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Phenol and Terpene Quenching of Singlet- and Triplet-Excited States of Riboflavin in Relation to Light-Struck Flavor Formation in Beer

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Phenolic compounds present in beer were shown by fluorescence spectroscopy and laser flash photolysis to deactivate both singlet- and triplet-excited states of riboflavin with bimolecular rate constants close to the diffusion control ranging from 2.8×10^9 to 1.1×10^{10} M^{-1} s^{-1} and from 1.1×10^9 to 2.6×10^9 M^{-1} s^{-1}, respectively. Enthalpies of activation were low (up to 33.2 kJ mol^{-1}), and entropies of activation were positive, ranging from 17 to 92 J mol^{-1} K^{-1}, as derived from temperature dependence, indicating a compensation effect. From a Stern−Volmer analysis of the singlet-excited riboflavin quenching by phenols it was found that high amounts of phenolic compounds (>0.3 M) would be needed to hinder triplet-excited riboflavin generation. On the other hand, a phenolic content of 0.36 mM is likely to quench 90% of the triplet-excited state. Phenol photodegradation was found to be complex, and using ESI-MS analysis it was not possible to identify specific photooxidation products of the phenolic compounds; only the photoproducts of riboflavin could be detected and structurally assigned. The rate of reaction of triplet-excited riboflavin with phenolic compounds in acetonitrile/citrate buffer (pH 4.6, 10 mM) is 550 times faster than the reaction with iso-α-acids from hops, indicating that triplet-excited quenchers such as phenols may be involved in the early steps in light-struck flavor formation in beer through radical formation. Terpenes present in herb-flavored beers were found to be nonreactive toward singlet- and triplet-excited-state riboflavin, and any protection depends on other mechanisms.

KEYWORDS: Beer; light-struck flavor; riboflavin; phenolics; terpenes

INTRODUCTION

Beer is consumed worldwide, and with an annual production of ≈1.22×10^6 hL and a global consumption of 60 mL per capita per day, beer is the most consumed alcoholic beverage (*1*, *2*). As for other foods and beverages, product quality of beer is subject to changes during storage, and the factors that affect the change in flavor are being studied in detail (*3*). It is well-known that beer exposed to light develops a particular "light-struck" off-flavor, for which the impact substance has been identified as 3-methylbut-2-ene-1-thiol (MBT), a powerful odor substance with a threshold of ≈4 ng L^{-1} of beer and an obnoxious odor generally referred to as a "skunky" odor (*1*, *4*).

It has now been recognized that MBT derives from the photodegradation of compounds in hop, the bittering agents of beer. Hops (*Humulus lupulus* L.) contain several hundreds of secondary metabolites, including the α-acids, predominantly humulone, cohumulone, and adhumulone (*5*−*8*). During wort boiling α-acids undergo a thermal isomerization to iso-α-acids (*trans*- and *cis*-isohumulone, isocohumulone, and isoadhumulone) (*5*−*7*). The characteristic bitter taste of beer is due to the presence of iso-α-acids in concentrations varying from 15 to 100 ppm and commonly around 15 ppm for larger beers. Iso-α-acids are light sensitive and play an essential role in light-struck flavor formation in beer, because the formation of MBT is observed in only hopped beer (*1*). Beer is commonly bottled in green glass, which is not transparent to UV-B light (280−320 nm). However, MBT is found to be formed when beer is irradiated with light in the spectral region between 350 and 500 nm, suggesting that riboflavin, naturally occurring in beer, acts as a photosensitizer (*8*, *9*). Recently, De Keukeleire et al. (*9*) proposed a mechanism by which MBT is formed in beer due to the quenching of triplet-excited riboflavin by electron transfer from iso-α-acids to triplet riboflavin, leading to riboflavin radical and triacylmethyl radicals derived from iso-α-acids. The complex reaction sequence includes inter- or intramolecular H-atom abstraction and subsequent α-cleavage yielding dehydrohumulinic acid and 3-methylbutyl-2-enyl radical, the main precursor of MBT in the presence of a suitable sulfur source (*8*−*11*). In a recent study, Goldsmith et al. (*12*) have identified

