroxybenzopyran					—	—	Detected* (Gerhauser *et al*[79])

— = Not determined; * = unstabilized beer; 4α = substituent in position 4 below the plane of the flavanoid; 4β = substituent in position 4 above the plane; and *ent*- = enantiomer (non-superimposable mirror images).

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Table 6
Trimers of flavan-3-ols found in malt, hop, and beer

Structures	Compounds	R1	R2	R3	R4	Malt (mg/kg)	Hop (mg/kg)	Beer (mg/l)
Procyanidins C								
	C2 (+)-Catechin-(4α-8)-(+)-catechin -(4α– 8)-(+)-catechin	H	OH	and R5 = H	and R6=OH	Detected (Mulkay *et al.*[59]) 7–110 (Jerumanis[47]) 29.6–119.4 (Zimmermann and Galensa[51])	287–875 (Jerumanis[47]) Detected (Li and Deinzer[48])	Detected (Callemien and Collin[53])
	(-)-Epicatechin-(4β-8)-(+)-catechin -(4α– 8)-(+)-catechin	OH	H	and R5 = H	and R6=OH	—	Detected (Stevens *et al.*[49]; Li and Deinzer[48])	—
	(-)-Epicatechin-(4β-8)-(-)-epicatechin -(4β– 8)-(+)-catechin	OH	H	and R6=OH	and R5=H	—	Detected (Li and Deinzer[48])	—
Prodelphinidins C								
	(-)-Gallocatechin-(4α–8)-(-)-gallocatechin-(4α-8)-(+)-catechin	OH	OH	H	/	135–244 (Jerumanis[47]) 32.5–81.7 (Zimmermann and Galensa[51])	Detected (Li and Deinzer[48])	Detected (Callemien and Collin[53])
	(-)-Gallocatechin-(4α–8)-(+)-catechin-(4α-8)-(+)-catechin	OH	H	H	/	114–245 (Jerumanis[47]) 47.2–104.3 (Zimmermann and Galensa[51])	—	Detected (Callemien and Collin[53])
	(+)-Catechin-(4α–8)-(-)-gallocatechin-(4α-8)-(+)-catechin	H	OH	H	/	59–138 (Jerumanis[47]) 26.1–64.8 (Zimmermann and Galensa[51])	Detected (Li and Deinzer[48])	—

— = Not determined; * = unstabilized beer; 4α = substituent in position 4 below the plane of the flavanoid; and 4β = substituent in position 4 above the plane. mirror images).

flavonoid oligomers were known in beer. The presence of three B-type dimers (one procyanidin and two prodelphinidins) and two prodelphinidins A dimers was mentioned.[55,57,58,60–62] No trace of tetramers and pentamers was found.[63] Very recently, thiolysis hyphenated to RP-HPLC-ESI(-)-MS/MS was optimized by our group to investigate beer polyphenolic oligomers.[54] Thiolysis indicated that most beer dimers are procyanidins B3 (two catechin units) whilst 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. Still higher mDPs were calculated for malt and hop, indicating selective extraction or depolymerization from raw materials to beer. Detailed structures were determined by RP-HPLC-ESI(-)-MS/MS.[53] Four dimers were identified : three procyanidins (B1, B3, and B4) and one prodelphinidin (B3). Previously detected in hop or malt, three trimers (the procyanidin C-4α-8-C-4α-8-C and two prodelphinidins, GC-4α-8-C-4α-8-C and GC-4α-8-GC-4α-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. Higher oligomers were extracted by polyamide SPE or dialysis. To obtain a more detailed composition of higher oligomers, the dialysate was thiolyzed. Results were compared to previous data obtained on the LH20 extract. It was concluded that "natural" beer oligomers exhibit a relatively low degree of polymerization whilst "chemically" synthesized oligomers account for most of the "heavy" flavonoid content.

Prenylchalcones and Derived Flavanones

More than twenty prenylchalcones and derived flavanones, studied mainly for their biological effects, have been identified in hop.[64] Concentrations higher than 0.6%, with a predominance of xanthohumol and desmethylxanthohumol, are usually found (Table 7).[65] Levels of 80 and 90 mg/kg 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.[66]

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.[67] Only 15 to 50% hop xanthohumol remains in the final beer,[68,69] leading to concentrations often below 1 ppm.[55,65,70,71] Stout- and Porter-style beers are characterized by slightly higher levels because dark malts contain compounds inhibiting xanthohumol isomerization.[67] 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 ppm).

Stilbenes

Our group recently discovered three stilbenes in hop: *trans*-resveratrol, *trans*-piceid, and *cis*-piceid.[43,72] Concentrations ranging from 0.7 to 11 mg/kg *trans*-piceid and from 0.03 to 2.3 mg/kg *trans*-resveratrol have been reported in hop cones (Table 8).[73] In grapes, these compounds are known as phytoalexins because they are synthesized in response to injury or fungal attack.[74,75] A strong influence of geographic origin and harvest year has been shown,[73] but American aroma cultivars like Willamette and Cascade emerge in all cases as the best sources of stilbenes. Resveratrol is very sensitive to heat and light.[43] Even during hop storage, a significant loss occurs, especially in highly oxygen-sensitive

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Table 7
Prenylchalcones and derived flavanones found in malt, hop, and beer

Structures	Compounds	R1	R2	R3	R4	Malt (mg/kg)	Hop (mg/kg)	Beer (mg/l)
PRENYLCHALCONES								
	Xanthohumol	OCH_3	H	OH	Isoprenyl	—	4800 (Stevens *et al.*[65,71])	Detected (Stevens *et al.*[80]) 0.002–0.69 (Stevens *et al.*[65,71]) 0.08 (Gerhauser *et al.*[55]) 0.1* (Biendl *et al.*[68]) <0.1–1.2** (Walker *et al.*[67]) <1* and 0.8–3.4* and ** (Biendl *et al.*[69])
	Xanthogalenol	OH	H	OCH_3	Isoprenyl	—	Detected (Stevens *et al.*[64])	—
	4′-O-Methylxanthohumol	OCH_3	H	OCH_3	Isoprenyl	—	Detected (Stevens *et al.*[64])	—
	4′,6′-Di-O-methylchalconaringenin	OCH_3	H	OCH_3	H	—	Detected (Stevens *et al.*[64])	—
	Desmethylxanthohumol	OH	H	OH	Isoprenyl	—	1200 (Stevens *et al.*[65,71])	Detected (Stevens *et al.*[80]) 0.043–4 (Stevens *et al.*[71])
	3′-Geranylchalconaringenin	OH	H	OH	Geranyl	—	Detected (Stevens *et al.*[64])	Detected (Stevens *et al.*[80])
	3′,5′-Diprenylchalconaringenin	OH	Isoprenyl	OH	Isoprenyl	—	Detected (Stevens *et al.*[64])	—
	5′-Prenylxanthohumol	OCH_3	Isoprenyl	OH	Isoprenyl	—	Detected (Stevens *et al.*[64])	Detected (Stevens *et al.*[80])
	Xanthohumol B or Dehydrocycloxanthohumol	/	/	/	/	—	Detected (Stevens *et al.*[64])	Detected (Stevens *et al.*[80])
	Xanthohumol C or Dehydrocycloxanthohumol hydrate	/	/	/	/	—	Detected (Stevens *et al.*[64])	Detected (Stevens *et al.*[80])
	Xanthohumol D	/	/	/	/	—	Detected (Stevens *et al.*[64])	—

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	Compound	R1	R2	R3	R4			
	Xanthohumol E	/	/	/	/	—	Detected (Stevens *et al.*[64])	—
with Isoprenyl:								
DERIVED FLAVANONES								
	Naringenin	OH	H	OH	H	—	—	Detected* (Gerhauser *et al.* (55))
	Isoxanthohumol	OCH_3	H	OH	Isoprenyl	—	80 (Stevens *et al.*[65,71])	Detected (Stevens *et al.*[80]) 0.04–3.44 (Stevens *et al.*[65,71]) 1.6 (Gerhauser *et al.*[55]) 6* (Biendl *et al.*[68]) 1.7–8.6* and 1.5–9.3* and ** (Biendl *et al.*[69])
	7-0-Methyl-6-prenylnaringenin	OH	Isoprenyl	OCH_3	H	—	Detected (Stevens *et al.*[64])	—
	7-0-Methyl-8-prenylnaringenin	OH	H	OCH_3	Isoprenyl	—	Detected (Stevens *et al.*[64])	—
	5,7-Di-0-methyl-8-prenylnaringenin	OCH_3	H	OCH_3	Isoprenyl	—	Detected (Stevens *et al.*[64])	—
	5,7-Di-0-methylprenylnaringenin	OCH_3	H	OCH_3	H	—	Detected (Stevens *et al.*[64])	—
	6-Prenylnaringenin	OH	Isoprenyl	OH	H	—	Detected (Stevens *et al.*[64])	Detected (Stevens *et al.*[80]) 0.001–0.56 (Stevens *et al.*[70])
	8-Prenylnaringenin or Hopein	OH	H	OH	Isoprenyl	—	20 (Stevens *et al.*[71]) 90 (Tekel' *et al.*[81])	Detected (Stevens *et al.*[80]) 0.001–0.24 (Stevens *et al.*[70,71]) 0–0.019 (Tekel' *et al.*[81])
	6,8-Diprenylnaringenin	OH	Isoprenyl	OH	Isoprenyl	—	Detected (Stevens *et al.*[64])	—
	6-Geranylnaringenin	OH	Geranyl	OH	H	—	Detected (Stevens *et al.*[64])	0.001–0.074 (Stevens *et al.*[70])
	8-Geranylnaringenin	OH	H	OH	Geranyl	—	Detected (Stevens *et al.*[64])	—
with Isoprenyl:							Geranyl:	

— = Not determined; * beer produced with a hop-enriched product; and ** Stout or Porter style beers.

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Table 8
Stilbenes found in malt, hop, and beer

Structures	Compounds	R1	R2	R3	R4	Malt (mg/kg)	Hop (mg/kg)	Beer (mg/l)
Stilbenes								
R1, R2, R3	*trans*-Resveratrol	OH	OH	OH	/	Not detected (Jerkovic and Collin[78])	0.5 (Callemien *et al.*[43]) 0.2–1 (Jerkovic *et al.*[77])	Detected (Jerkovic *et al.*[82])
	trans-Piceid	Glucose	OH	OH	/	Not detected (Jerkovic and Collin[78])	2 (Callemien *et al.*[43]) 4–8 (Jerkovic *et al.*[77])	Detected (Jerkovic *et al.*[82])
R1, R2, R3	*cis*-Piceid	Glucose	OH	OH	/	Not detected (Jerkovic and Collin[78])	0.9 (Callemien *et al.*[43]) 2–6 (Jerkovic *et al.*[77])	Detected (Jerkovic *et al.*[82])
with Glucose:			O, HO, HO, OH, O, OH					

varieties, leading to new analogs like *cis*-resveratrol and dimers.[76] Likewise, hop pelletization induces strong degradation.[76,77] *trans*-Resveratrol and glycosides are absent from malt,[78] so one should not be surprised to find only traces of stilbenes in beer. In order to increase the stilbene level, stilbene-enriched hop products and brewery process modifications are needed.

Extraction and Analysis

Global Assays: Coulometric Methods

Total Polyphenol Content

Folin-Ciocalteu assay. The Folin-Ciocalteu reagent is a mixture of phosphotungstic and phosphomolybdic acids (6+ valence state of the metal), which can be reduced by phenols to blue oxides of tungstene and molybdene (λmax = 725–760 nm, mixture of different valence states of the metals) (Fig. 1a). The detailed electronic structures of the blue reduction products are unclear. The oxidation reaction is enhanced under alkaline conditions, created here by sodium carbonate. To avoid the precipitation of sodium complex forms, lithium sulfate is added to the reaction medium.[83] Gallic acid and (+)-catechin are frequently exploited as references for calibration curves. This assay is often used to quantify polyphenols and proteins (with tyrosine residues), but it also measures other readily oxidizable compounds.[83,84] The contribution of ascorbic acid, for example, can be removed.[85]

Bishop assay. Mainly used by brewers, this assay is based on chelation of phenols with iron under alkaline conditions (pH 10 with ammonia), leading to a red color (λmax = 600 nm) (Fig. 1b).[86] Because most flavonoids are able to reduce Fe^{+3} to Fe^{+2}, chelation with Fe^{+3} or Fe^{+2} is observed. Three metal complexing sites are possible for flavanones: between their 3- or 5- hydroxyl and the 4-oxo group or between the *ortho*-hydroxyl groups (3′ and 4′) on the B-ring (Fig. 2). As for catechin, only the latter sites allow chelation.[87,88] Surprisingly, no influence of the polymerization degree has been observed (results obtained on fresh and oxidized flavan-3,4-diol).[89] No data are available for natural proanthocyanidins. Expectedly, Jerumanis[90] observed little to no color change with vanillic, syringic, and ferulic acids, which do not include such interaction sites (Table 10). Interference reactions with colorants (malt and sugar), melanoidins, reductones, cysteine, and ascorbic acid have been mentioned.[89–91] Carboxymethylcellulose is always added to the reaction media to improve transparency while EDTA is recommended as an antiseptic.[92] Polyphenols isolated from malt or hop are used as calibration curve references.

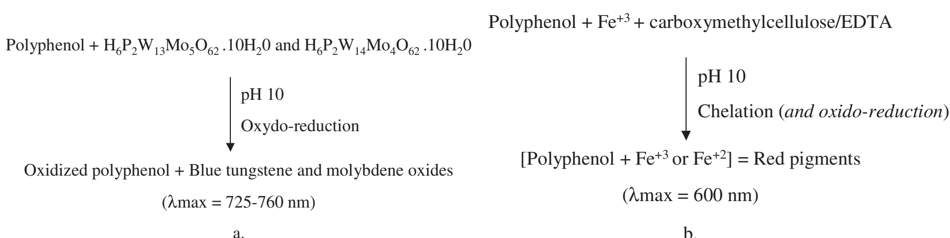

Figure 1. Chemistry of two total polyphenols measurement assays: a. Folin-Ciocalteu and b. Bishop.

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with $M^{n+} = Fe^{3+}$

Figure 2. Scheme of flavanoid chelation.

Table 9
Comparison between different polyphenol assays. Values obtained on nineteen fresh lager beers (adapted from [94])

	Bishop[a]	Folin ciocalteau[a]	Anthocyanogen[b]	p-Dimethylamino-cinnamaldehyde[a]	Vanillin[a]
Range	47–204	227–377	2.6–60	4.8–39.5	94–171
Average	138	309	33.8	26.3	144

[a]Catechin equivalents mg/L; and [b]flavan-3,4-diol equivalents mg/L.

Flavan-3-ol and proanthocyanidin contents

***p-D*imethylaminocinnamaldehyde assay for flavan-3-ols and proanthocyanidins.** A green color (λmax = 640 nm) is generated when *p*-dimethylaminocinnamaldehyde reacts with the A ring of flavan-3-ols or proanthocyanidins (Fig. 3a). Flavonoids with di- or tri-hydroxyl groups on the A ring (e.g., positions 5 and 7) and a single bond between the 2 and 3 positions of the C ring (higher nucleophilicity of the A ring) are needed for such a reaction.[93] (-)-Epicatechin (2,3-*cis*) gives higher color intensity than (+)-catechin (Table 10), and the color intensity depends strongly on the polymerization degree. Because at least one of the A-rings is involved in intermonomer linkage (C4-C8), the higher the DP, the weaker the response[94]. Figure 4 gives more precisely the relative sensitivity for each polyphenol. (+)-Catechin is used as reference for quantification.

Vanillin assay for flavan-3-ols and proanthocyanidins. Vanillin reacts with condensed tannins to produce red structures detected at 500 nm (Fig. 3b). As in the former assay, a single bond between the 2 and 3 C-ring positions and free *meta*-oriented hydroxyl groups on A are needed (Table 10).[95] (+)-Catechin is again used as standard. This assay is limited by acidity and water (H_2SO_4 is preferred to HCl, which contains more water), reaction time (15 min is optimal for monomers), and temperature (25–35°C for monomers vs. no influence for oligomers).[96]

Acid-butanol assay for proanthocyanidin content. Acid-catalyzed cleavage (by HCl) of interflavanoid bonds and oxidation convert proanthocyanidins to anthocyanidins (550 nm) (Fig. 3c). The auto-oxidation step is favored in the presence of Fe^{+3}. The limiting factor is conversion to cyanidins, which depends on the DP and water content.[97] Cyanidin chloride is used as standard. The ratio of the result of this assay to that of the vanillin assay has been often used to assess the polymerization degree of proanthocyanidins.

Anthocyanogen assay for proanthocyanidin content. It is often used for quantifying natural oligomeric flavanoids in beer (known as the anthocyanogen fraction in brewing

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Table 10
Response of various polyphenols in different assays

Compound	Bishop[a]	Folin-Ciocalteau[b]	Anthocyanogen[c]	Butanol[d]	p-Dimethylaminocinnamaldehyde[e]	Vanillin[f]
HYDROXYBENZOIC ACIDS						
4-Hydroxybenzoic acid	+++	—	—	—	0	—
Protocatechuic acid	+++	—	—	—	0	—
Gallic acid	+++	+++	—	—	0	—
Vanilic acid	0	—	—	—	0	—
Syringic acid	+/0	—	—	—	0	—
Gentisic acid	—	—	—	—	0	—
HYDROXYCINNAMIC ACIDS AND DERIVED COMPOUNDS						
Caffeic acid	+++	+++	—	—	0	—
Ferulic acid	0	—	—	—	0	—
Chlorogenic acid	+++	+++	—	—	0	—
Vanillin	—	+	—	—	—	—
FLAVONOLS						
Kaempferol	—	—	—	—	—	0
Quercetin	—	+++	—	—	0	0
Isoquercetin or Quercetin-3-O-glucoside	+++	—	—	—	—	—
Quercitrin or Quercetin-3-O-rhamnoside	—	+	—	—	—	0
Rutin or Quercetin-3-O-rutinoside	—	++	—	—	0	0
Myricetin	—	—	—	—	0	
FLAVANOIDS						
(+)-Catechin	+++	+++	—	—	++	+++
(-)-Epicatechin	+++	—	—	—	+++	—
Leucocyanidin	++	—	+++	+	—	—
Procyanidin B1	—	—	—	+++	—	—
Procyanidin B3	—	—	—	+++	+	—
Procyanidin B4	—	—	—	—	++	—
Prodelphinidin B3	—	—	—	—	—	—
Procyanidin C2	—	—	—	—	+	—
Procyanidin C (epi-epi-cat with 4β-8)	—	—	—	++	—	—

With +++ = reaction; ++ = middle reaction; + = few reaction; 0 no reaction; [a] (90); [b] (83),(85); [c] (89), [d] (97); [e] (93),(94); [f] (95),(96).

Figure 3. a. *para*-Dimethylaminocinnamaldehyde assay; b. vanillin assay (both Friedel-Crafts condensation); and c. acid-butanol assay.

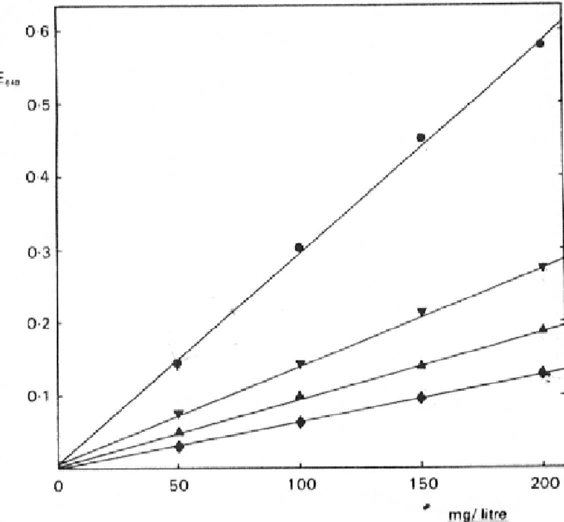

Figure 4. Dimethylaminocinnamaldehyde color yield (E_{640} nm vs mg/L) of different phenolics in 5% ethanol: • (+)-Catechin ; ▼ Procyanidin B4 ; ▲ Procyanidin B3 ; and ◆ a mixture of trimeric and tetrameric procyanidins mainly containing containing procyanidin C2.[(94)].

sciences), yielding anthocyanidins upon heating with HCl (like the acid-butanol assay). Polyphenol adsorption to PVPP is followed by desorption with N-methyl-2-pyrrolidone containing HCl and iron (which enhances the color measured at 550 nm). The yield of released cyanidin is lower when the DP is higher. Furthermore, the color will not be the same if cyanidins or delphinidins are released (λmax = 545 or 557 nm).[(91, 98–100)] Leucocyanidin is used as standard. Because the Bishop assay does not depend on the DP but the

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anthocyanogen assay does, Jerumanis has established a polymerization index obtained by calculating the ratio of the corresponding results.

Tannoid assay. Polyphenols involved in beer colloidal instability can be determined by titration with soluble PVP (turbidity measurement with a tannometer).[101,102] The so-quantified structures are most probably oxidized proanthocyanidins.[103]

Comparison of Global Polyphenol Assays. As depicted in Table 9, very dissimilar values are obtained with the different assays. However, a good correlation was found by Delcour and Janssens de Varebeke[94] between the *p*-dimethylaminocinnamaldehyde and the Bishop values of various beers, and between the former and the anthocyanogen assays ($R^2 = 0.903$ and $R^2 = 0.916$, respectively).

The highest concentrations are always determined with the Folin Ciocalteau assay, known to be less selective than the Bishop assay (interactions with proteins). Surprisingly, *p*-dimethylaminocinnamaldehyde and anthocyanogen values are much lower than those issued from the vanillin assay are. Concentrations obtained with the *p*-dimethylaminocinnamaldehyde assay are more in line with RP-HPLC quantifications.[104]

Selective Extraction

Polarity, acidity, volatility, and molecular size are four physicochemical properties of phenols that distinguish them from other co-constituents of hop, malt, and beer. Hop and malt are often ground before extraction, whilst beer just has to be degassed.

Polarity/Hydrophobicity of Phenols. Flavors derived from hydroxycinnamic acids are usually extracted by apolar solvents such as chloroform, diethylether, and Freon 11. Apolar resins like XAD-2 (elution with diethylether) can also be used (Table 11). Unlike most hop co-constituents, polyphenols show relatively high polarity. The solubility class[105] catechin or quercetin has been defined as S2 (soluble at 3.3% in water but not in diethylether), whereas stilbene aglycons and prenylchalcones should belong, rather, to the S1 class (soluble in both media). Therefore, although diethylether can be used to remove hydrophobic compounds from flavanoids or flavonols, solvents like cyclohexane or toluene should be preferred to keep xanthohumol or resveratrol analogs. The use of supercritical carbon dioxide is another interesting way to delipidate the matrix or to remove hop bitter acids (Table 15).

Counet *et al.*[106] have shown that acetone/water/acetic acid (70/28/2, v/v) is the best mixture for recovering flavanoids, especially higher oligomers (experiments conducted on delipidated chocolate). When methanol is used, no water should be added. Yet Jerumanis[107] indicates that proanthocyanidin can depolymerize in methanol. The same solvents are generally used to recover flavonols and prenylflavonoids. For the latter, prior extraction with chloroform is sometimes used. Surprisingly, despite its lower polarity, resveratrol is extracted better by ethanol/water (80/20, v/v, 60°C) than by aqueous acetone.[43]

Water-immiscible solvents do not extract polyphenols very efficiently[108], but ethyl acetate or butan-2-one has been used by some authors (Table 14).[47,90,109–111] In general, for beer, the use of solid phase extraction should thus be preferred (SEPACK C18, Sephadex LH20, Sephadex G25, Polyamide, or Nylon 66). Washing with water or aqueous methanol is frequently carried out before polyphenol elution with ethanol (for flavonols—Table 13), methanol (for flavonols and monomers of flavan-3-ols—Tables 13 and 14), or acetone (for oligomers of proanthocyanidins—Table 14), in all cases with or without water.