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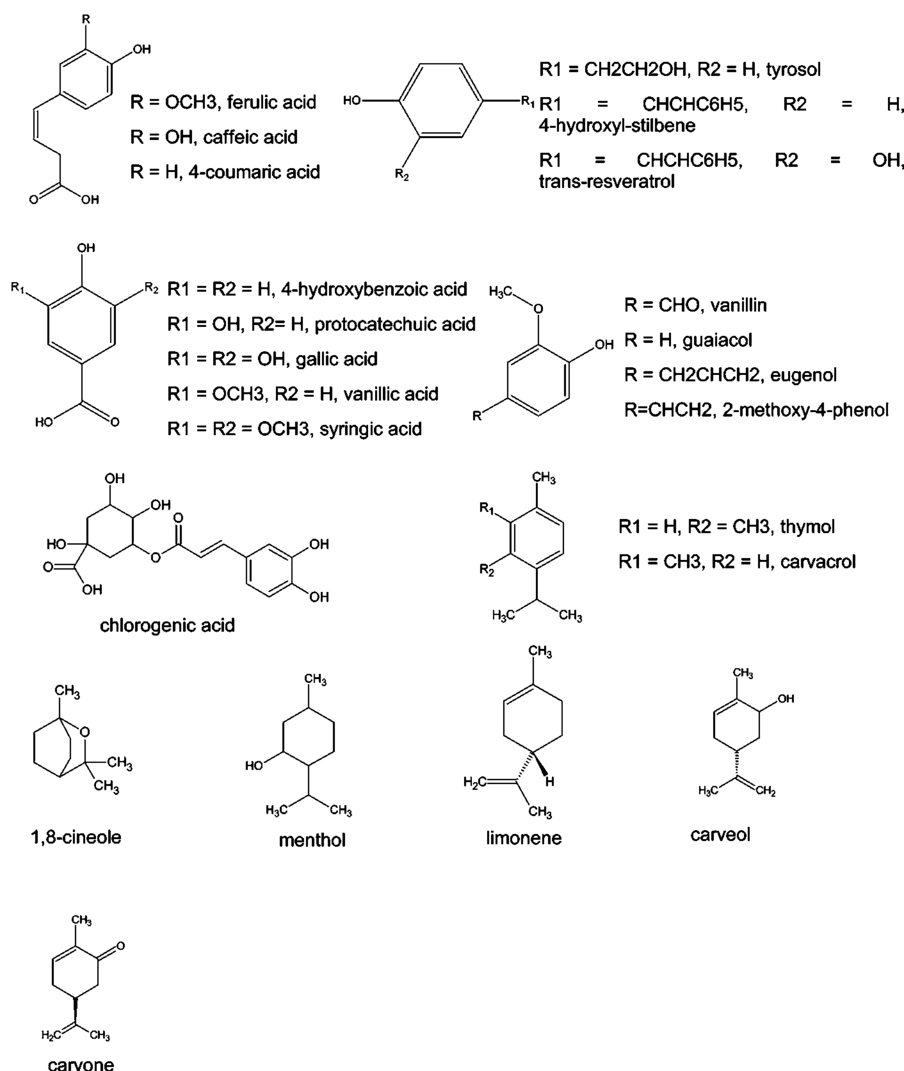
Chart 1. Chemical Structures of Phenols and Terpenes Studied in the Present Work



some natural triplet-excited riboflavin quenchers as inhibitors for light-struck flavor formation in beer and suggest the use of tryptophan, ascorbic acid, and catechin as additives to beer.

Beer contains many phenols, mainly (≈80%) from the malt and from hops (*13*). Phenols in beer are present in both monomeric and polymeric forms. Monomers are phenolic acids, flavanols and their glucosides, chalcones, isoflavones, and flavanones. They contribute to the antioxidant activity of beer and also are essential for determining beer flavor characteristics.

The aim of the present work is to examine the role of beer phenolics and terpenes from beer flavorings in the primary photochemical events leading to light-struck formation in order to clarify their role in the light-induced formation of MBT in beer sensitized by riboflavin. We report the singlet- and triplet-excited riboflavin deactivation rate constant by 18 phenols and 5 terpenes as studied by fluorescence spectroscopy and time-resolved absorption laser flash photolysis, respectively, combined with photoproduct analysis by ESI-MS. The chemical structures of the studied compounds are illustrated in **Chart 1**.

MATERIALS AND METHODS

Chemicals. Acetonitrile and methanol of HPLC grade were supplied by Lab Scan Analytical Sciences (Dublin, Ireland). Formic acid and ammonium hydroxide of ACS grade came from Sigma-Aldrich (Steinem, Germany). Coumarin 120, spectroscopy grade, was obtained from Lambda Physics (Göttingen, Germany). Caffeic acid, carvacrol, carveol, carvone, 4-coumaric, 1,8-cineole, chlorogenic acid, eugenol, ferulic acid, guaiacol, 4-hydroxybenzoic acid, limonene, menthol, protocatechuic acid, *trans*-resveratrol, riboflavin, syringic acid, thymol, tyrosol, vanillic acid, and vanillin were obtained from Sigma-Aldrich (Steinem, Germany). 2-Methoxy-4-vinylphenol was obtained from Alfa Aesar (Karlsruhe, Germany). Citric acid and sodium citrate were from Merck (Darmstadt, Germany). The water was purified using a Milli-Q system from Millipore (Bedford, MA).

Laser Flash Photolysis. The third harmonic, 355 nm, of a pulsed Q-switched Nd:YAG laser from Spectron Laser System (Rugby, U.K.) was used to pump the dye laser using Coumarin 120 (emission peak at 440 nm). The intensity of the laser pulse was ≈2.7 mJ cm^{-2}. Samples were excited at right angle by the laser pulse (8 ns) in 0.2 × 1.0 cm fluorescence quartz cells from Hellma (Mullein, Germany) and probed with a xenon arc pulse lamp from Applied Photophysics Ltd. (Leatherhead, U.K.) using appropriate UV cutoff filters to minimize the sample degradation by the monitoring light. The transmitted light entered a monochromator equipped with an R928 photomultiplier tube from Hamamatsu (Japan), and the signals were collected by a Phillips digital oscilloscope and transferred to the spectrometer workstation LKS.50 from Applied Photophysics Ltd. Experiments were carried out on thermostated fresh solutions purged with argon for 20 min. The main part of the experiment was performed at 298 K, but temperature dependence was investigated between 287 and 303 K. All of the measurements were performed in triplicate, and the results reported are the mean value and respective standard deviation.

Steady-State Fluorescence Spectroscopy. Fluorescence measurements were carried out using a Perkin-Elmer LS 55 luminescence

spectrometer (7.3 W pulsed xenon discharge lamp) mounted with a Hamamatsu R928 photomultiplier tube at 298 K in a thermostated cell holder. Emission spectra (4.0 nm band-pass on the excitation and 4.0 nm on the emission monochromator) were corrected for instrument response and were recorded for excitation at 445 nm. The static fluorescence quenching of singlet-excited riboflavin was studied only for those compounds that show the ability to quench the triplet-excited state. All of the measurements were performed in triplicate, and the results reported are the mean value and respective standard deviation.

Irradiation and ESI-MS Photoproduct Analyses. Samples were irradiated using a high-pressure Hg lamp with water filter and an interference filter, which was used to select light at 436 nm. Irradiations were performed in argon-purged (20 min) acetonitrile/Milli-Q water solution in 1 cm × 1 cm quarts cells (Hellma) for 60 min. The ESI-MS analyses were performed with an electrospray ionization sample inlet in an Agilent 1100 series LC-MSD ion trap (Agilent Technologies, Waldbronn, Germany). Samples were infused directly using a syringe pump with a flow rate of 17 μL min^{-1}. The general conditions were as follows: N_2 drying gas temperature, 325 °C; N_2 drying gas flow, 5 l min^{-1}; nebulizer pressure, 15 psi; capillary voltage, 3500 V. For ESI-(+)MS analysis, formic acid was added to a concentration of 0.1%; likewise, for ESI(−)MS, ammonium hydroxide was added. Mass spectra were acquired using ion charge control (ICC) with a maximum accumulation time of 60 s, and the spectra were recorded over the *m/z* 50−2000 range. An amplitude voltage of 1.0 V was typically used for fragmentation in the ion trap tandem MS experiments. The photoproduct analyses were carried out for photolysis of solutions containing riboflavin and phenol (4-hydroxybenzoic acid or ferulic acid) in anaerobic medium.