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Table 11

Extraction of hydroxybenzoic acids, hydroxycinnamic acids derived compounds from malt, hop, and beer

Sample	Fundamental basics	Successive steps	Reference
Malt	Polarity Volatility Acidity	1) 300 g Malt were frozen in liquid N_2 and ground. Powder was extracted three times with 400 mL diethyl ether by stirring for 4 h at room temperature 2) The combined extracts were concentrated to 100 mL by using a Vigreux column 3) To remove non volatile material, the concentrated was distilled *in vacuo* at room temperature 4) Acidic volatiles were separated from the neutral/basic volatile by treatment of the distillate with 150 mL sodium bicarbonate (0.5 mol/L) 5) The combined aqueous solutions were adjusted to pH 3 with HCl and then extracted with 150 mL diethylether 6) Neutral/basic and acidic extracts were dried with anhydrous Na_2SO_3 and concentrated to 300 μL by distilling off the solvent by means of a Vigreux column followed by microdistillation	Fickert and Schieberle [36]
Hop	Polarity Volatility Acidity	1) Dried (5 g) or fresh hop cones (22.5 g) were frozen in liquid N_2 and ground. To the sample of fresh hops was added anhydrous Na_2SO_3 (22.5 g). The powder was then extracted with 100 mL diethyl ether by stirring for 1 h at room temperature 2) To remove non volatile material, the combined extracts were distilled *in vacuo* 3) Acidic volatiles were separated from the neutral/basic volatile by treatment of the distillate with 200 mL sodium bicarbonate (0.5 mol/L) 4) The combined aqueous solutions were adjusted to pH 3 with HCl and then extracted with 150 mL diethylether to recover the acidic fraction 5) Neutral/basic and acidic extracts were dried with anhydrous Na_2SO_3 and concentrated to 1 mL by distilling off the solvent by means of a Vigreux column followed by microdistillation	Steinhaus and Schieberle [28]
Beer	Polarity Acidity	1) 10 mL Beer, 0.1 mL eugenol (100 ppm IS), 1.5 g NaCl, 0.4 mL $CHCl_3$ and 0.25 mL HCL 6N were shaken for 30 min at room temperature 2) Then the mix was centrifuged at 1000 g for 2 min and 3) Only the lower organic phase was analyzed	Thurston and Tubb [127]
Beer	Polarity Volatility (with derivatization)	1) 10 mL Degassed beer and 10 mL trichlorofluoromethane (Freon 11) were stirred for 10 min, the organic phase was then evaporated to dryness. The residue was dissolved in 10 mL benzene 2) 0.5 mL of the extract, 100 μL 0.1 M triethylamine and 10 μL heptafluorobutyric anhydride were mixed for 1 min and incubate for 10 min at 20°C 3) At the end, 0.5 mL sodium buffer solution pH 6 was added and the mixture stirred for 30 sec. The organic phase was separated by centrifugation for analysis	Villareal *et al.* [33]

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Beer	Polarity Volatility Acidity	1) 500 mL Beer was extracted with 1 L diethyl ether 2) The extract was concentrated to 150 mL using a Vigreux column and volatile isolated by sublimation *in vacuo* 3) By treatment of the distillate with 200 mL sodium bicarbonate (0.5 mol/L), acidic and of neutral/ basic fractions were obtained and concentrated by microdistillation	Schieberle [31]
Beer	Polarity Volatility Acidity	1) 500 mL Beer filtered through a paper filter (to avoid foaming during extraction) was extracted with 2.4 L diethyl ether 2) The extract was dried over sodium suplhate and finally concentrated to 100 mL by distilling off the solvent at 38°C using a Vigreux column 3) To remove the nonvolatile material, the concentrate was distilled using the solvent-assisted flavor evaporation method 4) By treatment of the distillate with 200 mL aqueous sodium bicarbonate (0.5 mol/L), acidic and neutral/ basic fractions were obtained 5) After drying over sodium suplhate, both fractions were concentrated by microdistillation to 250 mL	Fritsch and Schieberle [128]
Beer	(A) Polarity (B) Polarity and Acidity	**System (A):** 1) 50 mL Degassed beer was mixed with 2g Amberlite XAD2 resin at 200 rpm for 2 h at room temperature 2) The content of the flask was then transferred to a column (60 × 1 cm) and the column washed with 4 × 25 mL water 3) Apolar compounds were eluted with 2 × 20 mL diethyl ether, dried with anhydrous Na_2SO_3 and the extract concentrated to 0.5 mL **System (B):** 1) 50 mL Degassed beer, 5 ppm eugenol (IS), 1 mL of 37% HCL (v/v), and 6.45 g NaCl were mixed 2) After complete dissolution, 150 mL chloroform/methanol (3/1, v/v) was added and the mixture stirred for 10 min at 1500 rpm 3) The lower organic solvent layer was retained and the aqueous phase extracted a second time in the same manner 4) The 300-mL organic phase was then shaken with 50 mL of 10% potassium hydroxide solution for 10 min at 1500 rpm 5) The upper aqueous phase (pH 13) was recovered and the lower organic phase extracted a second time as described above 6) The pH of the aqueous phase was adjusted to 9.0 with HCl and extracted two times with 25 mL CH_2Cl_2 after stirring for 10 min at 1500 rpm 7) The combined organic phases were further concentrated to 0.5 mL	Callemien *et al* [112]

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Table 12
Extraction of hydroxybenzoic acids, hydroxycinnamic acids from malt and beer

Sample	Fundamental basics	Successive steps	Reference
Barley	Polarity	1) 5 g Grounded barley was sonicated for 1 h with 50 mL of acetone/water (80/20, v/v; for ferulic, caffeic, vanillic, *p*-coumaric acids), methanol/water (80/20, v/v; for syringic acid),ethanol/water (80/20, v/v), or water (for protocatechuic and gallic acid) under N_2 at 20°C 2) After centrifugation (10000 g for 10 min), the supernatant was collected and evaporated to dryness and dissolved in 50 mL methanol	Zhao *et al.*[108]
Wort	—	Degassed beer filtered on 0.22 µm and analyzed by HPLC	Madigan and McMurrough[30]
Beer		Wort was diluted 2 times with methanol, mixed and then centrifuged at 3000 g for 15 min. The supernatant was collected for analysis by HPLC	McMurrough *et al.*[17]
Wort Beer	—	Degassed beer and wort were filtered on 0.22 µm, diluted 2/5 (v/v) with 0.05 M KH_2PO_4 and 0.5 mm sodium lauryl sulfate and analysed by HPLC	Floridi *et al.*[6]
Wort Beer	—	Degassed beer filtered on 0.45 µm and analyzed while wort was centrifuged at 1400 g for 5 min and the supernatant collected for analysis by HPLC	Coghe *et al.*[21,129]
Wort	(A)–(B) Acidity (hydrolysis) and Polarity	**System (A) :** Degassed beer filtered on 0.45 µm and analyzed by HPLC	Vanbeneden *et al.*[18]
Beer		**System (B) :** 1) 0.5 mL Beer was subjected to alkaline hydrolysis with 4.5 ml 2N NaOH containing 10 mM EDTA and 1% ascorbic acid at 30°C for 30 min, with 1 µg isoferulic acid (IS); 2) At the end of incubation, 0.5 ml sample were acidified to pH 3.0 with 4 N HCl, added with 300 mg NaCl and extracted three times with ethyl acetate (4 volumes) by vortexing for 5 min. After each extraction, samples were centrifuged (3000 *g*, 10 min) and supernatants collected. The organic phase was dried under N_2; 3) The residue was dissolved up to 0.1 ml methanol and vortexed for 5 min; then 0.4 ml of buffer was added and samples centrifuged at 17 500 g for 5 min	
Beer	Acidity Polarity	1) 2 mL Beer acidified to pH 2 with 6M HCL stored previously at -35°C and saturated with NaCl were extracted three times with 2 mL ethyl acetate 2) The combined organic phases were dried with anhydrous Na_2SO_3, evaporated to dryness under N_2 and diluted in 2 mL 1M acetate buffer (pH 4.7)/ 0.25 M citric acid (pH 1.8)/water (1/1/8, v/v)	Kenyhercz and Kissinger[16]

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Beer	Acidity Polarity	1) 100 mL Degassed beer acidified to pH 2 with 2M HCL were extracted twice with 100 mL isooctane 2) The aqueous phase was then extract 4 times with 100 mL ethyl acetate and the combined organic phases were evaporated to 1–2 mL and diluted with methanol	Hayes *et al.*[9,130]
Beer	—	1) Filtration on 0.22 µm and diluted one hundred times in water and analysed by HPLC	Achilli *et al.*[4]
Beer	—	Beer was diluted then times with the mobile phase (34.7 µM sodium dodecyl sulfate, 0.1 M phosphate buffer (pH 3.35), 50 nM nitriloacetic acid in water/methanol (50/50, v/v)), filtered on 0.22 µm and analyzed by HPLC	Montanari *et al.*[12]
Beer	Polarity	1) 50 mL Beer extracted 3 times with 30 mL diethyl ether and then 3 times with 30 mL ethyl acetate. 2) The combined organic extracts were dried with anhydrous Na_2SO_3, filtered and evaporate to dryness 3) The residue was dissolved in 2 mL methanol/water (50/50, v/v)	Bartolome *et al.*[5]
Beer	Acidity Polarity	1) 25 mL Beer acidified to pH 1.5 with HCL 37% was load into a Sep-Pak C18 (500 mg) cartridge 2) Phenolic compounds were eluted with 12 mL acetonitrile (the best for protocatechuic, gentisic, caffeic, *p*-coumaric, ferulic salicylic acids) 3) The extract was evaporated to dryness and the residue dissolved in 1 mL methanol/water (50/50, v/v) acidified with 1% acetic acid	Garcia *et al.*[7]
Beer	(A) Acidity and Polarity (B) Acidity (hydrolysis) and Polarity	**System (A):** 1) 0.5 mL Beer was mixed with 1 µg isoferulic acid (IS) and acidifed with 1 N HCl to pH 3.0 2) After addition of 300 mg NaCl, samples were extracted three times with ethyl acetate (4 volumes) by vortexing for 5 min. After each extraction, samples were centrifuged (3000*g*, 10 min) and supernatants collected. The organic phase was dried under N_2; 3) The residue was dissolved up to 0.1 ml methanol and vortexed for 5 min; then 0.4 ml of buffer (91.75/7/1.25, water/methanol/acetic acid, v/v) was added and samples were centrifuged at 17 500 g for 5 min **System (B) :** 1) 0.5 mL Beer was subjected to alkaline hydrolysis with 4.5 ml 2N NaOH containing 10 mM EDTA and 1% ascorbic acid at 30°C for 30 min, with 1 µg isoferulic acid (IS); 2) At the end of incubation, 0.5 ml sample were acidified to pH 3.0 with 4 N HCl, added with 300 mg NaCl and extract as reported for (A) 3) The residue was dissolved up to 0.1 ml methanol and vortexed for 5 min; then 0.4 ml of buffer was added and samples were centrifuged at 17 500 g for 5 min	Nardini and Ghiselli[13]

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Table 13
Extraction of flavonols from malt, hop, and beer

Sample	Fundamental basics	Successive steps	Reference
Hop	Polarity	1) 10 g Hop were grounded and stirred with 100 mL acetone/water (80/20, v/v) at 10°C under gentle stirring and N_2 for 60 min (3 times) 2) The combined extract were filtered and the solvent evaporated to 10 mL 3) The extract was then saturated with NaCl and two liquid phase obtained 4) The upper liquid phase was mixed with an equal volume of hexane 5) The subsequent lower liquid phase obtained contains flavonoids 6) The extract was then concentrated to dryness, diluted in 50 mL ethanol and load in the Sephadex LH20 column (100 × 2.5 cm) for 5 days with ethanol (50 mL/h). Two fraction were obtained (flavonols and flavanols) 7) A second purification was lead by means of a Sephadex G25 Superfine (30 × 1.5 cm) with increasing concentration of methanol in water.	McMurrough and McDowell[93] McMurrough *et al.*[2,38]
Hop	Polarity and Acidity (hydrolysis)	1) 10 g Hop were grounded and then refluxed with 150 mL of methanol/4N HCL (75/25, v/v) for 2h 2) After cooling, the powder was washed with 75 mL methanol (twice) 3) The combined extracts were made up to 400 mL with water and shaken with 100 mL hexane 4) The red phase was then adjusted to 500 mL with methanol	McMurrough *et al.*[2,38]
Hop	Polarity	1) 0.5 g Hop grounded and stirred with 50 mL of toluene (3 times) then with cyclohexane (3 times). Hop powder dried at the end under vacuum 2) Extraction with 40 mL ethanol/water (75/25, v/v) at 60°C (3 times) 3) The combined extract were filtered and the solvent evaporated to dryness and the residue solubilized in 2 mL methanol/water (50/50, v/v)	Callemien *et al.*[43]
Beer	Polarity Acidity (hydrolysis) Volatility (with derivatization)	1) 200 mL Degassed beer mixed with 2 g Nylon 66 resin for 1 min 2) Washing of the resin with water (4 × 5 mL) and elution with acetone/water (75/25, v/v; 6 x 5 mL) then methanol/water (60/40, v/v; 2 × 5 mL) 3) Pooling of the organic phases containing flavonols and concentration to dryness 4) The residue was mixed with HCl 2N and 4 mL ethanol and maintained under reflux for 1h at 100°C 5) After cooling, the hydrolysate was extracted with 20 mL ethyl acetate and 2 mL water and then three times with 10 mL ethyl acetate 6) Combined extract were concentrated to dryness, diluted in methanol/water and transferred into the Amberlite CG-50 column (12.5 × 1 cm) 7) Washing with 40 Ml water and then elution of the flavonols with 25 Ml methanol/ water (80/20, v/v) and again concentration to dryness and dilution in 1 Ml methanol containing 1g/L gallic acid followed by a concentration to dryness 8) The residue was dissolved in 100 µL pyridine and 200 µL BSTFA and placed at 60°C for 30 min	Vancraenenbroeck *et al.*[44]
Beer	—	Filtration on 0.22 µm and diluted one hundred times in water and analysis by HPLC	Achilli *et al.*[4]
Beer	—	Degassing of beer and centrifugation if necessary and analysis by HPLC	Sagesser and Deinzer[39]

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Table 14
Extraction of flavanoids from malt, hop, and beer

Sample	Fundamental basics	Successive steps	Reference
Barley	Polarity	1) 10 g Barley were ground and stirred with 100 mL acetone/water (80/20, v/v) at 10°C under gentle stirring and N_2 for 60 min (3 times) 2) The combined extract were diluted two-fold in water and filtered on 0.22 µm filter	Madigan *et al.*[57]
Barley	Polarity	**System (A)** for dimers: 1) 50 g Ground barley were extracted with 150 mL methanol under CO_2 atmosphere for 1h 2) The extract was then filtered and evaporated to 10 mL **System (B)** for dimers and trimers: 1) 50 g Ground barley were extracted with 150 mL acetone/water (75/25, v/v) under CO_2 atmosphere for 1h 2) The extract was then filtered and shaken with NaCl (5 g) for 10 min and stand for 1-2h 3) The upper acetone phase was evaporated until 7 mL and filtered	McMurrough *et al.*[58]
Barley Malt	Polarity	1) 4 g Ground barley or malt were stirred with 40 mL acetone/water (75/25, v/v) at room temperature for 1h (2 times) 2) The combined extracts were concentrated to 5 mL and load on the Polyamide column (20 × 1.4 cm) 3) Washing twice with 25 mL water 4) Elution of monomeric flavanols with 30 mL methanol	Friedrich and Galensa[50]
Malt	Polarity	1) 3 g ground malt were stirred with 11 mL acetone/water (40/60, v/v) at 80°C under N_2 2) Mix of the extract with 40 mL water and loading into the Polyamide (1000 mg) column 3) Washing with 8 mL water and with 1 mL n,n-dimethylformamide/water (85/15, v/v) 4) Elution with 2.5 mL n,n-dimethylformamide/water (85/15, v/v)	Zimmermann and Galensa[51]
Barley Hop	Polarity	1) 10 g Hop were grounded and stirred with 100 mL acetone/water (80/20, v/v) at 10°C under gentle stirring and N_2 for 60 min (3 times) 2) The combined extract were filtered and the solvent evaporated to 10 mL 3) The extract was then saturated with NaCl and two liquid phase obtained 4) The upper liquid phase was mixed with an equal volume of hexane 5) The subsequent lower liquid phase obtained contains flavonoids 6) The extract was then concentrated to dryness and diluted in 50 mL ethanol and load in the Sephadex LH20 column (100 × 2.5 cm) for 5 days with ethanol (50 mL/h) and two fraction obtained (flavonols and flavanols) 7) A second purification was obtained by means of a Sephadex G25 Superfine (30 × 1.5 cm) with increasing concentration of methanol in water	McMurrough[38]

(*Continued*)

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Table 14
(*Continued*)

Sample	Fundamental basics	Successive steps	Reference
Hop	Polarity	1) 100 g Hop stirred for 1h in CH_2Cl_2 and again washed three times with 1.5 L CH_2Cl_2 2) Hop was dried and ground 3) 72.4 g Hop were extracted three times with 1 L acetone/water (70/30, v/v). The combined extracts were filtered and concentrated 5) The resulting extract was washed with hexane (0.5 L × 2) and CH_2Cl_2 (0.5 L × 2) and again concentrated 6) The extract was load onto Sephadex LH20 (30 × 4 cm) and eluted with water (500 mL), methanol/water (500 mL, 1/3, v/v), methanol/water (500 mL, 1/1, v/v), methanol/water (500 mL, 3/1, v/v), methanol (500 mL), and acetone/water (500 mL, 7/3, v/v) at a flow rate of 100 mL/h 7) Specific fractions were pooled and lyophilized 8) The crude extract was load onto the second Sephadex LH20 (45 × 4 cm) and eluted with water (1L), methanol/water (1L, 1/1, v/v), methanol (1L), and acetone/water (1L, 7/3, v/v) at a flow rate of 100 mL/h Fraction 1: glycosides, fraction 2: flavan-3-ols and dimers, fraction 3: oligomers and fraction 4: polymers	Li and Deinzer[48,131]
Malt Hop	Polarity and Acidity	1) Barley, malt (30 g) or hop (20 g) ground were mixed with 100 mL acetone/water (75/25, v/v), filtrated and the filtrated mixed with chloroform 2) The aqueous upper phase was then adjusted to 100 mL with water and 25 mg ascorbic acid 3) The extract was then mixed with 0.5 g Polyamid for 1 min, transferred to a column, washed with water and eluted with acetone/water (75/25, v/v; 2 × 10 mL and 1 × 5 mL) 4) The eluent was evaporated to dryness and diluted in methanol/water (50/50, v/v)	Jerumanis[47,107] Mulkay *et al.*[59]
Beer	Polarity and acidity	1) 100 mL Degassed beer are added to 100 mL of HCl 1N, 300 mL mQ H_2O and to 500 mL of iso-octaneto eliminate the isohumulones 2) Extraction 3 times with 300 mL ethyl acetate to extract proanthocyanidins 3) Concentration of the extract and freeze-drying	Owades *et al.*[110,111]
Beer	Polarity Acidity Volatility (with derivatization)	1) 1L Degassed beer was filtered 4 times through a bed of nylon 66 2) The nylon was then shaken with ethyl acetate/acetone (1/1, v/v; total 200 mL) overnight, filtered and evaporated 3) After four beer extraction, the combined residues were dissolved in 10 mL methanol and load onto the first Sephadex LH20 (41 × 5 cm) 4) The polyhenols were eluted with methanol 5) Specific fractions were pooled, concentrated and again load on the second LH20 column (50 × 2.5 cm) eluted as described previously 6) Dried polyphenol (1–5 mg) was mixed with bis-trimethylsilylacetamide (0.1 mL) and placed at 60°C for 30 min The same was lead with trifluoroacetic anhydride (0.2 mL), benzene (0.1 mL) and pyridine (0.02 mL) and mixed for 20 min	Eastmond[61]

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Beer	Polarity	1) 125 mL Degassed beer load onto a Sephadex LH20 (30 × 1.5 cm) at a rate of 90 mL/h 2) Column washed for four hours with 360 mL water and 125 mL water/methanol (70/30, v/v) 3) Elution with methanol/acetone (80/20, v/v) and eluted fraction evaporated to dryness, redissolved in 2 mL methanol 4) The extract was then saturated with ammonium sulfate and washed with 20 mL light petroleum and then with ethyl acetate (4 × 20 mL) 5) The final extract was concentrated and dissolved in 1.5 mL methanol	Kirby and Wheeler[56]
Beer	(A) Polarity (B) Moelcular size	System(A): 1) 1L Degassed beer load onto a Sephadex LH20 at a rate of 200 mL/h 2) Column washed with 200 mL water 3) Elution with 500 mL acetone/methanol (50/50, v/v) and the fraction evaporated to 10 mL 4) The extract was then load in the Sephadex LH20 column, eluted with methanol (100 mL/h) and two fraction were obtained (simple flavanols and polymeric flavanols) System (B): Dialysis lead at 4°C with 5 changes of distilled water	McMurrough *et al.*[104]
Beer	Polarity	1) 200 mL Degassed beer were extracted with 80 mL then 25 mL of benzene to eliminate isohumulones 2) Extraction with 150, 100, and 75 mL of butanone-2 to extract proanthocyanidins 3) Concentration and freeze-drying	Derdelinckx[132]
Beer	Polarity and Acidity	1) 750 mL Degassed beer agitated with 35 g Sephadex LH-20 resin and transferred into a column 2) Elution with 250 mL acetone/water/acetic ac. (75/30/0.1, v/v) to recover proanthocyanidins 3) Freeze-drying and dissolution in acetonitrile/water/acetic ac. (75/25/0.1, v/v)	Whittle *et al.*[63]
Beer	Polarity	1) 10 L Beer were injected into the LH20 column (85 × 2.5 cm) 2) Elution with ethanol/water (5/95, v/v) 3) Elution with acetone/methanol (75/25, v/v) to elute simple flavanols 4) Elution with acetone/water (75/25, v/v) to recover proanthocyanidins 5) Elution with 500 mL of methanol	McMurrough and Baert[62]
Beer	—	Degassing of beer, filtration through 0.22 µm filter and analysis by HPLC direct injection	Madigan *et al.*[57]
Beer	Polarity	1) 20 mL Beer were load into a Sephadex LH-20 column (3 g, 6 × 1.5 cm) 2) Washing with 40 mL methanol/water (30: 70) to elute sugars and other phenols 3) Elution with 70 mL acetone/water (70: 30) to recover proanthocyanidins 4) Dry drying and dissolution in solvent of extraction	Gu *et al.*[133]

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Acidity. With their pK_a close to 10, it is easy to solubilize phenols either in water or in organic solvents, according to the pH. In this way, 4-vinylsyringol has been selectively separated from all other beer aromas before being recovered in dichloromethane (Table 11).[112] Likewise, acidification of a water extract containing the phenolate form of prenylflavonoids allows their recovery with an organic solvent (Table 15).[71,113] Acidification at higher temperature allows release of any bound phenolic forms, ensuring that all compounds are present in their free state (e.g., hydroxybenzoic or hydroxycinnamic acids and flavonols - Tables 12 and 13).[2,7,9,13,16,18,38,44] For proanthocyanidins, acidified aqueous acetone increases the extractability while making the hydrogen bonds with fibrous polar matrices weaker (Table 14).[63,114,115]

Many polyphenols are unstable at extreme pH. Degradation is usually observed at pH values above 6 (autoxidation); the degradation products are A-type dimers (double-linked flavan-3-ols with a single C4-C8 bond and an additional ether bond between C2 and O-C7).[116] A low pH can be a disadvantage when glycosides are investigated (e.g., flavonols, flavanoids, or stilbenes). Moreover, oligomers of procyanidins are known to depolymerize at a pH below 4 (e.g., procyanidin B2 yields epicatechin and B5 isomer).[116] Besides, at pH values below 4, enzymatic oxidation can take place, yielding to dimers linked by C-O interflavanic linkage.[117] As for stilbenes, *trans*-resveratrol is stable in absence of light between pH 1 and pH 7, but at pH 10 very fast degradation is observed; *cis*-resveratrol is stable only at pH 7.[118]

Volatility. Phenolic aromas can be extracted from malt, hop, and beer by taking advantage of their relatively high volatility. Vacuum distillation, headspace techniques (static, dynamic, purge and trap, SPME…) or a Likens-Nickerson apparatus can thus be used (Table 11).[28,31,36,119] On the other hand, flavanoids, and chalcones are not volatile enough to be extracted in this way, unless derivatized before extraction. For instance, beer resveratrol has been analyzed by headspace SPME, after derivatization with N-O-bis(trimethylsilyl)trifluoroacetamide- BSTFA (Table 16).[82,120] A few flavonols and flavanoids and a prenylflavanone have also been studied by this means (Tables 13–15).

Molecular Size. Various methods have been developed to separate polyphenols according to molecular size. This can be very useful in studying proanthocyanidin oligomers. Dialysis with different cut-offs has been used to separate higher oligomers of beer from small analogs (Table 14).[104] Size-exclusion chromatography (SEC) with gel permeation columns (GPC, e.g., Sephadex LH20 or TSK gel HW40 columns) has also been applied to apple polyphenols. It allows fractionation according to increasing Mw.[121–123]

Other Methods. In the last decade, enzymes degrading cell-wall polysaccharides have been used with success to improve phenol recovery.[124] Optimizing temperature (25–60°C) is also useful for better recovery.[43,125,126] For instance, gentle heating (60°C) allows better recovery of hop stilbenes[43]. For some phenols, it is recommended to operate in the absence of light (e.g., stilbenes, hydroxybenzoic and hydroxycinnamic acids) to avoid degradation.

Chromatographic Separation

Gas Chromatography (GC). Gas chromatography is usually preferred for separating volatile compounds like hydroxycinnamic-acid-derived flavors (Table 17). Linear retention indexes (calculated by comparison to the retention times of *n*-alkanes) are often checked

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Table 15

Extraction of prenylchalcones and derived flavanones from hop and beer

Sample	Fundamental basics	Successive steps	Reference
Hop	Polarity	1) Ground hop was suspended in methanol/formic acid (99.99/0.01, v/v) 2) Vortex for 1 min and stirred for 1h, the supernatant was collected and filtered prior the analysis	De Keukeleire *et al.*[66]
Hop	Polarity	1) Hop (5 g) were extracted under reflux and N_2 with petroleum ether (3 times with 200, 125 and 80 mL) and *n*-hexane (3 times with 125 mL) 2) The residue was then extracted under reflux and N_2 with methanol/water (75/25, v/v) for 2 h (3 times with 80 mL) 3) The methanolic extract was then partitioned with petroleum ether (2 times with 100 mL) and *n*-hexane (2 times with 100 mL) 4) Injection into the semi-prep. Biosil C18 HL 90-10 (250 × 10 mm, 10 µm) with a linear gradient of water/formic ac. (95/5, v/v) and methanol/acetonitrile (95/5, v/v)	Milligan *et al.*[113,134,135]
Hop	Polarity Acidity	1) Immersion in 2L CH_2Cl_2 for 2h of 300 g hop, filtration and evaporation of the solvent 2) Addition of 300 mL methanol/water (70/30, v/v) and stored overnight at – 20°C (precipitation of resinous and other lipophilic constituents) 3) Elimination of the precipitate and concentration to dryness of the filtrate 4) Dissolution of the residue in ethanolic potassium hydroxide (95/5, v/v) and reflux under N_2 for 30 min 5) Then the solution was poured into 200 mL HCL 5% and extracted with ethyl acetate (2 times) 6) The combined extracts were dried with Na_2SO_4 and concentrated 7) Purification step with a silica gel column using ethyl acetate/cyclohexane (90/10, v/v) for the obtention of isoxanthohumol	Milligan *et al.*[113]
Hop	Polarity	1) Immersion in $CHCl_3$ for 1 min and 2) The extract was filtered and the solvent evaporated 3) Addition of methanol boiled under reflux for 5 min and storage overnight at – 20°C (precipitation of resinous and lipophilic constituents) 4) Elimination of the precipitate and concentration of the filtrate 5) Purification step on a Sephadex LH 20 column using methanol and then isolation on silica gel or preparative HPLC columns	Stevens *et al.*[80] Miranda *et al.*[136]

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Table 15
(*Continued*)

Sample	Fundamental basics	Successive steps	Reference
Hop	Polarity	1) Immersion in acetone (twice) and 2) The combined extracts were filtered and the solvent evaporated 3) Purification on a LH20 Sephadex column using methanol 4) Separation again on a Sephadex LH20 or an preparative Econosil C18 columns	Stevens *et al.*[64]
Hop	Polarity and Acidity	1) Hop was grounded and extracted with methanol (with and without formic acid 1%) and then filtration of the methanolic extract	Stevens *et al.*[64,65,70]
Ethanol hop extract	Polarity	1) Elimination of the alpha and beta acids with CO_2 at 50°C 280 bar 2) Mixing with Kieselguhr and obtention of an hop extract enriched product	Biendl *et al.*[69]
Ethanol hop extract	Polarity	1) Dissolution of the ethanolic hop extract in methanol/CH_2CL_2 (50/50, v/v) and injection into the Sephadex LH 20 column 2) Elution with methanol/CH_2CL_2 (80/20, v/v) 3) Separation of the different constituents on a silica gel column with hexane/EtOAc gradient	Gerhauser *et al.*[79]
Beer	Polarity Volatility (with derivatization)	1) Centrifugation of 20 mL beer at 3600 rpm and 2) Addition of acetate buffer and injection into the C18 column 3) Washing with water (twice) and then elution with methanol (3 mL twice) 4) Drying in a water bath at 40°C under N_2 and dissolution of the residue in water/methanol (96/4, v/v) 5) Loading into the second column and elution with diethyl ether/ethyl acetate (50/50, v/v) 6) Dry-drying and dissolution of the residue into methanol 7) Evaporation of the methanol at 60°C under N_2 and addition of BSTFA with 1% of trimethylchlorosilane 8) Heating for 1h at 60°C and then analysis of 8-prenylnaringenin	Tekel' *et al.*[81]
Beer	—	1) Beer was degassed, diluted in ethanol/water (5/95, v/v) and addition of the internal standard	Stevens *et al.*[65,70]
Beer	Polarity	1) 200 mL Degassed beer with *o*-phosphoric acid 85% injected into the Baker-bond SPE octadecyl C18 (1000 mg) column 2) Washing with water containing *o*-phosphoric acid (99.8/0.2, v/v) 3) Elution with methanol/water/ *o*-phosphoric acid (89.9/ 9.9/ 0.2, v/v)	Walker *et al.*[67] Buckee[137] (EBC method 7.8)

Table 16
Extraction of stilbenes from hop and beer

Sample	Fundamental basics	Successive steps	Reference
Hop	Polarity	1) 0.5 g Hop powder was stirred with 50 mL of toluene (3 times) then with cyclohexane (3 times). Hop powder dried at the end under vacuum 2) Extraction of the residue with 40 mL ethanol/water (75/25, v/v) at 60°C (3 times) 4) The combined extract were filtered and the solvent evaporated to dryness and the residue solubilized in 2 mL methanol/water (50/50, v/v)	Callemien *et al.* [43] Jerkovic *et al.* [77]
Beer	Polarity and volatility (with derivatization)	1) SPME fiber (polyacrylate 85 μm) immersed in 6 mL beer at ambient temperature under gentle stirring for 30 min 2) Fiber then transferred in the headspace of a 100 μL of a BSTFA solution for 20 min 3) Then fiber desorbed at 280°C for 7 min	Jerkovic *et al.*[82]

on apolar and polar columns to confirm identification. Less volatile compounds such as flavonols, flavanoids, prenylflavanones, or stilbenes can be derivatized with bis-trimethylsilylacetamide, trifluoroacetic acid, or N-O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) before GC analysis (Tables 19–22). Derivatization is also sometimes used for highly sensitive detections such as electron capture detection (Table 17).

High Pressure Liquid Chromatography (HPLC). HPLC is the method of choice for polyphenol separation (Tables 18–22). Reversed-phase columns can separate nearly all phenolic compounds. Binary elution systems are used with an aqueous acidified polar medium (aqueous acetic acid, phosphoric acid, or formic acid) as solvent A and a less polar organic system (methanol, acetonitrile, etc., possibly acidified) as solvent B. Occasionally, buffers are used. For hydroxybenzoic and hydroxycinnamic acids, isocratic systems are sometimes used. In reversed phase, most polar compounds elute first. Benzoic acid derivatives elute before cinnamic acid analogs (Table 18). An additional sugar moiety decreases the retention time (e.g., chlorogenic acid vs. caffeic acid—Table 18, rutin vs. quercetin—Table 19, *trans*-piceid vs. *trans*-resveratrol—Table 22), whereas methoxy groups slow down the elution (e.g., vanillic acid vs. syringic acid—Table 18). Catechin elutes before epicatechin (Table 20). Catechin is preceeded by 4α-8 and 4α-6 trimers and dimers of prodelphinidins and procyanidins but followed by dimers and trimers containing epicatechin (e.g., procyanidins B4, B2, and C1). Overlapping of heavy isomers having the same DP can generate broad peaks.[138] Although reversed-phase chromatography can be

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Table 17

Chromatographic analysis of hydroxybenzoic acids and hydroxycinnamic acids derived compounds from malt, hop, and beer

Sample	Method	Column	Vol (μl)	Gradient	Elution order	Reference
Malt	GC	SE-54 apolar (1) and FFAP polar (2) (25 m × 0.32 mm, 0.5 μm) 2 mL/min	0.5	Carrier gas helium Injector: 35°C (on-column) Oven program: (1) 35°C, 2 min; 35–150°C; 4°C/min; 150–240°C, 10°C/min (2) 35°C, 2min; 35–60°C, 40°C/min; 60–230°C, 6°C/min; 230°C, 10 min	4-Vinylguaiacol $RI_{SE\text{-}54} = 1312$ and $RI_{FFAP} = 2200$	Fickert and Schieberle[36]
Hop	GC	CPSIL-8CB apolar (1) and FFAP polar (2) (30 m × 0.32 mm, 0.5 μm) 2.2 mL/min	0.5	Carrier gas helium Injector: 35°C (on-column) Oven program: (1) 35°C, 2 min; 35–50°C; 40°C/min; 50°C, 2 min; 50–180°C, 6°C/min; 180–240°C, 10°C/min (2) 35°C, 2 min; 35–60°C; 40°C/min; 60°C, 2 min; 60–180°C, 6°C/min; 180–240°C, 10°C/min	4-Vinylguaiacol $RI_{CPSIL\text{-}8CB} = 1315$ and $RI_{FFAP} = 2200$	Steinhaus and Schieberle[28]
Beer	GC	Glass column 3% OV1 (1) (28 m × 2 mm) 30 mL/min Capillary column OV1 (2) (50 m × 0.3 mm)	1	(1) Carrier gas nitrogen Injector: 280°C Oven program: Isothermal at 105°C (2) Carrier gas helium Oven program: 80°C, 15 min; 80–200°C, 2°C/min	1. Ethylphenol, 2. 4-Methylguaiacol, 3. 4-Ethylguaiacol, 4. 4-Vinylguaiacol, 5. Eugenol (IS), 6. Vanillin	Thurston and Tubb[127]

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Beer	GC after derivatization	Glass column 5% SE-30 on Chromosorb (3.6 m × 2 mm) 30 mL/min	1	Carrier gas nitrogen Injector: 200°C Oven program:100°C, 8 min; 100–140°C, 10°C/min; 140°C, 12 min	After derivatization with heptafluorobutyric acid anhydride: 1. 4-Vinylguaiacol, 2. Eugenol	Villareal *et al.*[33]
Beer	GC	SE-54 and OV-1701 (apolar) (30 m × 0.32 mm, 0.3 µm) 2.2 mL/min	0.5	Carrier gas helium Injector: 35°C (on-column) Oven program: 35°C, 2 min; 35–50°C, 40°C/min; 50°C, 5 min; 50–240°C, 6°C/min	4-Vinylguaiacol $RI_{SE-54} = 1161$ and $RI_{OV-1701} = 1258$	Schieberle[31]
Beer	GC	CPSIL-5CB apolar (50 m × 0.32 mm, 1.2 µm) FFAP polar (25 m × 0.32 mm, 0.3 µm) 1 mL/min	1	Carrier gas helium Injector: 250°C Oven program: 36–85°C, 20°C/min; 85–145°C, 1°C/min; 145–250°C, 3°C/min; 250°C, 30 min	4-Vinylguaiacol $RI_{CPSIL-5CB} = 1289$ 4-Vinylsyringol $RI_{CPSIL-5CB} = 1532$ and $RI_{FFAP} = 2809$	Callemien *et al*[112]
Beer	GC	SE-54 apolar (1) and FFAP polar (2) (30 m × 0.32 mm, 0.25 µm) 2.5 mL/min	0.5	Carrier gas helium Injector: 35°C (on-column) Oven program: (1) 35°C, 2 min; 35-50°C; 40°C/min; 50°C, 2 min; 50–180°C, 6°C/min; 180–230°C, 20°C/min, 230°C, 10 min (2) 35°C, 2 min; 35–60°C; 40°C/min; 60°C, 2 min; 60–180°C, 6°C/min; 180–230°C, 10°C/min, 230°C, 10 min	4-Vinylguaiacol $RI_{SE-54} = 1317$ and $RI_{FFAP} = 2206$	Fritsch and Schieberle[128]

— = Not mentioned; and RI = Retention index.

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Table 18

Chromatographic analysis of hydroxybenzoic acids and hydroxycinnamic acids from malt and beer

Sample	Method	Column	Vol (µl)	Gradient	Elution order	Reference
Barley	RP-HPLC	Zorbax 300 SB-C18 (250 × 4.6 mm, 5 µm) 1 mL/min Column = 20°C	10	A) Water/ Acetic ac. (99.9/0.1, v/v) B) Methanol/ Acetic ac. (99.9/0.1, v/v) Gradient from A to B: 0–15 min, 5–20% B; 15–35 min, 20–40% B; 35–42 min, 40–65% B; 42–50 min, 65–80% B	1. Gallic acid, 2. Protocatechuic acid, 3. Vanillic acid, 4. Caffeic acid, 5. Syringic acid, 6. *p*-Coumaric acid, 7. Ferulic acid	Zhao *et al.*[108]
Wort Beer	RP-HPLC	Nucleosil C18 (250 × 4 mm, 10 µm) 1 mL/min Column = 20°C	25	Isocratic System 1: Water/Methanol/ Phosphoric ac. (540/450/10, v/v) with a run time of 25 min System 2: Water/Methanol/ Phosphoric ac. (640/350/10, v/v) with a run time of 60 min	Same elution order for both but retention times increased by two fold for 2: 1. Gallic acid, 2. Protocatechuic acid, 3. *p*-Hydroxybenzoic acid, 4. Vanillic acid, 5. Syringic acid, 6. Vanillin, 7. Phenol, 8. Guaiacol, 9. *p*-Coumaric acid, 10. Ferulic acid, 11. *m*-Coumaric acid, 12. *o*-Coumaric acid, 13. 4-Methylguaiacol, 14. 4-Vinylphenol, 15. 4-Vinylguaiacol, 16. Cinnamic acid, 17. 4-Ethylguaiacol	Madigan and McMurrough[30] McMurrough *et al.*[17]
Wort Beer	RP-HPLC	Nucleosil C18 (250 × 4 mm, 10 µm) 1 mL/min Column = 20°C	40	Isocratic: Water/Methanol/Phosphoric ac. (640/350/10, v/v)	1. Ferulic acid, 2. 4-Vinylguaiacol	Coghe *et al.*[21,129]

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Wort Beer	RP-HPLC	Nucleosil 100-10 C18 (250 × 4 mm) 1 mL/min	10	Isocratic Water/Methanol/Phosphoric ac. (745/245/10, v/v)	1. *p*-Hydroxybenzoic acid, 2. Vanillic acid, 3. Vanillin, 4. Acetovanillon, 5. *p*-Coumaric acid, 6. Ferulic acid, 7. Sinapic acid, 8. 4-Vinylphenol, 9. 4-Vinylguaiacol, 10. 4-Ethylguaiacol	Vanbeneden *et al.*[18]
Beer	Anion exchange HPLC	ZIPAX SAX (500 × 2.1 mm) 0.49 mL/min Column = 25°C	25	Isocratic 1M Acetate buffer (pH 4.7)/ 0.25 M Citric acid (pH 1.8)/Water (1/1/8, v/v)	1. Syringic acid, 2. Vanillic acid, 3. Gallic acid, 4. Sinapic acid, 5. Ferulic acid, 6. *p*-Coumaric acid, 7. Caffeic acid, 8. Gentisic acid	Kenyhercz and Kissinger[16]
Beer	RP-HPLC	Nucleosil 10 C18 (250 × 4.6 mm) 2 mL/min	25	A) Water/ Acetic ac. (96.5/3.5, v/v) B) Methanol Linear gradient from A to B: 0-30 min, 0-50% B	1. Gallic acid, 2. Protocatechuic acid, 3. *p*-Hydroxybenoic acid, 4. Vanillic acid, 5. Caffeic acid, 6. Syringic acid, 7. *p*-Coumaric acid, 8. Ferulic acid, 9. Sinapic acid	Hayes *et al.*[9,130]
Beer	RP-HPLC	HR 80 (80 × 4.6 mm, 3 μm) 1 mL/min Column = 37°C	10	A) 34.7 μM SDS, 0.1 M phosphate buffer, 50 nM nitrilotriacetic acid in Water/Methanol (50/50, v/v) pH 3.45 B) 173 μm SDS, 0.1 M phosphate buffer, 50 nM nitrilotriacetic acid in Water/Methanol (50/50, v/v) pH 3.45 Gradient from A to B: 0–10 min, 5% B; 10–15 min, 5–15% B; 15–20 min, 15–40% B; 20–25 min, 40% B; 25–35 min, 40–100% B; 35–40 min, 100% B	1. Gallic acid, 2. Gentisic acid, 3. Protocatechuic acid, 4. *p*-Hydroxybenzoic acid, 5. Vanillic acid, 6. Caffeic acid, 7. Chlorogenic acid, 8. Vanillin, 9. Syringic acid, 10. Syringaldehyde, 11. *p*-Coumaric acid, 12. Ferulic acid	Achilli *et al.*[4]

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Table 18
(*Continued*)

Sample	Method	Column	Vol (μl)	Gradient	Elution order	Reference
Beer	RP-HPLC	Inertsil ODS-3V (250 × 4.6 mm, 5 μm)	10	A) 34.7 μM SDS, 0.1 M phosphate buffer (pH 3.35), 50 nM nitrilo-acetic acid in Water/Methanol (50/50, v/v) B) 173 μM SDS, 0.1 M phosphate buffer (pH 3.35), 50 nM nitrilo-acetic acid in Water/Methanol (50/50, v/v) Gradient form A to B: 0–10 min, 6% B; 10–30 min, 6–24% B; 30–40 min, 24–70% B; 40–45 min, 100% B	-	Montanari *et al.*[12]
Beer	RP-HPLC	Nova-Pack C18 (300 × 3.9 mm) 1.2 mL/min	60	A) Water/Acetic ac. (98/2, v/v) B) Water/Acetonitrile/Acetic ac. (78/20/2, v/v) Gradient from A to B: 0–55 min, 80% B; 55–57 min, 90% B; 57–70 min, 90% B; 70-80 min, 95% B; 80-90 min, 100% B	1. *p*-Hydroxybenzoic acid, 2. Vanillic acid, 3. Caffeic acid, 4. Vanillin, 4. *p*-Coumaric acid, 5. Sinapic acid, 6. Ferulic acid	Bartolome *et al.*[5]

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Beer	RP-HPLC	Inertsil ODS-3V (250 × 4.6 mm, 5 μm) 0.4-0.9 mL/min	100	A) 0.05 M KH_2PO_4 and 0.5 mm SLS B) Phase A/Methanol/Acetonitrile (30/20/50, v/v) Both adjusted to pH 3.5 with 85% orthophosphoric acid Linear increase and decrease gradient from A to B: 0–5 min, 15–20% B; 5–8 min, 20% B; 8–15 min, 20–15% B; 15–25 min, 15% B; 25–45 min, 15–50% B; 45–46.1 min, 50–100% B; 46.1–53 min, 100% B And linear increase and decrease flow rate: 0–9 min, 0.9 mL/min; 9–9.1 min, 0.9–0.4 mL/min; 9.1–26.1 min, 0.4–0.9 mL/min; 26.1–26.5 min, 0.9 mL/min; 26.5–39.4 min, 0.9–0.5 mL/min; 39.4–39.5 min, 0.5 mL/min; 39.5–44 min, 0.5–0.9 mL/min; 44–53 min, 0.9 mL/min	1. Gallic acid, 2. Protocatechuic acid, 3. *p*-Hydroxybenzoic acid, 4. Gentisic acid, 5. Chlorogenic acid, 6. Vanillic acid, 7. Caffeic acid, 8. *m*-Hydroxybenzoic acid, 9. Syringic acid, 10. *p*-Coumaric acid, 11. Ferulic acid, 12. Sinapic acid, 13. *m*-Coumaric acid, 14. *o*-Coumaric acid	Floridi *et al.*[6]

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Table 18
(*Continued*)

Sample	Method	Column	Vol (µl)	Gradient	Elution order	Reference
Beer	RP-HPLC	Water Symmetry C19 (150 × 4.6 mm, 5 µm) 1 mL/min Column = 20°C	20	A) Water/Acetic ac. (99/1, v/v) B) Methanol/Acetic ac. (99/1, v/v) Gradient from A to B: 0–5 min, 10% B; 5–35 min, 50% B; 35–43min, 50% B	1. Gallic acid, 2. Protocatechuic acid, 3. Gentisic acid, 4. Caffeic acid, 5. *p*-Coumaric acid, 6. Ferulic acid	Garcia *et al.*[7]
Beer	RP-HPLC	Supelcosil LC-18 C18 (250 × 4.6 mm, 5 µm) 1 mL/min Column = 30°C	50	A) Water/ Acetic ac. (98.75/ 1.25, v/v) B) Methanol Gradient from A to B: 0–25 min, 2–24% B; 26–45 min, 24% B; 46–53 min, 24–45% B; 54–55 min, 45% B	1. Gallic acid, 2. Protocatechuic acid, 3. Hydroxybenzoic acid, 4. Vanillic acid, 5. Chlorogenic acid, 6. Caffeic acid, 7. Syringic acid, 8. *P*-Coumaric acid, 9. Ferulic acid, 10. Sinapic acid, 11. *O*-Coumaric acid	Nardini and Ghiselli[13]

— = Not mentioned.