RESULTS

Laser Flash Photolysis. Following excitation with 440 nm laser pulses of 8 ns, riboflavin (50 μM) in acetonitrile/citrate buffer (pH 4.6, I = 10 mM) was found to yield triplet-excited riboflavin (E° = 2.2 V vs NHE; $\Phi \approx 0.7$) as evidenced by the transient absorption spectra with maximum absorption in the visible region at 720 nm (**Figure 1**). The observed excited-state spectra were similar to those reported previously, and the excited triplet state had a comparable half-life as deduced from the decay of the transient absorption as previously reported (*14−16*).

When the experiment was repeated in the presence of carvone, carveol, 1,8-cineole, limonene, and menthol for up to 20 equivalents of terpene in excess relative to riboflavin (50 μM), no changes in the triplet-excited half-life were observed. The intensity of the transient absorption spectra was not affected, indicating that the terpenes do not interact with triplet-excited riboflavin. In contrast to the terpenes, phenols were found to decrease the lifetime of triplet-excited riboflavin as probed at 720 nm (**Figure 1A**). The decay could in all cases for excess of phenol be described by first-order kinetics. The pseudo-first-order rate constant for decay of triplet riboflavin was further found to depend linearly on phenol concentration, when in excess, and the second-order deactivation rate constant was obtained from the slope of such plots. Bimolecular rate constants for the series of 18 phenols naturally occurring in beer and investigated in the present study are given in **Table 1**.

The transient absorption spectra shown in **Figure 1** illustrate the changes in the time-resolved difference absorption spectra for a solution containing riboflavin alone (**A**), riboflavin and 4-hydroxybenzoic acid (**B**), and riboflavin and 4-hydroxystilbene (**C**).

As seen from the rate constants presented in **Table 1**, phenolic compounds deactivate the triplet-excited riboflavin with rates close to the diffusion control. In the case of the stilbene derivatives, the deactivation rate constant could not be determined due to the formation of a strong near-infrared band, which overlaps with the triplet-excited riboflavin absorption band centered at 720 nm (see **Figure 1C**). Instead, a strong absorption band centered at 420 nm was observed. This absorption is associated with radical formation from the stilbene derivative (*trans*-hydroxystilbene radical has an absorption maximum at 410 nm for pH 6) (*17*), indicating that the quenching process is taking place, however, not allowing the rate constant to be determined.