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Table 19

Chromatographic analysis of flavonols from hop and beer

Sample	Method	Column	Vol (µl)	Gradient	Elution order	Reference
Hop	RP-HPLC	µBondapack C18 (300 × 3.9 mm) 2 mL/min Column = 30°C	5–50	A) Water/Acetic ac. (97.5/2.5, v/v) B) Tetrahydrofuran Linear gradient from A to B: 0–30 min, 0–50% B	1. Quercetin triglycoside, 2. Quercetin-3-neohesperidoside, 3. Rutin (quercetin-3-O-rutinoside), 4. Isoquercitrin (quercetin-3-O-glucoside), 5. Kaempferol rutinoside, 6. Quercitrin (quercetin-3-O-rhamnoside), 7. Kaemferol glucoside, 8. Quercetin 1. Myricetin, 2. Quercetin, 3. Kaempferol	McMurrough *et al.*[2,38]
Hop	RP-HPLC	Protein and peptide C18 (250 × 1 mm, 5 µm) 40 µL/min	—	A) Water/Acetic ac. (99.9/0.1, v/v) B) Acetonitrile/Acetic ac. (99.9/0.1, v/v) Gradient from A to B: 0–5 min, 20% B; 5–35 min, 20–100% B	—	Sagesser and Deinzer[39]
Hop	RP-HPLC	Prevail C18 (150 × 2.1 mm, 2 µm) 0.2 mL/min Column = 30°C	10	A) Water/Acetonitrile/Formic ac. (98.9/1/0.1, v/v) B) Acetonitrile Linear gradient from A to B: 0–23 min, –45% B; 23–30 min, 45–100% B; 30–40, 100% B	1. Rutin, 2. Myricetin, 3. Quercetin, 4. Kaempferol	Callemien *et al.*[43]

(*Continued*)

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Table 19
(*Continued*)

Sample	Method	Column	Vol (µl)	Gradient	Elution order	Reference
Beer	GC after derivatization	Glass column 2.5% OV-1 Chromosorb GAW-DMCS (0.25″ × 6′) 30 mL/min	2	Carrier gas nitrogen Injector: 330°C Oven program: 200–350°C, 5°C/min	After derivatization with N-O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) 1. Gallic acid (IS), 2. Kaempferol, 3. Quercetin, 4. Myricetin	Vancraenenbroeck *et al.*[44]
Beer	RP-HPLC	HR 80 (80 × 4.6 mm, 3µm) 1 mL/min Column = 37°C	10	A) 34.7 µM SDS, 0.1 M phosphate buffer, 50 nM nitrilotriacetic acid in Water/Methanol (50/50, v/v) pH 3.45 B) 173 µm SDS, 0.1 M phosphate buffer, 50 nM nitrilotriacetic acid in Water/Methanol (50/50, v/v) pH 3.45 Gradient from A to B: 0–10 min, 5% B; 10–15 min, 5–15% B; 15–20 min, 15–40% B; 20–25 min, 40% B; 25–35 min, 40–100% B; 35–40 min, 100% B	1. Rutin, 2. Kaempferol	Achilli *et al.*[4]

— = Not mentioned.

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Table 20
Chromatographic analysis of flavanoids from malt, hop, and beer

Sample	Method	Column	Vol (µl)	Gradient	Elution order	Reference
Malt	RP-HPLC	Phenomenex Aqua RP-18 (150 × 4.6 mm, 3 µm) 1 mL/min/Column =30°C	20	A) 0.02 M NaH_2PO_4 at pH 3.4 with phosphoric acid B) 0.1M NaH_2PO_4 at pH 3/ Water/ Acetonitrile (1/1/4, v/v) Linear gradient from A to B: 0–35 min, 0–8% B; 35–50 min, 8–12% B; 50–60 min, 12–30% B; 60–65 min, 30–100% B; 65–75 min, 100% B	1. Prodelphinidin C (GC-4α-8-GC-4α-8-GC), 2. Prodelphinidin B3, 3. Prodelphinidin C (GC-4α-8-C-4α-8-C), 4. Prodelphinidin C (C-4α-8-GC-4α-8-C), 5. Procyanidin B3, 6. Catechin, 7. Procyanidin C2	Zimmermann and Galensa[51]
Hop	RP-HPLC	Synergi Hydro-RP-80 A (250 × 4.6 mm, 4 µm) 0.8 mL/min	—	A) Water/Acetic ac. (99/1, v/v) B) Methanol Linear gradient from A to B: 0–50 min, 5–50% B	1. Prodelphinidin C (GC-4α-8-GC-4α-8-C), 2. Prodelphinidin C (C-4α-8-GC-4α-8-C), 3. Prodelphinidin B3 (GC-4α-8-C), 4. Prodelphinidin B (GC-4α-6-C), 5. (+)-Gallocatechin, 6. Prodelphinidin B (C-4α-8-GC), 7. Prodelphinidin B (C-4α-6-GC), 8. Procyanidin C2, 9. Procyanidin B3, 10. Procyanidin B1, 11. Procyanidin B4, 12. (+)-Catechin 13. Propelargonidin B (afzelechin-4α-8-C), 14. Procyanidin B2, 15. Procyanidin C (EC-4α-8-EC-4α-8-C), 16. (-)-Epicatechin	Li and Deinzer[48,131]

(*Continued*)

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Table 20
(*Continued*)

Sample	Method	Column	Vol (µl)	Gradient	Elution order	Reference
Hop	RP-HPLC after acid-catalyzed cleavage	(1) Wakosil II 5 C18 (250 × 4.6 mm, 5 µm) 1 mL/min (2) Lichrospher RP-18 (250 × 3.2 mm, 5 µm) 0.65 mL/min	—	A) Water/Acetic ac. (99/1, v/v) B) Methanol Gradient from A to B: (1) 0–10 min, 5% B; 10–30 min, 5–20% B; 30–55 min, 20–40% B; 55–65 min, 100% B or (2) 0–35 min, 20–70% B; 35–40 min, 100% B	1) With phloroglucinol: 1. Ascor-bic acid, 2. Phloroglucinol, 3. Gallocat-echin-(4β-2)-phloroglu-cinol, 4. Cat-echin-(4α-2)-phloro-glucinol, 5. Catechin-(4β-2)-phloroglucinol, 6. (+)-Catechin, 7. Epicatechin gallate -(4β-2)- phloroglucinol, 8. (-)-Epicat-echin, 9. Epicatechin gallate (2) With benzylmercaptan: 1. (+)-Cat-echin, 2. (-)-Epicatechin, 3. Epicat-echin gallate, 4. Gallocat-echin-(4β-2)-benzylmercaptan, 5. Catechin-(4α-2)-benzylmer-captan, 6. Catechin-(4β-2)-ben-zylm-ercaptan, 7. Epicatechin gallate-(4β-2)-benzylmercaptan, 8. Benzyl mercaptan	Kennedy and Jones(142)
Hop	RP-HPLC after acid-cata-lyzed cleavage	Two Chromolith RP-18e (100 × 4.6 mm, 5 µm) con-nected in series 3 mL/min/Column = 30°C	—	A) Water/Acetic ac. (99/1, v/v) B) Acetonitrile/Acetic ac. (99/1, v/v) Gradient from A to B: 0–4 min, 3% B; 4–14 min, 3–18% B; 14–16 min, 80% B	1. Ascorbic acid, 2. Phloroglucinol, 3. Gallocatechin-(4β-2)-phlorogluci-nol, 4. Catechin-(4α-2)-phlorogluci-nol, 5. Catechin-(4β-2)-phloroglucinol, 6. (+)-Catechin, 7. Epicatechin gallate-(4β-2)-phloroglu-cinol, 8. (-)-Epicatechin, 9. Epicatechin gallate	Kennedy and Taylor[144] Taylor *et al.*[145]

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Hop	RP-HPLC after acid-catalyzed cleavage	Synergi C18 (250 × 4.6 mm, 4 μm) 0.8 mL/min	—	A) Water/Acetic ac. (99/1, v/v) B) Methanol Gradient from A to B: 0–10 min, 5% B; 10–30 min, 5–20% B; 30–55 min, 20–40% B; 55–65 min, 100% B	1. (+)-Catechin, 2. (-)-Epicatechin, 3. Epicatechin gallate, 4. Gallocatechin-(4β-2)-benzylmercaptan, 5. Catechin-(4α-2)-benzylmercaptan, 6. Catechin-(4β-2)-benzylmercaptan, 7. Epicatechin gallate-(4β-2)-benzylmercaptan, 8. Benzyl mercaptan	Li and Deinzer[48]
Hop	RP-HPLC after acid-catalyzed cleavage	Spherex (250 × 4.6 mm, 4 μm) 1 mL/min	—	A) Water/Acetic ac. (99/1, v/v) B) Methanol Linear gradient from A to B: 0–50 min, 0-40% B	1. (+)-Catechin, 2. (-)-Epicatechin, 3. Epicatechin gallate, 4. Gallocatechin-(4β-2)-benzylmercaptan, 5. Catechin-(4α-2)-benzylmercaptan, 6. Catechin-(4β-2)-benzylmercaptan, 7. Epicatechin gallate-(4β-2)-benzylmercaptan, 8. Benzyl mercaptan	Stevens *et al.*[49]
Malt	RP-HPLC	LiChroCART Superspher 100 (250 × 4 mm, 4 μm) 0.8 mL/min/Column = 30°C	—	A) 0.02 M NaH_2PO_4 at pH 3.4 with phosphoric acid B) 0.1M NaH_2PO_4 at pH 3/Water/Acetonitrile (1/1/4, v/v) Linear gradient from A to B: 0–40 min, 5–32% B; 40–45 min, 32–90% B	1. Catechin –7-O-β-D-glucopyranoside, 2. (+)-Catechin	Friedrich and Galensa[50]

(*Continued*)

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Table 20
(*Continued*)

Sample	Method	Column	Vol (µl)	Gradient	Elution order	Reference
Barley Hop	RP-HPLC	µBondapack C18 (300 × 3.9 mm) 2 mL/min / Column = 30°C	5–5	A) Water/Acetic ac. (97.5/2.5, v/v) B) Methanol Linear gradient from A to B: 0–20 min, 0–35% B	1. Prodelphinidin B3, 2. Procyanidin B3, 3. Properlargonidin, 4. (+)-Catechin, 5. (-)-Epicatechin	McMurrough[38]
Barley Beer	RP-HPLC	Nucleosil C_{18} (300 × 4.6 mm,10 µm) 1 mL/min	10	A) Water/Acetic ac. (97.5/2.5, v/v) B) Water/Acetic ac. (90/10, v/v) Linear gradient from A to B: 0–60 min, 0–100% B	1. Prodelphinidin B3, 2. Procyanidin B3, 3. (+)-Catechin, 4. (-)-Epicatechin	Madigan *et al.*[57] McMurrough *et al.*[58]
Malt Beer	RP-HPLC	Zorbax SB-C18 (150 × 4.6 mm, 5µm) 1 mL/min / Column = 22°C	—	A) Water/Acetic ac. (99.9/0.1, v/v) B) Acetonitrile/Acetic ac. (99.9/0.1, v/v) Linear gradient from A to B: 0–80 min, 2–10% B; 80–140 min, 10–25% B; 140–150 min, 25–100% B 150–160 min, 100% B	1. Catechin, 2. Epicatechin	Whittle *et al.*[63]
Barley Hop Beer	RP-HPLC	µBondapack C18 (300 × 3.9 mm) 2 mL/min / Column = 30°C	5–50	A) Water B) Water/Acetic ac. (90/10, v/v) Linear gradient from A to B: 0–60 min, 0–100% B	1. Prodelphinidin B3, 2. Procyanidin B3, 3. Properlargonidin, 4. (+)-Catechin, 5. (-)-Epicatechin	McMurrough *et al.*[104]

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Malt Hop Beer	RP-HPLC	Sil C_{18}HL (250 × 4 mm, 10 µm) 1 mL/min	20	A) Water B) Water/Acetic ac. (90/10, v/v) Linear gradient from A to B: 0–100 min, 50–100% B	1. Prodelphinidin C (GC-4α-8-GC-4α-8-C), 2. Prodelphinidin B3, 3. Prodelphinidin C (GC-4α-8-C-4α-8-C), 4. Prodelphinidin C (C-4α-8-GC-4α-8-C), 5. Procyanidin B3, 6. Procyanidin C2, 7. (+)-Catechin, 8. Procyanidin B6, 9. Procyanidin B2, 10. (-)-Epicatechin, 11. Procyanidin C1	Jerumanis[47,107] Mulkay *et al.*[59] Derdelinckx[132]
Beer	GC with derivatization	Glass column 25% silica oil (1) (2.5 ft × 0.25 in) (2) (9.0 ft × 0.25 in) 45 mL/min	5	Carrier gas nitrogen (1) Oven program: 250–350°C, 20°C/min (2) Oven program: 100°C, 5 min; 100–300°C, 20°C/min	(1) Derivatization with Bis-trimethylsilylacetamide: 1. Catechin 2. Procyanidin B3 (2) After derivatization with Trifluoroacetic acid: same elution	Eastmond[61]
Beer	RP-HPLC	Spherisorb S5-ODS (250 × 4 mm) 1 mL/min / Column = 26°C	20	A) 0.075 M Potassium dihydrogen orthophosphate B) Methanol Linear gradient from A to B: 0–35 min, 2–80% B	1. Prodelphinidin B3, 2. Procyanidin B3, 3. (+)-Catechin, 4. (-)-Epicatechin	Kirby and Wheeler[56]

(*Continued*)

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Table 20
(*Continued*)

Sample	Method	Column	Vol (µl)	Gradient	Elution order	Reference
Beer	RP-HPLC	Nucleosil C_{18} (300 × 4 mm, 10 µm) 1 mL/min	100	A) Water/Acetic ac. (97.5/2.5, v/v) B) Water/Acetic ac. (90/10, v/v) Linear gradient from A to B: 0–60 min, 0–100% B	1. Prodelphinidin B3, 2. Procyanidin B3, 3. (+)-Catechin, 4. (-)-Epicatechin	McMurrough and Baert[62]
Beer	RP-HPLC after acid-catalyzed cleavage	Luna C18 (250 × 4.6 mm, 5 µm) 1 mL/min / Column = 25°C	—	A) Water/Acetic ac. (98/2, v/v) B) Methanol Gradient from A to B: 0–45 min, 15–80% B	1. (+)-Catechin, 2. (-)-Epicatechin, 3. Epicatechin gallate, 4. Gallocatechin-(4β-2)-benzylmercaptan, 5. Catechin-(4α-2)-benzylmercaptan, 6. Catechin-(4β-2)-benzylmercaptan, 7. Epicatechin gallate-(4β-2)-benzylmercaptan, 8. Benzyl mercaptan	Gu *et al.*[133,140,146]
Beer	NP-HPLC	Phenomenex Luna Silica (250 × 4.6 mm, 5 µm) 1 mL/min	—	A) Dichloromethane B) Methanol C) Acetic ac./Water (1/1, v/v): 4% C constant Linear gradient from A to B: 0–20 min, 14–23.6% B; 20–50 min, 23.6–35% B; 50–55 min, 35–86% B; 55–65 min, 86% B	Order in function of the degree of oligomerization 1. Monomers, 2. Dimers, 3. Trimers, 4. Tetramers, etc…	Gu *et al.*[133]

— = Not mentioned; GC = gallocatechin; C = catechin; and E = epicatechin.

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Table 21
Chromatographic analysis of prenylchalcones and derived flavanones from hop and beer

Sample	Method	Column	Vol (μl)	Gradient	Elution order	Reference
Hop	RP-HPLC	Lichrospher RP-18 (250 × 4 mm, 5 μm) 1mL/min	20	A) Acetonitrile/Water (60/40, v/v) B) Acetonitrile/Formic ac. (99/1, v/v) Linear gradient from A to B: 0–40 min, 40–80% B	1. Isoxanthohumol, 2. 8-Prenylnaringenin, 3. Xanthohumol C, 4. Desmethylxanthohumol, 5. 6-Prenylnaringenin, 6. Xanthohumol, 7. 3′-Geranylnaringenin, 8. Xanthohumol B, 9. 5′-Prenylxanthohumol	Stevens *et al.*[80]
Hop	RP-HPLC	Omnisphere C18 (250 × 4.6 mm, 5μm) 1mL/min Column = 35°C	20	A) Water/Formic ac. (99.975/0.025, v/v) B) Methanol/Formic ac. (99.975/0.025, v/v) Gradient from A to B: 0–3min, 45% B; 3–32 min, 45–95% B; 32–37min, 95% B	1. Xanthohumol, 2. Desmethylxanthohumol, 3. Cohumulone, 4. Humulone+Adhumulone, 5. Colupulone, 6. Lupulone+Adlupulone	De Keukeleire *et al.*[66]
Hop Beer	RP-HPLC	Phenomenex C18 (250 × 4 mm, 5μm) 0.8 mL/min	20	A) Water/Formic ac. (99/1, v/v) B) Acetonitrile Linear gradient from A to B: 0–15 min, 0–100% B; 15–20 min, 100%	1. Isoxanthohumol, 2. 8-Prenylnaringenin, 3. Dexmethylxanthohumol, 4. 2′, 4-Dihydrochalcone, 5. 6-Prenylnaringenin, 6. Xanthohumol, 7. 8-Geranylnaringenin, 8. 3′-Geranylnaringenin, 9. 6-Geranylnaringenin	Stevens *et al.*[64,65,70,147] Walker *et al.*[67]
Beer	GC after derivatization	HP-5MS Fused silica capillary column (30 m × 0.25 mm, 0.25 μm) 1mL/min	2	Carrier gas helium Injector (280°C) Oven program: 70°C, 1 min; 70–200°C, 40°C/min; 200–280°C, 10°C/min	After derivatization with N-O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) 1. Kaempferol (IS), 2. 8-Prenylnaringenin	Tekel' *et al.*[81]
Beer	RP-HPLC	Nucleodur C18 (125 × 4 mm, 5μm) 1mL/min Column = 35°C	—	A) Methanol/Water/o-Phosphoric ac. (75/24/1, v/v) B) Methanol Linear gradient from A to B: 0–17 min, 0% B; 17–25min, 35% B	—	Walker *et al.*[67] Buckee[137] (EBC method 7.8)

— = Not mentioned.

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Table 22
Chromatographic analysis of stilbenes from hop and beer

Sample	Method	Column	Vol (µl)	Gradient	Elution order	Reference
Hop	RP-HPLC	Prevail C18 (150 × 2.1 mm, 2 µm) 0.2 mL/min Column = 30°C	10	A) Water/Acetonitrile/Formic ac. (98.9/1/0.1, v/v) B) Acetonitrile Linear gradient from A to B: 0–23 min, 5-45% B; 23–30 min, 45–100% B; 30–40, 100% B	1. *trans*-Piceid, 2. *cis*-Piceid, 3. *trans*-Resveratrol, 4. *cis*-Resveratrol	Callemien *et al.*[43] Jerkovic *et al.*[77]
Beer	GC after derivatization	CPSIL-5CB apolar (50 m × 0.32 mm, 1.2 µm) 1 mL/min	1	Carrier gas helium Injector: 250°C Oven program: 100–250°C, 10°C/min; 250°C, 30 min	After derivatization with BSTFA: 1. *cis*-Resveratrol, 2. *trans*-Resveratrol	Jerkovic *et al.*[82]

used to separate isomers of equal Mw, a normal phase is preferred for analyzing higher oligomers up to decamers.(106,133,139) (Table 20) In this case, elution sequence parallels the polymerization degree (monomers then dimers, and so forth). The solvent gradient is tertiary, including dichloromethane, methanol, and acid acetic(133) or acetonitrile, methanol, and water.(139) The nature of aglycons and sugars is often determined after acidic or β-glucosidase hydrolysis.(2,44) For proanthocyanidins, acid-catalyzed cleavage of the interflavanyl linkage in the presence of a nucleophilic reagent such as toluene-α-thiol(133, 140–143) or phloroglucinol (142) can be carried out before HPLC. Extension units combined with the nucleophile are released. Only the terminal unit is detected as a free flavan-3-ol (Fig. 5). Phloroglucinol adducts are more polar than the original structures, whereas benzylmercaptan adducts elute later on a reversed-phase column (Table 20). Thiolysis is often recommended because of a better depolymerization yield.(143) Yet several factors, such as impurities or reagent storage, can deteriorate the reaction yield.(141) Terminal and extension units are quantified and an average polymerization degree (mDP) is calculated.

Selective Detection

HPLC-UV Absorption and Fluorimetry. HPLC can be combined with UV-absorption or fluorimetry to detect phenols. Hydroxybenzoic and hydroxycinnamic acids are known to absorb at 254, 280, 306, and 330 nm. Their decarboxylated analog, 4-vinylguaiacol, has been also analyzed by UV-fluorescence (λexc = 259 nm, λem = 341 nm) (Table 23).

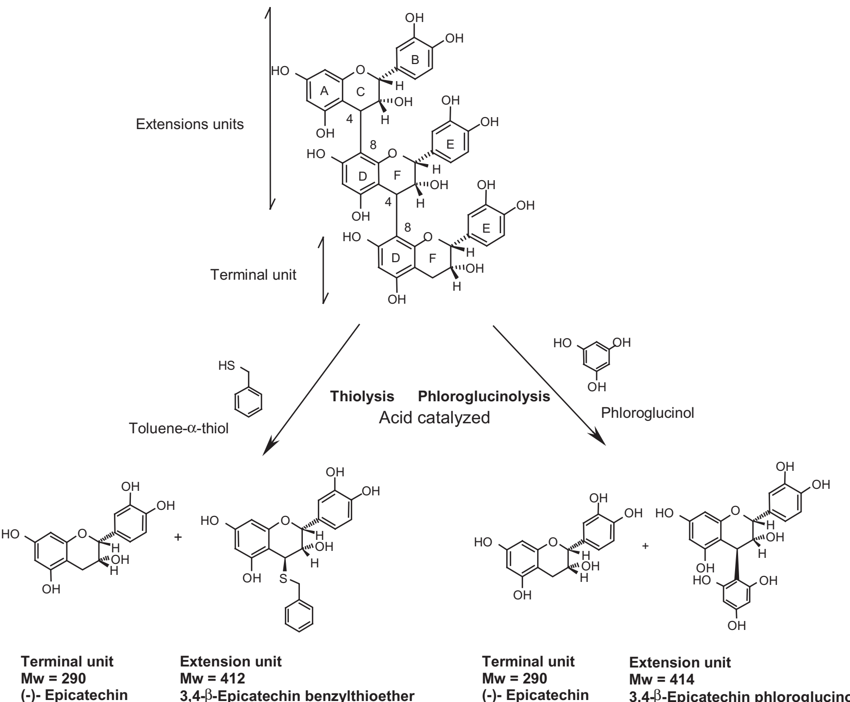

Figure 5. Chemistry of the phloroglucinol and thiolysis reactions.

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Table 23
Detectors used for the analysis of hydroxybenzoic acids, hydroxycinnamic acids derived compounds from malt, hop, and beer

Sample	Method	Detectors	Reference
Malt	GC	MS (EI and CI) FID Olfactometry/AEDA	Fickert and Schieberle[36]
Hop	GC	MS (EI and CI) FID Olfactometry/AEDA	Steinhaus and Schieberle[28]
Wort Beer	RP-HPLC	UV absorption at λ =280 nm Fluorescence λ exc = 259 nm and λem = 341 nm for 4-Vinylguaiacol ECD vs. Ag/AgCl as reference electrode: 900 mV (output range 20 nA) for 4-Vinylguaiacol and Ferulic acid	Madigan and McMurrough[30] McMurrough *et al.*[17]
Beer	GC	FID MS (EI)	Thurston and Tubb[127]
Beer	GC after derivatization	Ni electron-capture detector	Villareal *et al.*[33]
Beer	GC	MS (EI and CI) FID Olfactometry/AEDA	Schieberle[31]
Beer	GC	MS (EI and CI) FID Olfactometry/AEDA	Fritsch and Schieberle[128]
Beer	GC	MS (EI and CI) FID Olfactometry/AEDA	Callemien *et al.*[112]

Two absorption bands are characteristic of flavonoids: band II of the A-ring (max. 240-285 nm) and band I of the B-ring (max. 300–550 nm). Flavonols are quantified by UV absorption at 320 or 365 nm (Table 25). Flavan-3-ol monomers and oligomers have often been quantified at 280 nm (Table 26). It is generally accepted that the same weight of proanthocyanidin generates the same UV absorption, whatever the polymerization degree.[148] Chemical reaction with *p*-dimethylaminocinnamaldehyde, leading to colored adducts with the A-ring of flavan-3-ols, allows more sensitive detection at 640 nm[93]. Fluorescence has also been used to quantify flavan-3-ol derivatives (λexc = 276 nm, λem = 316 nm). UV-absorption has proved useful for distinguishing prenylchalcones (λ = 370 nm) from derived flavanones (λ = 270–295 nm) (Table 27). Stilbenes are analyzed at 306 nm (Table 28).

HPLC-Electrochemical Detection (ECD). ECD is a highly sensitive tool for the characterization of antioxidant phenols (good sensitivity and much greater selectivity).[57] ECD has been used extensively for the study of hydroxybenzoic acids, hydroxycinnamic acids, flavonols, and flavanoids (Tables 23–26).

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Table 24
Detectors used for the analysis of hydroxybenzoic acids, hydroxycinnamic acids from malt, hop, and beer

Sample	Method	Detectors	Reference
Barley	RP-HPLC	UV absorption at λ = 280 nm and 254 nm	Zhao *et al.*[108]
Wort	RP-HPLC	UV absorption at λ = 280 nm	Madigan and McMurrough[30]
Beer		Fluorescence λexc = 259 nm and λem = 341 nm for 4-Vinylguaiacol ECD vs. Ag/AgCl as reference electrode: 900 mV (output range 20 nA) for 4-Vinylguaiacol and Ferulic acid	McMurrough *et al.*[17]
Wort Beer	RP-HPLC	ECD: +900 mV vs. Ag/AgCl as reference electrode (output range 20 nA)	Coghe *et al.*[21,129]
Wort Beer	RP-HPLC	ECD: +1200 mV vs. Ag/AgCl as reference electrode	Vanbeneden *et al.*[18]
Beer	Anion exchange HPLC	ECD vs. SCE as reference electrode : + 800mV	Kenyhercz and Kissinger[16]
Beer	RP-HPLC	UV absorption at 254 nm ECD vs. Ag/AgCl as reference electrode = + 1000 mV	Hayes *et al.*[9,130]
Beer	RP-HPLC	ECD with CEAS = Coulochem electrode array system with sixteen coulometric electrodes vs. Pd as reference electrode: – 250 mV (electrode 1), + 60 mV (electrode 2) with an increment of + 60mV until + 840 mV at electrode 15 and -250 mV at electrode 16	Achilli *et al.*[4]
Beer	RP-HPLC	UV (DAD) absorption from 190 to 800 nm ECD with CEAS = Coulochem electrode array system with eight coulometric electrodes vs. Pd as reference electrode: 0 to + 905 mV with an increment of + 120 mV	Montanari *et al.*[12]
Beer	RP-HPLC	UV (DAD) absorption from 210 to 400 nm	Bartolome *et al.*[5]
Beer	RP-HPLC	UV-Vis (DAD) absorption from 190 to 800 nm ECD with CEAS = Coulochem electrode array system with eight coulometric electrodes vs. Pd as reference electrode: +100 to +905 mV with an increment of 105 mV	Floridi *et al.*[6]
Beer	RP-HPLC	UV absorption at λ =280 nm for Gallic acid 306 nm for Caffeic acid, *p*-Coumaric acid 330 nm for Gentisic acid	Garcia *et al.*[7]
Beer	RP-HPLC	ECD : +600mV (sensitive range 200 nA)	Nardini and Ghiselli [13]

Table 25
Detectors used for the analysis of flavonols from hop and beer

Sample	Method	Detectors	Reference
Hop	RP-HPLC	UV absorption at λ =365 nm	McMurrough *et al.*[2,38]
Hop	RP-HPLC	UV absorption at λ = 320 nm ESI(-)-MS/MS with a triple quadrupole	Sagesser and Deinzer[39]
Hop	RP-HPLC	APCI(+)-MS/MS with an ion trap	Callemien *et al.*[43]
Beer	GC after derivatization	FID	Vancraenenbroeck *et al.*[44]
Beer	RP-HPLC	ECD* (sixteen coulometric electrodes) : - 250 mV, + 60 mV with an increment of 60mV until 840 mV at electrode 15 and -250 mV at electrode 16	Achilli *et al.*[4]

GC and HPLC-Mass Spectroscopy (MS). GC and HPLC separation techniques are more and more frequently hyphenated to MS. The mass analyzers can be quadrupoles, ion traps, or time-of-flight systems. Various analysis modes exist such as Full-MS, SIM, MS/MS, and MRM. For very selective quantitative analyses, SIM or MRM is recommended (at least for a triple quadrupole), but MS/MS is more informative for structure determination.

Volatile compounds (e.g., 4-vinylguaiacol) can easily be identified and quantified after electron impact (EI, complete fragmentation pattern) or chemical ionization (CI, intense pseudomolecular ion) at the outlet of the GC column (Table 23).

Flavonols, flavanoids, prenylchalcones, and stilbenes require more recent techniques hyphenated to HPLC. Atmospheric pressure chemical ionization (APCI) is recommended for apolar and low-molecular-weight compounds, whereas electrospray ionization (ESI) is more convenient for polar and higher-molecular-weight structures. For flavonols, the positive mode in ESI or APCI has frequently been applied (Table 25). Flavanoids have been studied in the negative and positive modes by ESI and in positive mode by APCI (Table 26). MALDI (+) was applied with success to the analysis of proanthocyanidins in apple[149] and coffee pulp[150] with higher DP values. Prenylchalcones and flavanones cannot easily be distinguished by EI, CI, or APCI(+ or -) because of thermal isomerization of 2′-hydroxychalcones to their corresponding flavanones in the ion source.[80] Structural information is then obtained by specific fragmentation in MS/MS mode (Table 27). APCI in positive mode proves to be the most efficient way to analyze stilbenes (Table 28).[43]

GC-Olfactometry. For volatile phenols, GC-olfactometry is often applied before subsequent more usual GC-FID or GC-MS analyses. Traces of aroma-active compounds can indeed be detected in the bulk of “nonactive” volatiles, just by using the human nose at the outlet of the GC column. To obtain quantitative data, the AEDA (Aroma Extract Dilution Analysis) strategy can be applied (Table 23).

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Table 26
Detectors used for the analysis of flavanoids from malt, hop, and beer

Sample	Method	Detectors	Reference
Barley	RP-HPLC	UV absorption at 280 nm	McMurrough[38]
Malt	RP-HPLC	UV (DAD) absorption at $\lambda = 280$ nm	Zimmermann and Galensa[51]
Malt	RP-HPLC	UV absorption at $\lambda = 280$ nm ECD with CEAS = Coulochem electrode array system with eight coulometric electrodes vs. Pd as reference electrode: 0-770 mV (increment 110 mV)	Friedrich and Galensa[50]
Hop	RP-HPLC after acid catalysed cleavaged	UV absorption at $\lambda = 280$ nm Fluorescence λexc = 276 nm and λem = 316 nm ESI(-)-MS/MS with an ion trap	Gu *et al.*[133]
Hop		UV absorption at $\lambda = 280$ nm ESI(+)-MS/MS with a triple quadrupole	Kennedy and Jones[142] Kennedy and Taylor[144] Taylor *et al.*[145]
Hop		UV absorption at $\lambda = 280$ nm	Li and Deinzer[48]
Hop		ESI(+)-MS/MS with a triple quadrupole	Stevens *et al.*[49]
Hop	RP-HPLC	UV absorption at $\lambda = 280$ nm APCI(+)-MS/MS with a triple quadrupole ESI(+)-MS/MS with a triple quadrupole	Li and Deinzer[48,131]
Barley Beer	RP-HPLC	ECD (dual) : + 350 mV and - 650 mV	Madigan *et al.*[57] Mc Murrough *et al.*[58]
Malt Beer	RP-HPLC	UV absorption at $\lambda = 280$ nm ECD (dual): + 350 mV and – 650 mV ESI(+)-MS/MS with a triple quadrupole	Whittle *et al.*[63]
Hop Barley Beer	RP-HPLC	UV absorption at 280 nm	McMurrough *et al.*[104]
Hop Malt Beer	RP-HPLC	UV absorption at $\lambda = 280$ nm	Jerumanis[47,107] Mulkay *et al.*[59] Derdelinckx[132]
Beer	GC after derivatization	FID	Eastmond [61]
Beer	RP-HPLC	UV absorption at 283 nm	Kirby and Wheeler[56]

(*Continued*)

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Table 26
(*Continued*)

Sample	Method	Detectors	Reference
Beer	RP-HPLC	ECD (single) : 850 m VECD (dual) : + 10 mV and + 350 mV	McMurrough and Baert[62]
Beer	NP-HPLC	UV absorption at λ = 280 nm Fluorescence λ exc = 276 nm and λem = 316 nm ESI(-)-MS/MS with an ion trap	Gu *et al.*[133,140,146]

Table 27
Detectors used for the analysis of prenylchalcones and derived flavanones from hop and beer

Sample	Method	Detectors	Reference
Hop	RP-HPLC	UV absorption (DAD) λ xanthohumol and desmethylxanthohumol = 370 nm λ derived flavanones = 270-295 nm λα- and β- acids = 314 nm	De Keukeleire *et al.*[66]
Hop	RP-HPLC	Direct MS (EI) via a solid probe APCI(+ and -)-MS/MS with a triple quadrupole	Stevens *et al.*[80]
Hop beer	RP-HPLC	APCI(+)-MS/MS (MRM mode) with a triple quadrupole Ions selected for 1. Isoxanthohumol *m/z* 355 → 179, 2. 8-Prenylnaringenin *m/z* 341 → 165, 3. Dexmethylxanthohumol *m/z* 341 → 165, 4. 2′, 4-Dihydrochalcone *m/z* 241 → 121, 5. 6-Prenylnaringenin *m/z* 341 → 165, 6. Xanthohumol *m/z* 355 → 179, 7. 8-geranylnaringenin *m/z* 409 → 165, 8. 3′-geranylnaringenin *m/z* 409 → 165, 9. 6-geranylnaringenin *m/z* 409 → 165	Stevens *et al.*[64,65,70,147] Walker *et al.*[67]
Beer	GC with deriva-tization	MS (EI) in SIM mode Ions selected for Kaemferol (IS): 559, 487, 458, 415 *m/z* and for 8-Prenylnaringenin : 556, 541, 513, 485 *m/z*	Tekel' *et al.*[81]
Beer	RP-HPLC	UV absorption (DAD) λxanthohumol = 370 nm, λ isoxanthohumol = 290 nm, λα- and β- acids = 270 nm	Walker *et al.*[67] Buckee[137] (EBC method 7.8)

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Table 28
Detectors used for the analysis of stilbenes from hop

Sample	Method	Detectors	Reference
Hop	RP-HPLC	UV absorption $\lambda = 306$ nm APCI(+)-MS/MS with an ion trap	Callemien *et al.*[43] Jerkovic *et al.*[151]
Beer	GC after derivatization	MS (EI) in SIM mode on 444 *m/z*	Jerkovic *et al.*[82]

Organoleptic Properties, Antioxidant Activity, Colloidal Instability and Health-Impact of Phenolic compounds: Fate of them through Beer Aging

Aroma

Hydroxybenzoic and hydroxycinnamic acids are characterized by relatively high flavor thresholds (> ppm, mainly bitter taste and astringency—see Table 30).[152] On the other hand, their decarboxylated derivatives can impart very strong phenolic/clove/smoked flavors to beer, because of their low threshold values (ppb order—Table 29). 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).[29,31,34,153]

Table 29
Organoleptic properties and thresholds of hydroxycinnamic acids derived compounds

Phenolic compounds	Organoleptic characteristics	Threshold in beer (mg/l)
4-Vinylguaiacol	Clove, phenolic, bitter	0.25*; 0.30** and ***
4-Ehylguaiacol	Clove, phenolic, sweet	0.13* and **
4-Methylguaiacol	Medicinal, burned	0.20*
Guaiacol	Phenolic, burned	0.70*
Eugenol or 4-Allylguaiacol	Clove, dental, disinfectant	0.20*
Isoeugenol or 4-Propenylguaiacol	Clove, dental, disinfectant	0.10*
Vanillin	Vanilla	0.50*
Acetovanillone	Vanilla	0.50*
4-Vinylphenol	Phenolic, bitter, astringent	0.20*
4-Ethylphenol	Cresol	0.10*
4-Methylphenol	Medicinal, phenolic	0.20*
Phenol	Phenolic, cresol	0.30*
4-Vinylsyringol	Smoked, burned	0.50*
4-Ethylsyringol	Smoked, burned	0.50*
4-Methylsyringol	Smoked, burned	0.50*
4-Propylsyringol	Smoked, burned	0.50*
4-Allylsyringol	Smoked, burned	0.50*
4-Propenylsyringol	Smoked, burned	0.25*
Syringol and syringaldehyde	—	—

— = Not determined; * [3]; ** [152]; *** [11].

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Table 30
Organoleptic properties and thresholds of various phenolic compounds in beer*, in 5% aqueous ethanol** (adapted from[188]) and in water ***[148]

Phenolic compounds	Organoleptic characteristics	Threshold (mg/L)
Hydroxybenzoic acids		
4-Hydroxybenzoic acid	Bitter	20* and 2**
Protocatechuic acid	Bitter, astringent	>50* and 5**
Gallic acid	Astringent	50* and 5**
Vanilic acid	Astringent	20* and 10**
Syringic acid	Bitter	10* and 5**
Gentisic acid	Bitter, astringent	20* and 2**
Hydroxycinnamic acids		
p-Coumaric acid	Astringent	50* and 5**
Caffeic acid	Astringent, bitter	20* and 2**
Ferulic acid	Astringent	20* and 10**
Sinapic acid	Bitter, bitter-sweet	20* and 5**
Flavonols		
Quercetin	Bitter	20* and 10**
Kaempférol	—	50* and 5**
Myricetin	—	10* and 10**
Flavanoids		
(+)-Catechin	Bitter, astringent	20*, 5**, 1***
(-)-Epicatechin	Bitter, astringent	1***
Stilbenes		
trans-resveratrol	Bitter,astringent	1***

— = Not determined.

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According to its concentration, 4-vinylguaiacol can lead either to strong pharmaceutical off-flavor defects[14] or to pleasant clove flavors,[19] whilst 4-vinylphenol is always considered to be an off-flavor.[19]

Many papers have stressed the importance of a few compounds responsible for aged-beer off-flavors. Special attention has focused on *trans*-2-nonenal.[154–156] Yet depending on the beer type and storage conditions, other defects may be more pronounced, such as the typical onion-odor imparted by dimethyltrisulfide[157] or the "light-skunky" off-flavor caused by 3-methyl-2-butene-1-thiol.[158] More rarely, β-damascenone (red fruit-like[159–161]), methional (potato-wort odor [157,162,163]), 2-furfuryl ethyl ether (solvent-like[164–166]) and 2-mercaptoethylacetate (roasted flavour [167]) can be responsible for consumer disappointment.

Regarding phenolic flavors in aged beer, very few data are available. Degradation of 4-vinylguaiacol through natural aging (25% after 20 days) or at 40°C (50% after 20 days) has been reported.[17,31,162] This compound could be partially transformed to 4-ethylguaiacol, vanillin, and guaiacol.[19,168]

Recently, we used AEDA methodology applied to aged lager beers to investigate an interesting old-beer-like phenolic odorant (FD value as high as that of *trans*-2-nonenal in aged beer).[112,160] Phenol-specific extractions, GC cold trapping, and mass spectrometry enabled us to identify it as 4-vinylsyringol. 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 (Fig. 6).

Higher-weight beer phenols like flavonoids most probably also influence beer aging by acting as antioxidants against reactive oxygen species (ROS).[169] Yet hydroxyl radicals react mainly with ethanol, the second most abundant compound in beer, to produce 1-hydroxyethyl radicals.[170] The level of free radicals in aged beer increases with increasing temperature and increasing iron/copper ion and oxygen concentrations.[171] In beer containing sulfites, free radicals are generated after a definite time period, called the ''lagtime''.[172–174]

The impact of phenols in the bottle is very ambiguous. Of course they protect sensitive fractions such as isohumulones and sulfites from oxidation.[109] Unfortunately, they are also progressively degraded to oxidized analogs with unexpected properties.[175] A decrease in (+)-catechin, (-)-epicatechin, prodelphinidin, and procyanidin B3 concentrations has been reported by Moll *et al.*,[46] McMurrough *et al.*,[176] Vanderhaeghen *et al.*,[175] and Callemien and Collin.[52] It was observed either after accelerated aging at 37°C or after a 6-month natural aging period. The loss was higher during the first four to five weeks but continued at a decreased rate for a long time. Dimeric flavanols disappeared more rapidly than monomers.[46,176]

In contrast, after a lag period of about 5 weeks, levels of tannoids (phenols titrated by PVP) began to increase.[102] Polymerization of small flavonoids to tannoids could be induced by acetaldehyde (excreted by yeast or issued from ethanol oxidation) through formation of ethyl bridges between flavanols.[177] Opening of oxidized phenol rings has been proposed as an alternative mechanism of degradation.[178] In one study, lager beer aging experiments conducted with a stable non-radioactive oxygen isotope ($^{18}O_2$) made it possible to visualize the incorporation of oxygen. ^{18}O was present in 6.5% of the polyphenols after 5 days at 40°C but in only 0.6% after 9 months at 20°C.[109] Especially in natural aging, huge amounts of ^{18}O isotope were recovered in the water fraction, indicating that polyphenols were also oxidized to quinones. In the same way, recent studies show a strong decrease in xanthohumol (48%) and isoxanthohumol (6%) concentrations in beer after an 8-month dark storage at 22°C.[179]

The protective capacity of phenols is much more obvious upstream in the brewing process. Many papers have demonstrated the huge impact of flavanoid enrichment when used to prevent enzymatic and radicalar linoleic acid oxidation in wort. Appropriate selection of raw materials (e.g., lipoxygenase-free barley) or exogenous polyphenol enrichment makes it possible to control synthesis of *trans*-2-nonenal and nonenal potential (*trans*-2-nonenal bound to proteins: a yeast-resistant molecule) during mashing and boiling. In this way, the cardboard off-flavor can be minimized in aged lager beers.[156, 180–182]

Figure 6. Potential synthesis pathways leading to 4-vinylsyringol in beer (adapted from [112]).

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Bitterness and Astringency

Bitterness (mainly brought by hop iso-α-acids in the case of beer) is perceived on the tongue, whereas astringency is characterized by drying, roughing, and puckering of the mucosal surface in the mouth. Astringency is not a taste but a tactile feeling due to non-adaptation.[183,184] It is not always perceived immediately, but evolves after swallowing [183] and increases upon repeated ingestion.[185] Saliva contains proline-rich proteins that lubricate the mouth. A wide range of compounds can bind to these proteins: salts of multivalent metallic cations, dehydrating agents, acids, and polyphenols.[184] This leads to formation of insoluble complexes, a decrease in salivary lubrication properties, and the perception of astringency.[184,186,187] In beer, phenolic acids, flavonols, flavanoids, and stilbenes could be responsible for astringency.[148,188] No data are available in the literature concerning prenylchalcone astringency. Polyphenol thresholds lie between 1 and 50 ppm,[148,188] with often higher values for the beer matrix (Table 30).

Sugars reduce polyphenol astringency[184,189] whilst oxidation may enhance it by increasing the polymerization degree (DP) of polyphenols.[190,191] If the DP is too high, however, precipitation may occur, making the beverage less astringent. Astringency is intensified at low pH, especially near 4.0–4.2,[192] but a higher astringency has been measured by François *et al.*[193] in beers with a pH close to 5. In this case, it was suspected that the pH of the samples fell in the mouth to a value of 4.4 before polyphenol/protein interactions occurred. As regards the effect of beer temperature, astringency may be higher at 21°C than at 7°C,[194] but a more recent paper mentions no significant difference.[189]

As depicted in Table 31, the perception descriptors used for (+)-catechin and (-)-epicatechin are quite contradictory, either bitter or astringent.[188,195–198]

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 (Fig. 7).[175] 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).[193]

In both cases, an increase in DP (global assay) and a decrease in total flavanoids was mentioned, especially at higher temperature or pH, in the presence of air (Fig. 8).[175,193]

Table 31
Descriptors used for flavan-3-ols

			Perceptions	
	Matrix	Spiked Concentrations (ppm)	Bitter	Astringent
	Wine	1200[a]	+	-
		1500[b]	-	+
(+)-Catechin	Water	46[c]	+	-
		100[d]	+	-
	Gazeous water	100[d]	+	+
(-)-Epicatechin	Wine	*500[e]	+	+
		900[f]	+	+

* = Natural concentration (ppm); + = sensation perceived; - = sensation not perceived; a.[197]; b.[196]; c.[195]; d. unpublished results of our group; e.[188]; f.[198].

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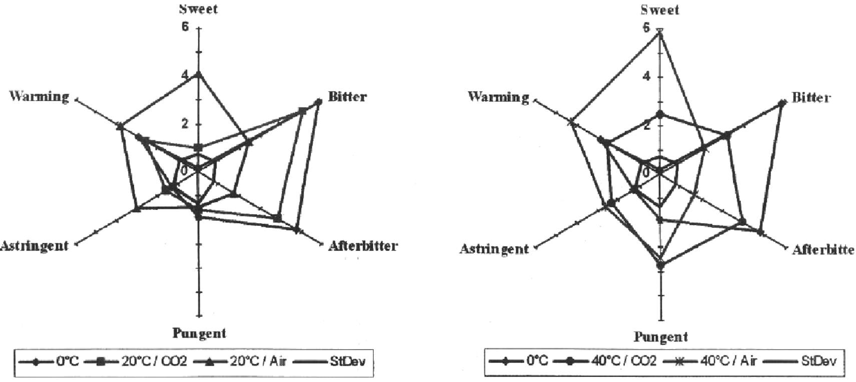

Figure 7. Sensory profiles of a beer stored for 6 months.[175]

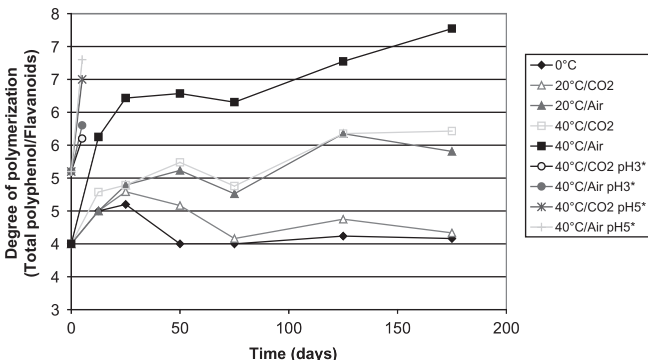

Figure 8. Evolution of the degree of polymerization (total polyphenols/total flavanoids ratio) during beer aging (based on the results of [175, 193*]).

Antioxidant Activity

Polyphenols contribute up to 60% of the endogenous reducing capacity of unstabilized beers.[176] They can react with reactive oxygen species (superoxide anion $O_2^{-\cdot}$<hydroxyl radical $OH^{\cdot}$) to produce phenoxy radicals which will further react by radical coupling reactions to give stable compounds. They can also act as antioxidants through their capacity to complex metal ions. Unfortunately, some polyphenols can act as pro-oxidants by transferring electrons to metal ions.[199] There is some controversy concerning the relevance of polyphenolic antioxidants in beer. In two ESR lag phase studies,[200,201] polyphenols appeared to have no significant effect on free radical formation. This was attributed to the extreme reactivity of hydroxyl radicals with prominent beer compounds like ethanol.

As depicted in Fig. 9, flavonols (e.g., quercetin), flavan-3-ols (e.g., catechin), and hydroxycinnamic acids (e.g., caffeic acid and ferulic acid) are significantly more potent than ascorbic acid (vitamin C) as inhibitors of AAPH (2,2′-azobis(2-amidinopropane)dihydrochloride) – induced peroxidation of an aqueous linoleic acid dispersion.[106,202] Resveratrol displays a lesser antioxidant activity. Procyanidin oligomers are by far the most interesting compounds in this respect, although different authors have obtained somewhat conflicting

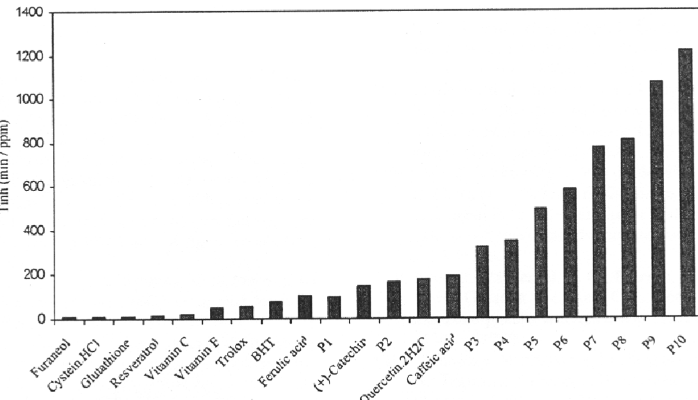

Figure 9. Antioxidant efficiency of ten purified chocolate procyanidin fractions and twelve commercial antioxidants.[106]. P1 to P10 = monomer to decamer of (+)-catechin and/or (-)-epicatechin.

data regarding the effect of the DP on the antioxidant efficiency. Some have found the antioxidant activity per gram of these compounds to increase with increasing DP in both aqueous [106] and lipidic systems.[203] In the aqueous phase, for instance, 1 g hexamer displayed a 6-times higher antioxidant activity than 1 g monomer.[106] In another study,[204] however, the antioxidant efficiency was found to decrease with increasing DP in lipidic medium, whilst in aqueous phase it increased from monomer to trimer, then dropped for the tetramer.