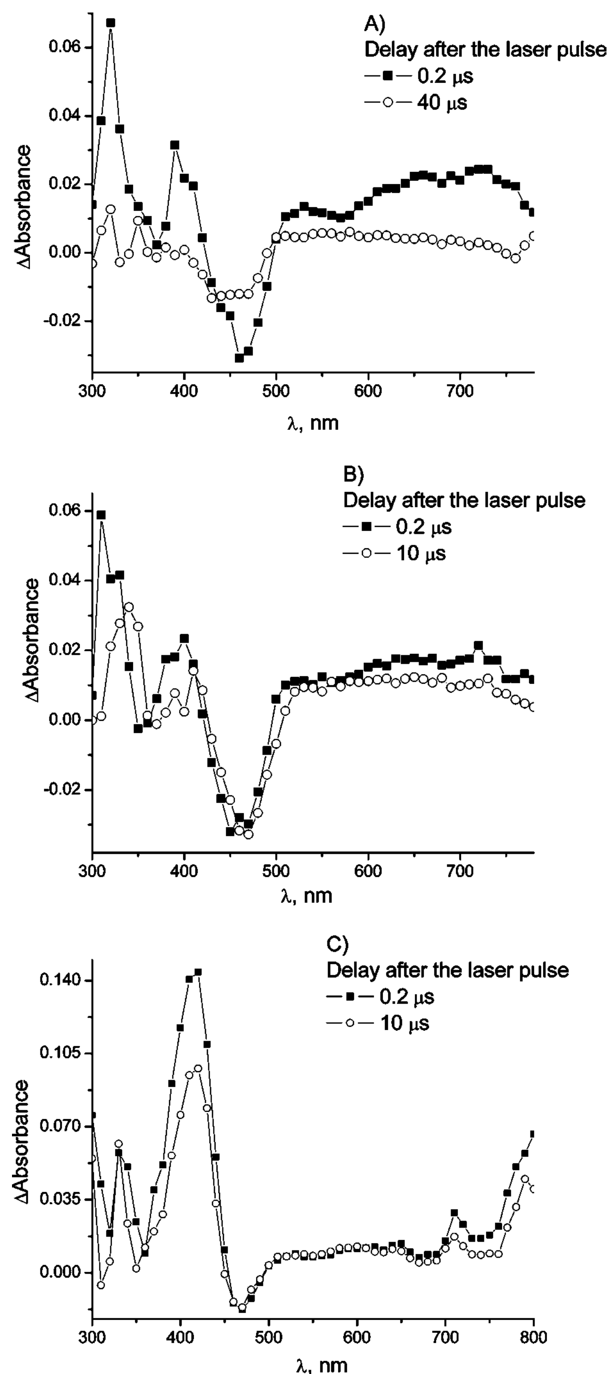


Figure 1. Transient difference absorption spectrum of (**A**) riboflavin (50 μM), (**B**) riboflavin (50 μM) plus added 4-hydroxybenzoic acid (0.5 mM), and (**C**) riboflavin (50 μM) plus added 4-hydroxystilbene (0.4 mM) in anaerobic acetonitrile/citrate (pH 4.6, 10 mM, 1:1 v/v).

The temperature dependence of the rate constants for triplet quenching was determined between 282 and 303 K. Using transition state theory, the activation parameters were derived and are reported in **Table 1**. For the reactions of triplet-excited riboflavin with phenolic compounds leading to quenching, relatively low activation enthalpies were found, ranging from

Table 1. Activation Parameter Bimolecular Rate Constant, ^3k_q, for the Triplet-Excited Riboflavin Deactivation by Phenols in (1:1) Acetonitrile/Citrate Buffer (pH 4.6, 10 mM) at 298 K

phenol	^3k_q (M^{-1} s^{-1})	$\Delta H^{\ddagger}$ (kJ mol^{-1})	$\Delta S^{\ddagger}$ (J mol^{-1} K^{-1})	[Q][a] (M)
caffeic acid	$(2.2 \pm 0.3) \times 10^9$	26.5	+66	3.0×10^{-4}
carvacrol	$(1.9 \pm 0.3) \times 10^9$	26.6	+70	3.5×10^{-4}
4-coumaric acid	$(1.8 \pm 0.3) \times 10^9$	25.4	+65	3.7×10^{-4}
chlorogenic acid	$(1.7 \pm 0.2) \times 10^9$	16.8	+38	4.0×10^{-4}
eugenol	$(2.2 \pm 0.2) \times 10^9$	22	+37	3.0×10^{-4}
ferulic acid	$(2.2 \pm 0.1) \times 10^9$	27	+72	3.0×10^{-4}
gallic acid	$(2 \pm 0.1) \times 10^9$	33.2	+92	3.4×10^{-4}
guaiacol	$(2.6 \pm 0.3) \times 10^9$	19.1	+47	2.6×10^{-4}
4-hydroxy-*trans*-stilbene	ND[b]	ND	ND	ND
4-hydroxybenzoic acid	$(1.1 \pm 0.2) \times 10^9$	12.3	+17	6.1×10^{-4}
2-methoxy-4-vinylphenol	$(1.3 \pm 0.5) \times 10^9$	19.4	+42	5.2×10^{-4}
protocatechuic acid	$(2.4 \pm 0.4) \times 10^9$	26.6	+71	2.8×10^{-4}
trans-resveratrol	ND	ND	ND	ND
syringic acid	$(1.6 \pm 0.3) \times 10^9$	18.2	+40	4.2×10^{-4}
thymol	$(1.8 \pm 0.3) \times 10^9$	20	+46	3.7×10^{-4}
tyrosol	$(1.7 \pm 0.2) \times 10^9$	21.7	+52	3.9×10^{-4}
vanillic acid	$(2.06 \pm 0.06) \times 10^9$	19	+44	3.3×10^{-4}
vanillin	$(2.2 \pm 0.05) \times 10^9$	21	+52	3.0×10^{-4}

[a] Concentration of a quencher, phenol, needed for 90% quenching of the triplet-excited riboflavin assuming a triplet half-life of 13.4 μs (*16*). [b] Not determined.