In lipidic systems, proanthocyanidin gallates and glycosides show lower antioxidant activity than in aqueous medium.[204,205] The antioxidant activity of 4-6 linked B dimers is higher than that of 4-8 linked dimers,[206] while A-type dimers are less antioxidant than B-type dimers.[204]

As regards prenylchalcones, the compounds xanthohumol, desmethylxanthohumol, and 3′-geranylchalconaringenin appear to protect LDL effectively from *in vitro* oxidation. Prenylflavanones show poor antioxidant activity while non-isoprenylated chalcones and flavanones act as pro-oxidants.[136]

Source of Colloidal Instability

Colloidal instability due to interactions between polyphenols and proteins limits the shelf life of beer. Fig. 10 shows the haze scale established for brewers.

Beer contains less haze-active polyphenols than haze-active proteins [207]. Derived from barley hordeins, haze-active proteins (10 kD-30 kD) are acidic hydrophilic polypeptides, rich in both proline and glutamic acid [208] and glycosylated.[209]

Flavan-3-ol monomers do not induce haze as strongly as higher polymers (Table 32). Among dimers, procyanidin B3 and prodelphinidin B3 are very strong haze inducers, especially the latter.[58,176,210,211] Procyanidin trimer C2 is even more haze-active. The polymerization degree appears to be more determinant than the number of hydroxyl groups [211]. Phenolic acids and flavonols do not participate in beer haze formation (Table 32).[212,213] No similar data are available in the literature for stilbenes, prenylchalcones, or derived flavanones.

When haze-active proteins and haze-active polyphenols are combined in a buffered model system,[207,214] haze rises, peaks, and then declines as the concentration of haze-active agents increases. The pH also has a huge impact. Much more haze is produced near

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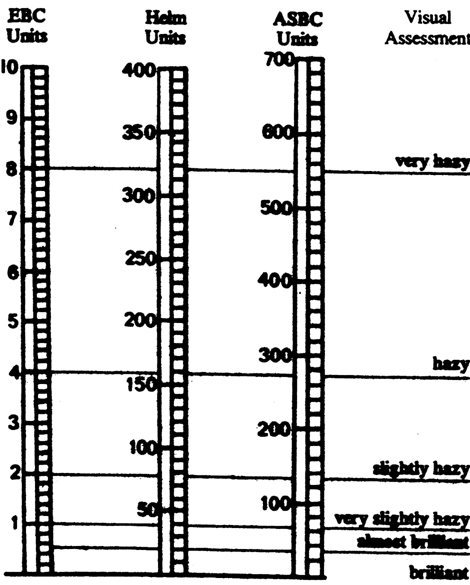

Figure 10. Equivalent scales of haze measurement in beer (1EBC = 4 NTU = 40 Helm = 69 ASBC).

Table 32
Haze-forming capacities of various phenolic compounds (adapted from[212])

Phenolic compound	Haze-forming capacity (EBC F.U.)
Hydroxybenzoic acids	
4-Hydroxybenzoic acid	0
Protocatechuic acid	0
Gallic acid	0
Vanilic acid	0
Syringic acid	0
Gentisic acid	0
Hydroxycinnamic acids	
Caffeic acid	0
Ferulic acid	0
Chlorogenic acid	0
Flavonols	
Rutin or Quercetin-3-O-rutinoside	0
Flavanoids	
(+)-Catechin	0.02
(-)-Epicatechin	0.42
Procyanidin B3	5.5
Prodelphinidin B3	18.2
Procyanidin C2	79.3
Proanthocyanidin tetramer	26.5
Proanthocyanidin pentamer	38.2

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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, whilst strong haze is observed at higher concentrations.[214]

To preserve beer colloidal stability, brewers usually remove haze-active materials.[215] To get rid of haze-active proteins, precipitation with tannic acid, hydrolysis with papain, and adsorption to bentonite[216] or silica gel[217,218] 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[219] (Fig. 11), 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.[209,220] Another innovative way is the use of flavan-3-ol and proanthocyanidin-free malt which allows affording an excellent colloidal stability.[221]

A lag phase is usually observed in lager beers before chill-haze development.[210,222,223] As depicted in Fig. 12 for different batches[1,2,3], 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.

As explained above, 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).[208,213,224] Free radicals are known to enhance haze.[225] Tannoids have been defined by Chapon [103] as intermediates in the oxidation of simple flavanoids to tannins, forming complexes with proteins. On the other

Polyproline segment PVPP segment

Figure 11. Comparison between a segment of protein rich in proline and PVPP.

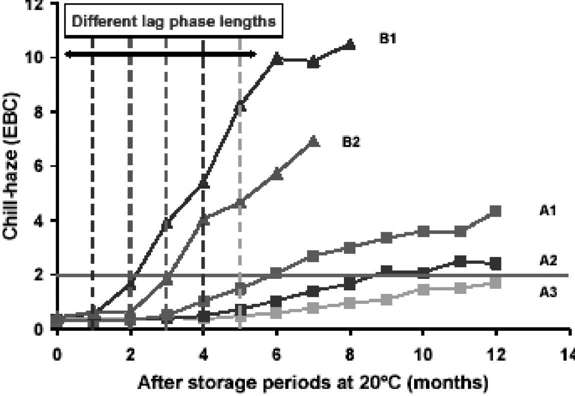

Figure 12. Evolution of the chill-haze during storage at 20°C in two larger beers (A and B) from different batches (A1, A2, etc.).[223]

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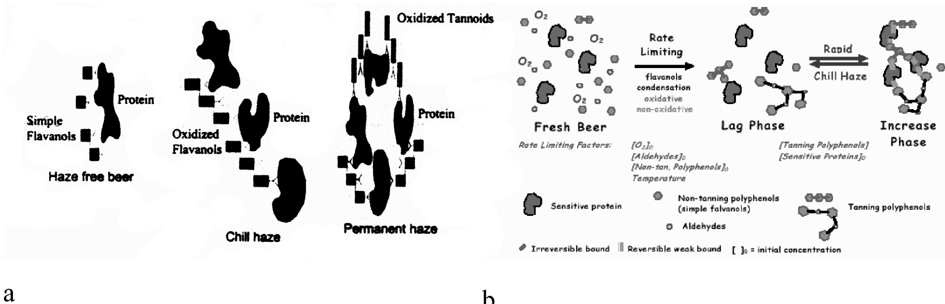

a b

Figure 13. a. Model of chill and permanent haze in beer [(224)]; and b. theorical chill-haze formation mechanism.[(223)]

hand, according to O'Rourke *et al.*,[(224)] oxidized flavanols cause chill haze but only subsequent polymerization leads to tannoids and permanent haze (Fig. 13a).

Leemans *et al.*[(223)] have recently proposed a model in which aldehydes and oxygen play key roles in tanning polyphenol formation (Fig. 13b). The time needed to form critical amounts of tanning polyphenols leading to visible chill-haze particles corresponds to the lag phase. Not only dissolved oxygen but also shaking, higher temperature, polyphenol-rich raw materials, light, and heavy metals will significantly increase colloidal instability.[(223)]

Impact on Color Stability

Beer color increases through storage, especially in the presence of oxygen and at higher temperature (Fig. 14). Most probably, Maillard reactions are partly involved [(175,176)]. Yet a yellow-brown color can also come from oxidized polyphenols.[(52,188)]

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. 15).[(117,226)] Recently, Callemien, and Collin[(52)] detected dehydrocatechin A, in a beer spiked with catechin after storage.

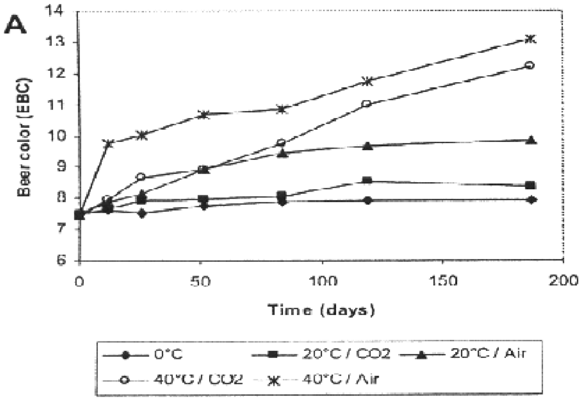

Figure 14. Evolution of the color during the aging of beer.[(175)]

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Figure 15. Proposed degradation schemes of (+)-catechin to form colorless compounds with MW = 578 and yellow compounds with MW = 576.[117])

The formation of yellow pigments is favored in the presence of iron, but their structure differs in this case (Abs $_{\text{with iron}}$ = 440–460 nm vs. Abs $_{\text{without iron}}$ = 385–412 nm).[227] In wine, colored polyphenols have been better studied. It is well known that condensations between anthocyanidins and flavanols (sometimes mediated by acetaldehyde) give rise to xanthylium salts, also characterized by a yellow-brown color. Other analogs including pyruvic acid, vinylphenol, cinnamic acid, glyoxylic acid, furfural, and 5-(hydroxymethyl)furfural, have also been described as potential pigments.[228–232] In Port wines, two molecules issued from the reaction between acetoacetic acid and anthocyanidins may contribute to the orange-red color of aged wines.[233]

Health Properties

Although ethanol is recognised as a major contributor to cancer diseases, moderate consumption of red wine is known to have some health benefits. The decrease in coronary heart disease observed among wine drinkers despite a diet very rich in saturated fat is known as the "French paradox".[234] Wine polyphenols and alcohol most probably contribute to this protective effect.[235,236] Beer contains much less polyphenols than wine, although one of its raw materials, hop, is much richer than grapes. The two beverages also show very different polyphenol distributions (e.g., no anthocyanidins in beer but more prenylchalcones). As the health properties claimed for polyphenols have been reviewed by many authors, just a few references are given below. Few data are available concerning the human biodisponibility of beer polyphenols. The presence in beer of high levels of proteins could be a limiting-factor.

Cardiovascular protection. Flavonols and flavan-3-ols induce cardioprotective effects, including antioxidant effects (protection against LDL oxidation) and inhibition of

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platelet activity and vasodilatation.[205,237] *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).[238] Although less potent, *cis*-resveratrol, *trans*- and *cis*-piceid also improve the antioxidant activity [239,240]. Piceid absorption is enhanced by the presence of its sugar.[241]

Anticancer activity. 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.[55,71,79,242,243] *trans*-Resveratrol inhibits the initiation and growth of tumors. It inhibits cyclooxygenase, ornithine decarboxylase, and angiogenesis.[244,245] *trans*-Piceid is a weaker inhibitor of ROS production.[246] Very little information is available on potential anticancer effects of flavonols and flavan-3-ols. Flavonoids might reduce the risk of cancer, although some procarcinogenic activities have also been reported.[115,205]

Anti-inflammatory activity. Flavonoids alter the synthesis of eicosanoids (mediators of inflammation). They decrease the leukotriene/prostacyclin ratio by modifying lipoxygenase activity.[247,248] Immune regulation has also been observed.[249] *trans*-Resveratrol shows similar effects.[245,250]

Estrogenic activity. 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.[71,134,242] 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.[135]

Estrogenic activity has recently been reported for some stilbenes, especially *trans*-resveratrol. *cis*-Resveratrol appears less potent.[245,251]

Impact on neurodegenerative diseases. Hop proanthocyanidins can help prevent nitric-oxide-related disorders such as Alzheimer's and Parkinson's diseases.[49]

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Abbreviations

AAPH	2,2′-Azobis(2-amidinopropane)-dihydrochloride
AEDA	Aroma extract dilution analysis
APCI	Atmospheric pressure chemical ionization
BSA	Bis-trimethylsilylacetamide
BSTFA	N-O-bis(trimethylsilyl)trifluoroacetamide
C	Catechin
CEAS	Coulomchem electrode array system
CI	Chemical ionization
CV	Coefficient of variation

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DAD Diode array detector
E Epicatechin
EBC European brewery convention
ECD Electrochemical detection
EGC and GCEpigallocatechin and gallocatechin
EI Electronic impact ionization
ESI Electrospray ionization
GC Gas chromatography
GC-FID GC hyphenated to flame ionization detection
GC-O GC hyphenated to olfactometric detection
GPC Gel permeation column
HFAA HeptaFluorobutyric acid anhydride
HPLC or LCHigh performance liquid chromatography
HRF Heterocyclic ring fission
INRA Institut National de la Recherche Agronomique
MALDI Matrix assisted laser desorption
MRM Multiple reaction monitoring
MS Mass spectroscopy
MS/MS Tandem mass spectroscopy
mDP mean Degree of polymerization
NP Normal phase
P1 to P10 Procyanidins from monomers to decamers
PVP Polylvinylpyrrolidone
PVPP Polyvinylpolypyrrolidone
QM Quinone methide cleavage
RDA Retro-diels-alder
RP Reversed phase
RI Retention index
RT Retention time
SDS Sodium dodecyl sulfate
SEC Size exclusion chromatography
SLS Sodium lauryl sulfate
SPME Solid phase microExtraction
TFAA Trifluoroacetic acid
Tinh Inhibition time
TOFMS Time-of-flight mass spectrometry
UV Ultraviolet

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References

1. Dalgliesh, C.E. Flavour stability. *Proceedings of the European Brewery Convention (Amsterdam)* **1977**, 623–659.
2. McMurrough, I.; Henningan, G.P.; Loughrey, M.L. Quantitative analysis of hop flavonols using high-performance liquid chromatography. *J. Agric. Food Chem.* **1982**, *30*, 1102–1106.
3. Wackerbauer, K.; Kramer, P.; Siepert, J. Phenolic aromatic substances in beer. Phenolic carboxylic acid and phenols in the raw material and beer preparation. *Brauwelt* **1982**, *122* (15), 618–20, 6622, 624, 626.
4. Achilli, G.; Cellerino, G.P.; Gamache, P.H.; Deril, G.V.M. Identification and determination of phenolic constituents in natural beverages and plant-extracts by means of a coulometric electrode array system. *Journal of Chromatography* **1993**, *632* (1–2), 111–117.
5. Bartolome, B.; Pena-Neira, A.; Gomez-Cordoves, C. Phenolics and related substances in alcohol-free beers. *European Food Research and Technology* **2000**, *210* (6), 419–423.

6. Floridi, S.; Montanari, L.; Marconi, O.; Fantozzi, P. Determination of free phenolic acids in wort and beer by coulometric array detection. *J. Agric. Food Chem.* **2003**, *51* (6), 1548–1554.
7. Garcia, A.A.; Grande, B.C.; Gandara, J.S. 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. *Journal of Chromatography A* **2004**, *1054* (1–2), 175–180.
8. Gorinstein, S.; Caspi, A.; Zemser, M.; Trakhtenberg, S. Comparative contents of some phenolics in beer, red and white wines. *Nutrition Research* **2000**, *20* (1), 131–139.
9. Hayes, P.J.; Smyth, M.R.; McMurrough, I. 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* **1987**, *112* (9), 1205–1207.
10. McMurrough, I.; Roche, G.P.; Cleary, K.C. Phenolics acids in beers and worts. *Journal of the Institute of Brewing* **1984**, *90*, 181–187.
11. Moll, M. *Beers & Coolers: definition, production, and composition.* Collection Sciences of Techniques Agro-alimentaries, Apria, TEC&DOC Lavoiser, 1991.
12. Montanari, L.; Perretti, G.; Natella, F.; Guidi, A.; Fantozzi, P. Organic and phenolic acids in beer. *Food Science and Technology-Lebensmittel-Wissenschaft & Technologie* **1999**, *32* (8), 535–539.
13. Nardini, M.; Ghiselli, A. Determination of free and bound phenolic acids in beer. *Food Chemistry* **2004**, *84* (1), 137–143.
14. Wackerbauer, K.; Kossa, T.; Tressl, R. Phenol formation by yeasts. *Monatsschrift fuer Brauerei* **1978**, *31* (2), 52–55.
15. Stewart, D.; Robertson, G.W.; Morrison, I.H. Phenolic-acids dimers in the cell-walls of barley. *Biological Mass Spectrometry* **1994**, *23* (2), 71–74.
16. Kenyhercz, T.M.; Kissinger, P.T. A new approach to the phenolic components in beer. Application to the determination of sinapic, ferulic, and *p*-coumaric acids. *J. Agric. Food Chem.* **1977**, *25* (4), 959–961.
17. McMurrough, I.; Madigan, D.; Donnelly, D.; Hurley, J.; Doyle, A.M.; Hennigan, G.; McNulty, N.; Smyth, M.R. Control of ferulic acid and 4-vinyl guaiacol in brewing. *Journal of the Institute of Brewing* **1996**, *102* (5), 327–332.
18. Vanbeneden, N.; Delvaux, F.; Delvaux, F.R. Determination of hydroxycinnamic acids and volatile phenols in wort and beer by isocratic high-performance liquid chromatography using electrochemical detection. *Journal of Chromatography A* **2006**, *1136* (2), 237–242.
19. Tressl, R.; Renner, R. Gas chromatographic-mass spectrometric study of some lactones in beer. *Monatsschrift fuer Brauerei* **1975**, *28* (8), 195–197.
20. Vanbeneden, N.; Delvaux, F.; Delvaux, F.R. Release of phenolic flavour precurors during mashing. *Proceedings of XIIth De Clerck Chair (Leuven)* **2006**.
21. Coghe, S.; Benoot, K.; Delvaux, F.; Vanderhaegen, B.; Delvaux, F.R. Ferulic Acid Release and 4-Vinylguaiacol Formation during Brewing and Fermentation: Indications for Feruloyl Esterase Activity in Saccharomyces cerevisiae. *J. Agric. Food Chem.* **2004**, *52* (3), 602–608.
22. Fiddler, W.; Parker, W. E.; Wasserman, A. E.; Doerr, R. C. Thermal decomposition of ferulic acid. *J. Agric. Food Chem.* **1967**, *15* (5), 757–761.
23. Tressl, R.; Kossa, T.; Renner, R. 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. *Proceedings of the Congress—European Brewery Convention* **1975**, *15*, 737–756.
24. Clausen, M.; Lamb, C.J.; Megnet, R.; Doerner, P.W. PAD1 encodes phenylacrylic acid decarboxylase which confers resistance to cinnamic acid in *Saccharomyces cerevisiae. Gene* **1994**, *142* (1), 107–112.
25. Meaden, P.G.; Taylor, N.R. Cloning of a yeast gene which causes phenolic off-flavors in beer. *Journal of the Institute of Brewing* **1991**, *97* (5), 353–357.
26. Chatonnet, P.; Dubourdieu, D.; Boidron, J. N.; Pons, M. The origin of ethylphenols in wines. *Journal of the Science of Food and Agriculture* **1992**, *60* (2), 165–178.

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27. Lindsay, R.F.; Priest, F.G. Decarboxylation of substituted cinnamic acids by enterobacteria: the influence on beer flavour. *Journal of Applied Bacteriology* **1975**, *39* (2), 181–187.
28. Steinhaus, M.; Schieberle, P. 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.* **2000**, *48* (5), 1776–1783.
29. Kieninger, H.; Narziss, L.; Miedaner, H.; Hecht, S. Changes of quality-determining compounds in the preparation of Bavarian wheat beers. *Monatsschrift fuer Brauwissenschaft* **1984**, *37* (1), 9–18, 55.
30. Madigan, D.; McMurrough, I. Rapid determination of 4-vinyl guaiacol and ferulic acid in beers and worts by high-performance liquid chromatography. *Journal of the American Society of Brewing Chemists* **1994**, *52* (4), 152–155.
31. Schieberle, P. Primary odorants of pale lager beer - differences to other beers and changes during storage. *Zeitschrift fur Lebensmittel-Untersuchung Und-Forschung* **1991**, *193* (6), 558–565.
32. Tressl, R.; Renner, R.; Apetz, M. Volatile phenolic components in beer, smoked beer and sherry. *Zeitschrift fuer Lebensmittel-Untersuchung und -Forschung* **1976**, *162* (2), 115–122.
33. Villarreal, R.; Sierra, J.A.; Cardenas, L. A rapid and sensitive method for the determination of 4-vinyl guaiacol in beer by electron-capture gas-liquid chromatography. *Journal of the American Society of Brewing Chemists* **1986**, *44* (3), 114–117.
34. Wackerbauer, K.; Kramer, P. Bavarian wheat beer - an alternative. Production and composition. *Brauwelt* **1982**, *122* (18), 758–760, 762.
35. Tressl, R.; Kossa, T.; Renner, R. Formation of aroma compounds in malt, wort, and beer. *Monatsschrift fuer Brauerei* **1974**, *27* (5), 98–102.
36. Fickert, B.; Schieberle, P. Identification of the key odorants in barley malt (caramalt) using GC/MS techniques and odour dilution analyses. *Nahrung-Food* **1998**, *42* (6), 371–375.
37. Kieninger, H.; Boeck, D. Gas chromatographic determination of certain volatile phenols in barley, green malt, kilned malt, wort and beer. *Brauwissenschaft* **1977**, *30* (12), 357–360.
38. McMurrough, I. High-performance liquid chromatography of flavonoids in barley and hops. *Journal of Chromatography A* **1981**, *218*, 683–693.
39. Sagesser, M.; Deinzer, M. HPLC-ion spray tandem mass spectrometry of flavonol glycosides in hops. *Journal of the American Society of Brewing Chemists* **1996**, *54* (3), 129–134.
40. Vancraenenbroeck, R.; Vanclef, A.; Lontie, R. Characterization of flavonols and glucoside of phloroisobutyrophenone in hops. *Proceedings of the Congress—European Brewery Convention* **1965**, 360–371.
41. Vancraenenbroeck, R.; Callewaert, W.; Gorissen, H.; Lontie, R. Chromatographic separation and identification of hop flavonols and flavans. *Proceedings of the Congress - European Brewery Convention* **1970**, *12*, 29–43.
42. Vancraenenbroeck, R.; Callewaert, W.; Lontie, R. Identification of flavonol glycosides in hops. *Cerevisia (1976–1990)* **1980**, *5* (2), 69–73.
43. Callemien, D.; Jerkovic, V.; Rozenberg, R.; Collin, S. 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.* **2005**, *53* (2), 424–429.
44. Vancraenenbroeck, R.; De Brackeleire, C.; Willems, Y.; Devreux, A. Quantitative determination of flavonols in beer. *Cerevisia (1976–1990)* **1983**, *8* (1), 13–19.
45. Lermusieau, G.; Liégeois, C.; Collin, S. Reducing power of hop cultivars and beer ageing. *Food Chemistry* **2001**, *72* (4), 413–418.
46. Moll, M.; Fonknechten, G.; Carnielo, M.; Flayeux, R. Changes in polyphenols from raw materials to finished beer. *Technical Quarterly - Master Brewers Association of the Americas* **1984**, *21* (2), 79–87.
47. Jerumanis, J. Quantitative analysis of flavanoids in barley, hops and beer by high-performance liquid chromatography (HPLC). *Journal of the Institute of Brewing* **1985**, *91*, 250–252.
48. Li, H. J.; Deinzer, M. L. Structural identification and distribution of proanthocyanidins in 13 different hops. *J. Agric. Food Chem.* **2006**, *54* (11), 4048–4056.

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49. Stevens, J.F.; Miranda, C.L.; Wolthers, K.R.; Schimerlik, M.; Deinzer, M.L.; Buhler, D. R. Identification and *in vitro* biological activities of hop proanthocyanidins: Inhibition of nNOS activity and scavenging of reactive nitrogen species. *J. Agric. Food Chem.* **2002**, *50* (12), 3435–3443.
50. Friedrich, W.; Galensa, R. Identification of a new flavanol glucoside from barley (*Hordeum vulgare* L.) and malt. *European Food Research and Technology* **2002**, *214* (5), 388–393.
51. Zimmermann, B.F.; Galensa, R. 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* **2007**, *224* (3), 385–393.
52. Callemien, D.; Collin, S. Involvement of flavanoids in beer color instability during storage. *J. Agric. Food Chem.* **2007**, *55* (22), 9066–9073.
53. Callemien, D.; Collin, S. Use of RP-HPLC-ESI(-)-MS/MS to differentiate various proanthocyanidin isomers in lager beer extracts. *Journal of the American Society of Brewing Chemists* **2008**, *66* (2), 109–115.
54. Callemien, D.; Guyot, S.; Collin, S. Use of thiolysis hyphenated to RP-HPLC-ESI(-)-MS/MS for the analysis of flavanoids of fresh lager beers. *Food Chemistry* **2008**, *110*, 1012–1018.
55. Gerhauser, C.; Alt, A.; Heiss, E.; Gamal-Eldeen, A.; Klimo, K.; Knauft, J.; Neumann, I.; Scherf, H.R.; Frank, N.; Bartsch, H.; Becker, H. Cancer chemopreventive activity of Xanthohumol, a natural product derived from Hop. *Molecular Cancer Therapeutics* **2002**, *1* (11), 959–969.
56. Kirby, W.; Wheeler, R. E. The extraction of beer polyphenols and their assay by H.P.L.C. *Journal of the Institute of Brewing* **1980**, *86* (1), 15–17.
57. Madigan, D.; McMurrough, I.; Smyth, M.R. Determination of proanthocyanidins and catechins in beer and barley by high-performance liquid chromatography with dual-electrode electrochemical detection. *Analyst (Cambridge, United Kingdom)* **1994**, *119* (5), 863–868.
58. McMurrough, I.; Madigan, D.; Smyth, M.R. Semipreparative chromatographic procedure for the isolation of dimeric and trimeric proanthocyanidins from barley. *J. Agric. Food Chem.* **1996**, *44* (7), 1731–1735.
59. Mulkay, P.; Touillaux, R.; Jerumanis, J. Proanthocyanidins of barley: separation and identification. *Journal of Chromatography* **1981**, *208* (2), 419–423.
60. Delcour, J.A.; Tuytens, G.M. Structure elucidation of three dimeric proanthocyanidins isolated from a commercial Belgian pilsner beer. *Journal of the Institute of Brewing* **1984**, *90* (3), 153–161.
61. Eastmond, R. Separation and identification of a dimer of catechin occurring in beer. *Journal of the Institute of Brewing* **1974**, *80* (2), 188–192.
62. McMurrough, I.; Baert, T. Identification of proanthocyanidins in beer and their direct measurement with a dual electrode electrochemical detector. *Journal of the Institute of Brewing* **1994**, *100* (6), 409–416.
63. Whittle, N.; Eldridge, H.; Bartley, J.; Organ, G. Identification of the polyphenols in barley and beer by HPLC/MS and HPLC/electrochemical detection. *Journal of the Institute of Brewing* **1999**, *105* (2), 89–99.
64. Stevens, J.F.; Taylor, A.W.; Nickerson, G.B.; Ivancic, M.; Henning, J.; Haunold, A.; Deinzer, M.L. Prenylflavonoid variation in *Humulus lupulus: distribution and taxonomic significance of xanthogalenol and 4′-O-methylxanthohumol. Phytochemistry* **2000**, *53* (7), 759–775.
65. Stevens, J.F.; Taylor, A.W.; Deinzer, M.L. Quantitative analysis of xanthohumol and related prenylflavonoids in hops and beer by liquid chromatography-tandem mass spectrometry. *Journal of Chromatography A* **1999**, *832* (1–2), 97–107.
66. De Keukeleire, J.; Ooms, G.; Heyerick, A.; Roldan-Ruiz, I.; Van Bockstaele, E.; De Keukeleire, D. Formation and accumulation of alpha-acids, beta-acids, desmethylxanthohumol, and xanthohumol during flowering of hops (*Humulus lupulus* L.). *J. Agric. Food Chem.* **2003**, *51* (15), 4436–4441.
67. Walker, C.J.; Lence, C.F.; Biendl, M. Studies on xanthohumol levels in Stout/Porter beer. *Brauwelt* **2003**, *143* (50), 1709–1712.
68. Biendl, M.; Mitter, W.; Peters, U.; Methner, F.J. Use of a xanthohumol-rich hop product in beer production. *Brauwelt International* **2002**, *I*, 39–42.

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69. Biendl, M.; Methner, F.J.; Stettner, G.; Walker, C. Brewing trials with a xanthohumol-enriched hop product. *Brauwelt International* **2004**, *III*, 182–184.
70. Stevens, J.F.; Taylor, A.W.; Clawson, J.E.; Deinzer, M.L. Fate of xanthohumol and related prenylflavonoids from hops to beer. *J. Agric. Food Chem.* **1999**, *47* (6), 2421–2428.
71. Stevens, J.F.; Page, J.E. Xanthohumol and related prenylflavonoids from hops and beer: to your good health! *Phytochemistry* **2004**, *65* (10), 1317–1330.
72. Callemien, D.; Counet, C.; Cawet, Q.; Collin, S. Hop as a determinant nutrition key for health? *Proceedings of the Congress - European Brewery Convention* **2003**, *29th*, 137-1-137/8.
73. Jerkovic, V.; Collin, S. Occurrence of resveratrol and piceid in American and European hop cones. *Journal of the American Society of Brewing Chemists* **2007**, *55* (8754), 8758.
74. Jeandet, P.; Bessis, R.; Sbaghi, M.; Meunier, P. Production of the phytoalexin resveratrol by grapes as a response to Botrytis attack under natural conditions. *Journal of Phytopathology* **1995**, *143* (3), 135–139.
75. Langcake, P.; Pryce, R.J. The production of resveratrol by *vitis vinefera* and other members of the vitacea as a response to infection or injury. *Physiological Plant Pathology* **1976**, *9*, 77–86.
76. Jerkovic, V.; Collin, S. Fate of resveratrol and piceid through different hop processings and storage times. *J. Agric. Food Chem.* **2008**, *56* (584), 590.
77. Jerkovic, V.; Callemien, D.; Collin, S. Determination of Stilbenes in Hop Pellets from Different Cultivars. *J. Agric. Food Chem.* **2005**, *53* (10), 4202–4206.
78. Jerkovic, V.; Collin, S. Resveratrol analogues in hop, malt, and adjuncts. *Proceedings of the 31th European Brewery Convention* **2007**, *76*, 1–6.
79. Gerhauser, C.; Alt, A.; Klimo, K.; Knauft, J.; Frank, N.; Becker, H. Isolation and potential cancer chemoprenventive activities of phenolic compounds of beer. *Phytochemistry Reviews* **2002**, *1*, 369–377.
80. Stevens, J.F.; Ivancic, M.; Hsu, V.L.; Deinzer, M.L. Prenylflavonoids from *Humulus lupulus*. *Phytochemistry* **1997**, *44* (8), 1575–1585.
81. Tekel', J.; De Keukeleire, D.; Rong, H.; Daeseleire, E.; Van Peteghem, C. Determination of the hop-derived phytoestrogen, 8-prenylnaringenin, in beer by gas chromatography/mass spectrometry. *J. Agric. Food Chem.* **1999**, *47* (12), 5059–5063.
82. Jerkovic, V.; Nguyen, F.; Timmermans, A.; Collin, S. Comparison of procedures for resveratrol analysis in beer. Assessment of stilbene stability through wort fermentation and beer aging. *Journal of the Institute of Brewing* **2008**, *114* (2), 143–149.
83. Singleton, V.L.; Rossi, J.A., Jr. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture* **1965**, *16* (3), 144–158.
84. Singleton, V.L.; Orthofer, R.; Lamuela-Raventos, R.M. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. *Methods in Enzymology* **1999**, *299* (Oxidants and Antioxidants, Part A), 152–178.
85. George, S.; Brat, P.; Alter, P.; Amiot, M.J. Rapid determination of polyphenols and vitamin C in plant-derived products. *J. Agric. Food Chem.* **2005**, *53* (5), 1370–1373.
86. Bishop, L.R. Analysis committee of the European brewery convention. Measurement of total polyphenols in worts and beers. *Journal of the Institute of Brewing* **1972**, *78* (1), 37–38.
87. Fernandez, M.T.; Mira, M.L.; Florencio, M.H.; Jennings, K.R. Iron and copper chelation by flavonoids: an electrospray mass spectrometry study. *Journal of Inorganic Biochemistry* **2002**, *92* (2), 105–111.
88. Mira, L.; Fernandez, M.T.; Santos, M.; Rocha, R.; Florencio, M.H.; Jennings, K.R. Interactions of flavonoids with iron and copper ions: A Mechanism for their antioxidant activity. *Free Radical Research* **2002**, *36* (11), 1199–1208.
89. Jerumanis, J. Polymerization of polyhydric phenols. *Bulletin de l'Association Royale des Anciens Etudiants en Brasserie de l'Universite de Louvain* **1969**, *65* (4), 169–190.
90. Jerumanis, J. Simplification and improvement of the method of total polyphenol determination in brewing. *Bulletin de l'Association Royale des Anciens Etudiants en Brasserie de l'Universite de Louvain* **1968**, *64* (4), 223–240.

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91. Jerumanis, J. Methods of determination of polyphenols in the brewery. *Bulletin de l'Association Royale des Anciens Etudiants en Brasserie de l'Universite de Louvain* **1973**, *69* (1), 1–14.
92. De Clerck, J.; Jerumanis, J. Determination of polyphenols in brewery products. *Bulletin de l'Association Royale des Anciens Etudiants en Brasserie de l'Universite de Louvain* **1967**, *63* (4), 137–161.
93. McMurrough, I.; McDowell, J. Chromatographic separation and automated analysis of flavanols. *Analytical Biochemistry* **1978**, *91* (1), 92–100.
94. Delcour, J.A.; Janssens de Varebeke, D. A new colorimetric assay for flavanoids in pilsner beers. *Journal of the Institute of Brewing* **1985**, *91* (1), 37–40.
95. Sarkar, S. K.; Howarth, R. E. Specificity of the vanillin test for flavanols. *J. Agric. Food Chem.* **1976**, *24* (2), 317–320.
96. Sun, B.S.; Ricardo-da-Silva, J.M.; Spranger, I. Critical factors of vanillin assay for catechins and proanthocyanidins. *J. Agric. Food Chem.* **1998**, *46* (10), 4267–4274.
97. Porter, L.J.; Hrstich, L.N.; Chan, B.G. The conversion of procyanidins and prodelphinidins to cyanidin and delphinidin. *Phytochemistry* **1986**, *25* (1), 223–230.
98. Harris, G.; Ricketts, R. W. Nonbiological hazes of beers. VIII. Rapid estimation of anthocyanogens in beer. *Journal of the Institute of Brewing* **1959**, *65*, 331–333.
99. McFarlane, W.D. Determination of anthocyanogens. *Journal of the Institute of Brewing* **1961**, *67*, 502–506.
100. McFarlane, W.D.; Vader, M.J. Determination of anthocyanogens. II. Further studies on the analysis of beer. *Journal of the Institute of Brewing* **1962**, *68*, 254–257.
101. Chapon, L.; Chapon, S.; Seyer, R.; Louis, C. Model experiments illustrating some aspect of the mechanism of formation of chill haze. *Proceedings of the European Brewery Convention, Fachverlag Hans Carl, Nürnberg, Germany* **1975**, *5*, 423–438.
102. McMurrough, I.; Madigan, D.; Kelly, R. J. Evaluation of rapid colloidal stabilization with polyvinylpolypyrrolidone (PVPP). *Journal of the American Society of Brewing Chemists* **1997**, *55* (2), 38–43.
103. Chapon, L. The mechanics of beer stabilisation. *Brewers' Guardian* **1994**, *123* (46), 50.
104. McMurrough, I.; Hennigan, G.P.; Loughrey, M.J. Contents of simple, polymeric and complexed flavanols in worts and beers and their relationship to haze formation. *Journal of the Institute of Brewing* **1983**, *89* (1), 15–23.
105. Schriner R.L.; Hermann C.K.F.; Morril, T.C.; Curtin D.Y.; Fuson R.C. *The systematic identification of organic compounds*; 1998.
106. Counet, C.; Collin, S. Effect of the number of flavanol units on the antioxidant activity of procyanidin fractions isolated from chocolate. *J. Agric. Food Chem.* **2003**, *51* (23), 6816–6822.
107. Jerumanis, J. Separation and identification of flavanoids by high-performance liquid chromatography (HPLC). *Proceedings of the Congress - European Brewery Convention* **1979**, *17th*, 309–319.
108. Zhao, H.F.; Dong, J.J.; Lu, J.; Chen, J.; Li, Y.; Shan, L.J.; Lin, Y.; Fan, W.; Gu, G.X. Effects of extraction solvent mixtures on antioxidant activity evaluation and their extraction capacity and selectivity for free phenolic compounds in barley (*Hordeum vulgare* L.). *J. Agric. Food Chem.* **2006**, *54* (19), 7277–7286.
109. Noël, S.; Metais, N.; Bonte, S.; Bodart, E.; Peladan, F.; Dupire, S.; Collin, S. The use of Oxygen 18 in appraising the impact of oxidation process during beer storage. *Journal of the Institute of Brewing* **1999**, *105* (5), 269–274.
110. Owades, J.L.; Rubin, G.; Brenner, M.W. Determination of food tannins by ultraviolet spectrophotometry. *J. Agric. Food Chem.* **1958**, *6*, 44–46.
111. Owades, J.L.; Rubin, G.; Brenner, M.W. Determination of tannins in beer and brewing materials by ultraviolet spectrophotometry. *Proceedings of the American Society of Brewing Chemists* **1958**, 66–73.
112. Callemien, D.; Dasnoy, S.; Collin, S. Identification of a stale-beer-like odorant in extracts of naturally aged beer. *J. Agric. Food Chem.* **2006**, *54* (4), 1409–1413.
113. Milligan, S.; Kalita, J.; Pocock, V.; Heyerick, A.; De Cooman, L.; Rong, H.; De Keukeleire, D. Oestrogenic activity of the hop phyto-oestrogen, 8-prenylnaringenin. *Reproduction* **2002**, *123* (2), 235–242.

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114. Kantz, K.; Singleton, V.L. Isolation and determination of polymeric polyphenols using Sephadex Lh-20 and analysis of grape tissue-extracts. *American Journal of Enology and Viticulture* **1990**, *41* (3), 223–228.
115. Prior, R.L.; Gu, L.W. Occurrence and biological significance of proanthocyanidins in the American diet. *Phytochemistry* **2005**, *66* (18), 2264–2280.
116. Zhu, Q.Y.; Holt, R.R.; Lazarus, S.A.; Ensunsa, J.L.; Hammerstone, J.F.; Schmitz, H.H.; Keen, C.L. Stability of the flavan-3-ols epicatechin and catechin and related dimeric procyanidins derived from cocoa. *J. Agric. Food Chem.* **2002**, *50* (6), 1700–1705.
117. Guyot, S.; Vercauteren, J.; Cheynier, V. Structural determination of colourless and yellow dimers resulting from (+)-catechin coupling catalysed by grape polyphenoloxidase. *Phytochemistry* **1996**, *42* (5), 1279–1288.
118. Trela, B.C.; Waterhouse, A.L. Resveratrol: Isomeric molar absorptivities and stability. *J. Agric. Food Chem.* **1996**, *44* (5), 1253–1257.
119. Fritsch, H.; Schieberle, P. Changes in key aroma compounds during boiling of unhopped and hopped wort. *Proceedings of the Congress - European Brewery Convention* **2003**, *29th*, 22-1-22/9.
120. Luan, T.G.; Li, G.K.; Zhang, Z.X. Gas-phase postderivatization following solid-phase microextraction for rapid determination of trans-resveratrol in wine by gas chromatography-mass spectrometry. *Analytica chimica Acta* **2000**, *424* (1), 19–25.
121. Yanagida, A.; Kanda, T.; Shoji, T.; Ohnishi-Kameyama, M.; Nagata, T. Fractionation of apple procyanidins by size-exlcusion chromatography. *Journal of Chromatography A* **1999**, *855*, 181–190.
122. Yanagida, A.; Shoji, T.; Kanda, T. Characterization of polymerized polyphenols by size-exclusion HPLC. *Bioscience Biotechnology and Biochemistry* **2002**, *66* (9), 1972–1975.
123. Yanagida, A.; Shoji, T.; Shibusawa, Y. Separation of proanthocyanidins by degree of polymerization by means of size-exclusion chromatography and related techniques. *Journal of Biochemical and Biophysical Methods* **2003**, *56* (1–3), 311–322.
124. Pinelo, M.; Arnous, A.; Meyer, A.S. Upgrading of grape skins: Significance of plant cell-wall structural components and extraction techniques for phenol release. *Trends in Food Science & Technology* **2006**, *17* (11), 579–590.
125. Andres-Lacueva, C.; Lamuela-Raventos, R.M.; Jaurgui, O.; Casals, I.; Izquierdo-Pulido, M.; Permanyer, J. An LC method for the analysis of cocoa phenolics. *LC-GC Europe* **2000**, *13* (12), 902–904.
126. Pinelo, M.; Rubilar, M.; Jerez, M.; Sineiro, J.; Nunez, M.J. Effect of solvent, temperature, and solvent-to-solid ratio on the total phenolic content and antiradical activity of extracts from different components of grape pomace. *J. Agric. Food Chem.* **2005**, *53* (6), 2111–2117.
127. Thurston, P.A.; Tubb, R.S. Screening yeast strains for their ability to produce phenolic off-flavours: a simple method for determining phenols in wort and beer. *Journal of the Institute of Brewing* **1981**, *87*, 177–179.
128. Fritsch, H.T.; Schieberle, P. Identification based on quantitative measurements and aroma recombination of the character impact odorants in a Bavarian Pilsner-type beer. *J. Agric. Food Chem.* **2005**, *53* (19), 7544–7551.
129. Coghe, S.; Martens, E.; D'Hollander, H.; Dirinck, P.J.; Delvaux, F.R. Sensory and instrumental flavour analysis of wort brewed with dark specialty malts. *Journal of the Institute of Brewing* **2004**, *110* (2), 94–103.
130. Hayes, P.J.; Smyth, M.R.; McMurrough, I. Comparison of electrochemical and ultraviolet detection methods in High-Performance Liquid-Chromatography for the determination of phenolic-compounds commonly found in beers .1. Optimization of operating parameters. *Analyst* **1987**, *112* (9), 1197–1204.
131. Li, H. J.; Deinzer, M.L. Tandem mass spectrometry for sequencing proanthocyanidins. *Analytical Chemistry* **2007**, *79* (4), 1739–1748.
132. Derdelinckx, G.; Jerumanis, J. Depolymerization of the proanthocyanidins: indentification of intermediate compounds. *Proceedings of the Congress—European Brewery Convention* **1987**, *21st.*, 577–582.

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133. Gu, L. W.; Kelm, M.A.; Hammerstone, J.F.; Beecher, G.; Holden, J.; Haytowitz, D.; Prior, R.L. Screening of foods containing proanthocyanidins and their structural characterization using LC-MS/MS and thiolytic degradation. *J. Agric. Food Chem.* **2003**, *51* (25), 7513–7521.
134. Milligan, S.R.; Kalita, J.C.; Heyerick, A.; Rong, H.; De Cooman, L.; De Keukeleire, D. Identification of a potent phytoestrogen in hops (*Humulus lupulus* L.) and beer. *Journal of Clinical Endocrinology and Metabolism* **1999**, *84* (6), 2249–2252.
135. Milligan, S.R.; Kalita, J.C.; Pocock, V.; Van de Kauter, V.; Stevens, J.F.; Deinzer, M.L.; Rong, H.; De Keukeleire, D. The endocrine activities of 8-prenylnaringenin and related hop (*Humulus lupulus* L.) flavonoids. *Journal of Clinical Endocrinology and Metabolism* **2000**, *85* (12), 4912–4915.
136. Miranda, C.L.; Stevens, J.F.; Ivanov, V.; McCall, M.; Frei, B.; Deinzer, M. L.; Buhler, D.R. Antioxidant and prooxidant actions of prenylated and nonprenylated chalcones and flavanones in vitro. *J. Agric. Food Chem.* **2000**, *48* (9), 3876–3884.
137. Buckee, G.K. Determination of Iso-Alpha-Acids, Alpha-Acids and Beta-Acids in hop and isomerized hop extracts - a collaborative trial. *Journal of the Institute of Brewing* **1994**, *100* (3), 151–153.
138. Guyot, S.; Doco, T.; Souquet, J.M.; Moutounet, M.; Drilleau, J.F. Characterization of highly polymerized procyanidins in cider apple (*Malus sylvestris* var kermerrien) skin and pulp. *Phytochemistry* **1997**, *44* (2), 351–357.
139. Kelm M.A.; Johnson J.C.; Robbins R.J.; Hammerstone J.F; Schmitz H.H. High-performance liquid chromatography separation and purification of cacao (*theobroma cacao* L.) procyanidins according to degree of polymerization using a diol stationary phase. *J. Agric. Food Chem.* **2006**, *54* (5), 1571–1576.
140. Gu, L.W.; Kelm, M.; Hammerstone, J.F.; Beecher, G.; Cunningham, D.; Vannozzi, S.; Prior, R.L. Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method. *J. Agric. Food Chem.* **2002**, *50* (17), 4852–4860.
141. Guyot, S.; Marnet, N.; Sanoner, P.; Drilleau, J.F. Direct thiolysis on crude apple materials for high-performance liquid chromatography characterization and quantification of polyphenols in cider apple tissues and juices. *Flavanoids and other polyphenols. Methods in enzymology* **2001**, *335*, 57–70.
142. Kennedy, J.A.; Jones, G.P. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* **2001**, *49* (4), 1740–1746.
143. Matthews, S.; Mila, I.; Scalbert, A.; Pollet, B.; Lapierre, C.; Herve du Penhoat, C.L. M.; Rolando, C.; Donnelly, D. M. X. Method for estimation of proanthocyanidins based on their acid depolymerization in the presence of nucleophiles. *J. Agric. Food Chem.* **1997**, *45* (4), 1195–1201.
144. Kennedy, J.A.; Taylor, A.W. Analysis of proanthocyanidins by high-performance gel permeation chromatography. *Journal of Chromatography A* **2003**, *995* (1–2), 99–107.
145. Taylor, A.W.; Barofsky, E.; Kennedy, J.A.; Deinzer, M.L. Hop (*Humulus lupulus* L.) proanthocyanidins characterized by mass spectrometry, acid catalysis, and gel permeation chromatography. *J. Agric. Food Chem.* **2003**, *51* (14), 4101–4110.
146. Gu, L.W.; Kelm, M.A.; Hammerstone, J.F.; Zhang, Z.; Beecher, G.; Holden, J.; Haytowitz, D.; Prior, R.L. Liquid chromatographic/electrospray ionization mass spectrometric studies of proanthocyanidins in foods. *Journal of Mass Spectrometry* **2003**, *38* (12), 1272-–1280.
147. Stevens, J.F.; Miranda, C.L.; Buhler, D.R.; Deinzer, M.L. Chemistry and biology of hop flavonoids. *Journal of the American Society of Brewing Chemists* **1998**, *56* (4), 136–145.
148. Counet, C. Détermination des structures chimiques des polyphénols du chocolat et étude de leurs principales propriétés fonctionnelles. Thesis of INBR,UCL, Belgium, 2004.
149. Ohnishi-Kameyama, M.Y. A. K. T. a. N. T. 1. Identification of catechin oligomers from apple (*Malus pulima* cv. Fuji) in Matrix Assisted Laser Desorption/Ionisation Time-of-Flight. *Rapid Communication in Mass spectrometry* **1997**, *11*, 31–36.

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150. Ramirez-Coronel, M.A.; Marnet, N.; Kuam Kolli, V.S.; Roussos, S.; Guyot, S.; Augur, C. Characterization and estimation of proanthocyanidins and other phenolics in coffee pulp (*coffea arabica*) by thiolysis-high-performance liquid chromatography. *J. Agric. Food Chem.* **2004**, *52*, 1344–1349.
151. Jerkovic, V.; Callemien, D.; Dubois, D.; Collin, S. Determination of stilbenes content in various hop cultivars and conditionings. *Proceedings of the Congress—European Brewery Convention* **2005**, *30th*, 111-1-111/5.
152. Meilgaard, M.C. Flavor chemistry of beer. II: Flavor and threshold of 239 aroma volatiles. *Technical Quarterly - Master Brewers Association of the Americas* **1975**, *12*, 5–13.
153. Narziss, L.; Miedaner, H.; Nitzsche, F. The formation of 4-vinylguaiacol during the manufacture of Bavarian wheat beers (Weizenbier). *Monatsschrift fuer Brauwissenschaft* **1990**, *43* (3), 96–100.
154. Drost, B.W.; Van den Berg, R.; Frejee F.J.M.; Van der Velde, E.G.; Hollemans, M. Flavor stability. *Journal of American Society of Brewing Chemists* **1990**, *48* (124), 131.
155. Lermusieau, G.; Noël, S.; Liégeois, C.; Collin, S. Nonoxidative mechanism for development of trans-2-nonenal in beer. *Journal of the American Society of Brewing Chemists* **1999**, *57* (1), 29–33.
156. Noël, S.; Liégeois, C.; Lermusieau, G.; Bodart, E.; Badot, C.; Collin, S. Release of deuterated nonenal during beer aging from labeled precursors synthesized in the boiling kettle. *J. Agric. Food Chem.* **1999**, *47* (10), 4323–4326.
157. Gijs, L.; Perpète, P.; Timmermans, A.; Collin, S. 3-Methylthiopropionaldehyde as precursor of dimethyl trisulfide in aged beers. *J. Agric. Food Chem.* **2000**, *48* (12), 6196–6199.
158. Burns, C.S.; Heyerick, A.; De Keukeleire, D.; Forbes, M.D.E. Mechanism for formation of the lightstruck flavor in beer revealed by time-resolved electron paramagnetic resonance. *Chemistry-A European Journal* **2001**, *7* (21), 4553–4561.
159. Chevance, F.; Guyot-Declerck, C.; Dupont, J.; Collin, S. Investigation of the beta-damascenone level in fresh and aged commercial beers. *J. Agric. Food Chem.* **2002**, *50* (13), 3818–3821.
160. Gijs, L.; Chevance, F.; Jerkovic, V.; Collin, S. How low pH can intensify beta-damascenone and dimethyl trisulfide production through beer aging. *J. Agric. Food Chem.* **2002**, *50* (20), 5612–5616.
161. Theddy, V.; Bononi, M.; Tateo, F. Identificazione di un composto carbonilico in birre “invecchiate” che presentano “staling flavour”: ricerca del damascenone. *Birra e Malto* **1997**, (67), 9–24.
162. Schieberle, P.; Komarek, D. Changes in key aroma compounds during natural beer aging. *FRESHNESS AND SHELF LIFE OF FOODS ACS symposium series* **2003**, 70–79.
163. Vesely, P.; Lusk, L.; Basarova, G.; Seabrooks, J.; Ryder, D. Analysis of aldehydes in beer using solid-phase microextraction with on-fiber derivatization and gas chromatography/mass spectrometry. *J. Agric. Food Chem.* **2003**, *51* (24), 6941–6944.
164. Harayama, K.; Hayase, F.; Kato, H. Contribution to stale flavor of 2-furfuryl ethyl ether and its formation mechanism in beer. *Bioscience Biotechnology and Biochemistry* **1995**, *59* (6), 1144–1146.
165. Vanderhaegen, B.; Neven, H.; Daenen, L.; Verstrepen, K.J.; Verachtert, H.; Derdelinckx, G. Furfuryl ethyl ether: Important aging flavor and a new marker for the storage conditions of beer. *J. Agric. Food Chem.* **2004**, *52* (6), 1661–1668.
166. Vanderhaegen, B.; Neven, H.; Verstrepen, K.J.; Delvaux, F.R.; Verachtert, H.; Derdelinckx, G. Influence of the brewing process on furfuryl ethyl ether formation during beer aging. *J. Agric. Food Chem.* **2004**, *52* (22), 6755–6764.
167. Lejeune I.; Maudoux M.; Collin S. Fate of polyfunctional thiols through beer ageing. *Proceedings of the 31th European Brewery Convention* **2007**, *52*, 1–6.
168. Moir, M. The 1990 Laurence Bishop Silver Medal Lecture the Desideratum for Flavor Control. *Journal of the Institute of Brewing* **1992**, *98* (3), 215–220.
169. Bamforth, C.W.; Parsons, R. New procedures to improve the flavor stability of beer. *Journal of the American Society of Brewing Chemists* **1985**, *43* (4), 197–202.

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170. Andersen, M.L.; Skibsted, L.H. Electron spin resonance spin trapping identification of radicals formed during aerobic forced aging of beer. *J. Agric. Food Chem.* **1998**, *46* (4), 1272–1275.
171. Kaneda, H.; Kano, Y.; Koshino, S.; Ohyanishiguchi, H. Behavior and role of iron ions in beer deterioration. *J. Agric. Food Chem.* **1992**, *40* (11), 2102–2107.
172. Liégeois, C.; Collin, S. Reduction power test in malt, wort and beer. *Proceedings of IXth De Clerck Chair (Louvain-la-Neuve, Belgium)* **2000**.
173. Uchida, M.; Suga, S.; Ono, M. Improvement for oxidative flavor stability of beer - Rapid prediction method for beer flavor stability by electron spin resonance spectroscopy. *Journal of the American Society of Brewing Chemists* **1996**, *54* (4), 205–211.
174. Uchida, M.; Ono, M. Improvement for oxidative flavor stability of beer - Role of OH-radical in beer oxidation. *Journal of the American Society of Brewing Chemists* **1996**, *54* (4), 198–204.
175. Vanderhaegen, B.; Neven, H.; Coghe, S.; Verstrepen, K. J.; Verachtert, H.; Derdelinckx, G. Evolution of chemical and sensory properties during aging of top-fermented beer. *J. Agric. Food Chem.* **2003**, *51* (23), 6782–6790.
176. McMurrough, I.; Madigan, D.; Kelly, R.J.; Smyth, M.R. The role of flavanoid polyphenols in beer stability. *Journal of the American Society of Brewing Chemists* **1996**, *54* (3), 141–148.
177. Delcour, J.A.; Dondeyne, P.; Trousdale, E.K.; Singleton, V.L. The reactions between polyphenols and aldehydes and the influence of acetaldehyde on haze formation in beer. *Journal of the Institute of Brewing* **1982**, *88*, 234–243.
178. Cilliers, J.J.L.; Singleton, V.L. Autoxidative phenolic ring-opening under alkaline conditions as a model for natural polyphenols in food. *J. Agric. Food Chem.* **1990**, *38* (9), 1797–1798.
179. Forster, A.; Massinger, S.; Schmidt, R. Breakdown of hop bitter substances during storage of beer. *Brauwelt International* **2004**, *VI*, 426.
180. Liégeois, C.; Meurens, N.; Badot, C.; Collin, S. Release of deuterated (E)-2-nonenal during beer aging from labeled precursors synthesized before boiling. *J. Agric. Food Chem.* **2002**, *50* (26), 7634–7638.
181. Noël, S.; Collin, S. Trans-2-nonenal degradation products during mashing. *Proceedings of the Congress - European Brewery Convention* **1995**, *25th*, 483–490.
182. Collin, S.; Noel, S.; Bonte, S.; Metais, N.; Bodart, E.; Peladan, F.; Dupire, S. The use of $^{18}O_2$ in appraising the impact of oxidation processes during mashing and beer storage. *Proceedings of the Congress—European Brewery Convention* **1997**, *26th*, 535–544.
183. Guinard, J.X.; Pangborn, R.M.; Lewis, M.J. Preliminary studies on acidity-astringency interactions in model solutions and solutions. *Journal of the Science of Food and Agriculture* **1986**, *37*, 811–817.
184. Siebert, K.J.; Chassy, A.W. An alternate mechanism for the astringent sensation of acids. *Food Quality and Preference* **2004,** *15* (1), 13–18.
185. Courregelongue, S.; Schlich, P.; Noble, A.C. Using repeated ingestion to determine the effect of sweetness, viscosity and oiliness in temporal perception of soymilk astringency. *Food Quality and Preference* **1999**, *10*, 273–279.
186. Gawel, R.; Iland, P.G.; Francis, I.L. Characterizing the astringency of red wine: a case study. *Food Quality and Preference* **2001**, *12*, 83–94.
187. Thomas, C.J.C.; Lawless, H.T. Astringent subqualities in acids. *Chemical Senses* **1995**, *20* (6), 593–600.
188. Dadic, M.; Belleau, G. Polyphenols and beer flavors. *Proceedings of the American Society of Brewing Chemists* **1973**, *4*, 107–114.
189. Valentova, H.; Skrovankova, S.; Panovska, Z.; Pokorny, J. Time-intensity studies of astringent taste. *Food Chemistry* **2002**, *78* (1), 29–37.
190. Noble, A.C. Astringency and bitterness of flavonoid phenols. *CHEMISTRY OF TASTE: MECHANISMS. ACS symposium series* **2002**, 192–201.
191. Peleg, H.; Gacon, K.; Schlich, P.; Noble, A.C. Bitterness and astringency of flavan-3-ol monomers, dimers and trimers. *Journal of the Science of Food and Agriculture* **1999**, *79*, 1123–1128.
192. Siebert, K.J. pH and colloidal stability. *Proceedings of XIth De Clerck Chair (Louvain-la-Neuve, Belgium)* **2004**.

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193. Francois, N.; Guyot-Declerck, C.; Hug, B.; Callemien, D.; Govaerts, B.; Collin, S. Beer astringency assessed by time-intensity and quantitative descriptive analysis: Influence of pH and accelerated aging. *Food Quality and Preference* **2006**, *17* (6), 445–452.
194. Pangborn, R.M.; Lewis, M.J.; Tanno, L.S. Sensory quantification of bitterness and flavor of beer during storage. *Journal of the Institute of Brewing* **1977**, *83* (4), 244–250.
195. Delcour, J. A.; Vanderberghe, M. M.; Corten, P. F.; Dondeyne, P. Flavor tresholds of polyphenolics in water. *American Journal of Enology and Viticulture* **1984**, *35* (3), 134–136.
196. Kielhorn, S.; Thorngate, J. H. Oral sensations associated with the flavan-3-ols (+)-catechin and (-)-epicatechin. *Food Quality and Preference* **1999**, *10*(2), 109–116.
197. Robichaud, J.L.; Noble, A.C. Astringency and Bitterness of Selected Phenolics in Wine. *Journal of the Science of Food and Agriculture* **1990**, *53* (3), 343–353.
198. Thorngate, J.M.; Noble, A.C. Sensory evaluation of bitterness and astringency of 3 R(-)-epicatechin and 3s(+)-catechin. *Journal of the Science of Food and Agriculture* **1995**, *67*, 531–535.
199. Buggey, L.A. A review of polyphenolic antioxidants in hops, brewing and beer. *The Brewer International* **2001**, *4*, 21–25.
200. Andersen, M. L.; Outtrup, H.; Skibsted, L. H. Potential antioxidants in beer assessed by ESR spin trapping. *J. Agric. Food Chem.* **2000**, *48* (8), 3106–3111.
201. Andersen, M.L.; Skibsted, L.H. Modification of the levels of polyphenols in wort and beer by addition of hexamethylenetetramine or sulfite during mashing. *J. Agric. Food Chem.* **2001**, *49* (11), 5232–5237.
202. Liégeois, C.; Lermusieau, G.; Collin, S. Measuring antioxidant efficiency of wort, malt, and hops against the 2,2′-azobis(2-amidinopropane)dihydrochloride-induced oxidation of an aqueous dispersion of linoleic acid. *J. Agric. Food Chem.* **2000**, *48* (4), 1129–1134.
203. Lotito, S.B.; Actis-Goretta, L.; Renart, M.L.; Caligiuri, M.; Rein, D.; Schmitz, H.H.; Steinberg, F.M.; Keen, C.L.; Fraga, C.G. Influence of oligomer chain length on the antioxidant activity of procyanidins. *Biochemical and Biophysical Research Communications* **2000**, *276* (3), 945–951.
204. Plumb, G.W.; Pascual-Teresa, S.; Santos-Buelga, C.; Cheynier, V.; Williamson, G. Antioxidant properties of catechins and proanthocyanidins: Effect of polymerisation, galloylation and glycosylation. *Free Radical Research* **1998**,*29* (4), 351–358.
205. Santos-Buelga, C.; Scalbert, A. Proanthocyanidins and tannin-like compounds - nature, occurrence, dietary intake and effects on nutrition and health. *Journal of the Science of Food and Agriculture* **2000**, *80* (7), 1094–1117.
206. Wang, J.N.; Chen, Y.J.; Hano, Y.; Taro, N.; Tan, R.X. Antioxidant activity of polyphenols from seeds of Vitis amurensis *in vitro*. *Acta Pharmacologica Sinica* **2000**, *21* (7), 633–636.
207. Siebert, K. J.; Troukhanova, N. V.; Lynn, P. Y. Nature of polyphenol-protein interactions. *J. Agric. Food Chem.* **1996**, *44* (1), 80–85.
208. Asano, K.; Shinagawa, K.; Hashimoto, N. Characterization of haze-forming proteins of beer and their roles in chill haze formation. *Journal of the American Society of Brewing Chemists* **1982**, *40* (4), 147–154.
209. Leiper, K.A.; Stewart, G.G.; McKeown, I.P. Beer polypeptides and silica gel - Part I. Polypeptides involved in haze formation. *Journal of the Institute of Brewing* **2003**, *109* (1), 57–72.
210. McMurrough, I.; Kelly, R.; Byrne, J.; O'Brien, M. Effect of the removal of sensitive proteins and proanthocyanidins on the colloidal stability of lager beer. *Journal of the American Society of Brewing Chemists* **1992**, *50* (2), 67–76.
211. Mulkay, P.; Jerumanis, J. Effect of molecular weight and the hydroxy groups in proanthocyanidins on the colloidal stability of beer. *Cerevisia (1976–1990)* **1983**, *8* (1), 29–35.
212. Asano, K.; Ohtsu, K.; Shinagawa, K.; Hashimoto, N. 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. *Agricultural and Biological Chemistry* **1984**, *48* (5), 1139–1146.
213. Eastmond, R.; Gardner, R.J. Effect of various polyphenols on the rate of haze formation in beer. *Journal of the Institute of Brewing* **1974**, *80* (2), 192–200.
214. Siebert, K.J.; Carrasco, A.; Lynn, P.Y. Formation of protein-polyphenol haze in beverages. *J. Agric. Food Chem.* **1996**, *44* (8), 1997–2005.

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215. Siebert, K. J. Effects of protein-polyphenol interactions on beverage haze, Stabilization and analysis. *J. Agric. Food Chem.* **1999**, *47* (2), 353–362.
216. De Clerck, J. The use of proteolytic enzymes for the stabilization of beer. *Technical Quarterly—Master Brewers Association of the Americas* **1969**, *6*, 136–140.
217. Siebert, K.J.; Lynn, P.Y. Mechanisms of adsorbent action in beverage stabilization. *J. Agric. Food Chem.* **1997**, *45* (11), 4275–4280.
218. Siebert, K.J.; Lynn, P.Y. Mechanisms of beer colloidal stabilization. *Journal of the American Society of Brewing Chemists* **1997**, *55* (2), 73–78.
219. Siebert, K.J.; Lynn, P.Y. Comparison of polyphenol interactions with polyvinylpolypyrrolidone and haze-active protein. *Journal of the American Society of Brewing Chemists* **1998**, *56* (1), 24–31.
220. Leiper, K.A.; Stewart, G.G.; McKeown, I.P.; Nock, T.; Thompson, M.J. Optimising beer stabilisation by the selective removal of tannoids and sensitive proteins. *Journal of the Institute of Brewing* **2005**, *111* (2), 118–127.
221. Erdal, K.; Outtrup, H.; Ahrenst-Larsen, B. Barley and hop proanthocyanidins and the colloidal stability of beer. *Proceedings of the Congress—European Brewery Convention* **1985**, *20th*, 459–466.
222. Gardner, R.J.; McGuinness, J.D. Complex phenols in brewing - a critical survey. *Technical Quarterly—Master Brewers Association of the Americas* **1977**, *14* (4), 250–261.
223. Leemans, C.; Pellaud, J.; Melotte, L.; Dupire, S. Opportunities for lag phase prediction: a new tool to assess beer colloidal stability. *Proceedings of the Congress—European Brewery Convention* **2003**, *29th*, 88-1-88/11.
224. O'Rourke, T.; Ianniello, R.; McMurrough, I.; Springle, A. The role of tannoids in the colloidal stabilization of beer. *Proceedings of the Convention—Institute of Brewing (Asia Pacific Section)* **1998**, *25th*, 143–146.
225. Kaneda, H.; Kano, Y.; Osawa, T.; Kawakishi, S.; Kamimura, M. Effect of free-radicals on haze formation in beer. *J. Agric. Food Chem.* **1990**, *38* (10), 1909–1912.
226. Young, D.A.; Young, E.; Roux, D.G.; Brandt, E.V.; Ferreira, D. Synthesis of condensed tannins .19. phenol oxidative coupling of (+)-catechin and (+)-mesquitol - conformation of bis-(+)-catechins. *Journal of the Chemical Society-Perkin Transactions 1* **1987**, (11), 2345–2351.
227. Oszmianski, J.; Cheynier, V.; Moutounet, M. Iron-catalyzed oxidation of (+)-catechin in model systems. *J. Agric. Food Chem.* **1996**, *44* (7), 1712–1715.
228. Es-Safi, N. E.; Le Guerneve, C.; Fulcrand, H.; Cheynier, V.; Moutounet, M. New polyphenolic compounds with xanthylium skeletons formed through reaction between (+)-catechin and glyoxylic acid. *J. Agric. Food Chem.* **1999**, *47* (12), 5211–5217.
229. Es-Safi, N.E.; Fulcrand, H.; Cheynier, V.; Moutounet, M. Studies on the acetaldehyde-induced condensation of (-)-epicatechin and malvidin 3-O-glucoside in a model solution system. *J. Agric. Food Chem.* **1999**, *47* (5), 2096–2102.
230. Es-Safi, N.E.; Cheynier, V.; Moutounet, M. 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.* **2000**, *48* (12), 5946–5954.
231. Es-Safi, N.E.; Le Guerneve, C.; Fulcrand, H.; Cheynier, V.; Moutounet, M. Xanthylium salts formation involved in wine colour changes. *International Journal of Food Science and Technology* **2000**, *35* (1), 63–74.
232. Fulcrand, H.; Cheynier, V.; Oszmianski, J.; Moutounet, M. An oxidized tartaric acid residue as a new bridge potentially competing with acetaldehyde in flavan-3-ol condensation. *Phytochemistry* **1997**, *46* (2), 223–227.
233. He, J.; Santos-Buelga, C.; Silva, A.M.S.; Mateus, N.; De Freitas, V. Isolation and structural characterization of new anthocyanin-derived yellow pigments in aged red wines. *J. Agric. Food Chem.* **2006**, *54* (25), 9598–9603.
234. Renaud, S.; Debord, S. Wine, alcohol, platelets and the french paradox for coronary heart disease. *The lancet* **1992**, *339*, 1523–1526.
235. Frémont, L. Biological effects of resveratrol. *Life Sciences* **2000**, *66* (8), 663–673.

236. Rimm, E.B.; Klatsky, A.; Grobbee, D.; Stampfer, M.J. Review of moderate alcohol consumption and reduced risk of coronary heart disease: Is the effect due to beer, wine, or spirits? *British Medical Journal* **1996**, *312* (7033), 731–736.
237. Prior, R.L.; Lazarus, S.A.; Cao, G.; Muccitelli, H.; Hammerstone, J.F. Identification of procyanidins and anthocyanins in blueberries and cranberries (*vaccinium* spp.) using high-performance liquid chromatography/mass spectrometry. *J. Agric. Food Chem.* **2001**, *49*, 1270–1276.
238. Delmas, D.; Jannin, B.; Latruffe, N. Resveratrol: Preventing properties against vascular alterations and ageing. *Molecular Nutrition & Food Research* **2005**, *49* (5), 377–395.
239. Fauconneau, B.; WaffoTeguo, P.; Huguet, F.; Barrier, L.; Decendit, A.; Merillon, J. M. Comparative study of radical scavenger and antioxidant properties of phenolic compounds from *Vitis vinifera* cell cultures using *in vitro* tests. *Life Sciences* **1997**, *61* (21), 2103–2110.
240. Orallo, F. Comparative studies of the antioxidant effects of *cis*- and *trans*-resveratrol. *Current Medicinal Chemistry* **2006**, *13* (1), 87–98.
241. Hollman, P. C. H. Bioavailability of flavonoids. *European Journal of Clinical Nutrition* **1997**, *51*, S66–S69.
242. De Keukeleire, D.; Milligan, S.R.; Kalita, J.; Pocock, V.; De Cooman, L.; Heyerick, A.; Rong, H.; Roelens, F. Prenylated hop flavonoids are key agents in relation to health-beneficial properties of beer. *Proceedings of 28th European Brewery Convention (Budapest), Fachverlag Hans Carl, Nürnberg, Germany* **2001**, 566–575.
243. Gerhauser, C. Beer constituents as potential cancer chemopreventive agents. *European Journal of Cancer* **2005**, *41* (13), 1941–1954.
244. Aggarwal, B. B.; Bhardwaj, A.; Aggarwal, R. S.; Seeram, N. P.; Shishodia, S.; Takada, Y. Role of resveratrol in prevention and therapy of cancer: Preclinical and clinical studies. *Anticancer Research* **2004**, *24* (5A), 2783–2840.
245. Baur, J.A.; Sinclair, D.A. Therapeutic potential of resveratrol: the *in vivo* evidence. *Nature Reviews Drug Discovery* **2006**, *5* (6), 493–506.
246. Jang, D.S.; Kang, B.S.; Ryu, S.Y.; Chang, I.M.; Min, K.R.; Kim, Y. Inhibitory effects of resveratrol analogs on unopsonized zymosan-induced oxygen radical production. *Biochemical Pharmacology* **1999**, *57* (6), 705–712.
247. Schewe, T.; Kuhn, H.; Sies, H. Flavonoids of cocoa inhibit recombinant human 5-lipoxygenase. *Journal of Nutrition* **2002**, *132* (7), 1825–1829.
248. Schramm, D.D.; Wang, J.F.; Holt, R.R.; Ensunsa, J.L.; Gonsalves, J.L.; Lazarus, S. A.; Schmitz, H.H.; German, J.B.; Keen, C.L. Chocolate procyanidins decrease the leukotriene-prostacyclin ratio in humans and human aortic endothelial cells. *American Journal of Clinical Nutrition* **2001**, *73* (1), 36–40.
249. Sanbongi, C.; Suzuki, N.; Sakane, T. Polyphenols in chocolate, which have antioxidant activity, modulate immune functions in humans *in vitro*. *Cellular Immunology* **1997**, *177* (2), 129–136.
250. De la Lastra, C.A.; Villegas, I. Resveratrol as an anti-inflammatory and anti-aging agent: Mechanisms and clinical implications. *Molecular Nutrition & Food Research* **2005**, *49* (5), 405–430.
251. Basly, J. P.; Marre-Fournier, F.; Le Bail, J.C.; Habrioux, G.; Chulia, A. J. Estrogenic/antiestrogenic and scavenging properties of (E)- and (Z)-resveratrol. *Life Sciences* **2000**, *66* (9), 769–777.

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