Table 2. Bimolecular Rate Constant, ^1k_q, for the Singlet-Excited Riboflavin Deactivation by Phenols in Methanol at 298 K

phenol	^1k_q (M^{-1} s^{-1})	[Q][a] (M)
caffeic acid	$(8.1 \pm 0.2) \times 10^9$	0.19
carvacrol	$(5.2 \pm 0.07) \times 10^9$	0.30
4-coumaric acid	$(4.5 \pm 0.27) \times 10^9$	0.35
chlorogenic acid	$(5.3 \pm 0.42) \times 10^9$	0.29
eugenol	$(6.7 \pm 0.18) \times 10^9$	0.23
ferulic acid	$(5.0 \pm 0.05) \times 10^9$	0.31
gallic acid	$(5.0 \pm 0.13) \times 10^9$	0.31
guaiacol	$(6.3 \pm 0.35) \times 10^9$	0.25
4-hydroxystilbene	$(1.1 \pm 0.05) \times 10^{10}$	0.14
4-hydroxybenzoic acid	$(2.8 \pm 0.13) \times 10^9$	0.56
2-methoxy-4-vinylphenol	$(4.5 \pm 0.1) \times 10^9$	0.35
protocatechuic acid	$(3.7 \pm 0.3) \times 10^9$	0.42
syringic acid	$(4.9 \pm 0.2) \times 10^9$	0.32
thymol	$(4.8 \pm 0.18) \times 10^9$	0.33
tyrosol	$(4.6 \pm 0.23) \times 10^9$	0.34
vanillic acid	$(4.1 \pm 0.25) \times 10^9$	0.38
vanillin	$(4.5 \pm 0.22) \times 10^9$	0.35

[a] Concentration of a quencher, phenol, needed for 90% quenching of the singlet-excited riboflavin.

Table 3. Chemical Component and Observed Ions in Mass Spectra of Precursors and Photoproducts Obtained for the Triplet Riboflavin Reaction with Ferulic Acid or 4-Hydroxybenzoic Acid

name	peak (*m/z*)
riboflavin	377.2 $(M + H)^+$
	399.1 $(M + Na)^+$
lumicrome	243.1 $(M + H)^+$
	265.1 $(M + Na)^+$
	507.1 $(M + Na + M)^+$
formyllumiflavin	285.1 $(M + H)^+$
lumiflavin-hydroxy-acetaldehyde	315.1 $(M + H)^+$
$R_1 = O$, $R_2 = OH$; 2′-ketoriboflavin	375.4 $(M + H)^+$
$R_1 = OH$, $R_2 = O$; 4′-ketoriboflavin	375.4 $(M + H)^+$
ferulic acid	195.2 $(M + H)^+$
	193.2 $(M - H)^-$
ferulic acid–riboflavin adduct	569.0 $(M - H)^-$
4-hydroxybenzoic acid	139.0 $(M + H)^+$
	137.0 $(M - H)^-$
4-hydroxybenzoic acid–riboflavin adduct	513.2 $(M - H)^-$

12.3 (4-hydroxybenzoic acid) to 33.2 kJ mol^{-1} (gallic acid), concomitant with positive activation entropies. The systematic variation in activation enthalpy and activation entropy as observed for the series of phenols is indicative of what has been termed compensation effect.

Steady-State Fluorescence. The interaction of phenols and the singlet-excited state of riboflavin was further studied by steady-state fluorescence quenching of the singlet-excited riboflavin emission in methanol as a function of phenol concentration. The quenching rate constant was determined from these steady-state fluorescence measurements by employing the Stern−Volmer equation

$$I_0/I = 1 + K_{sv}[\text{phenol}]$$

where I_0 and I are the fluorescence intensities in the absence and in the presence of phenol, respectively, and $K_{sv} = {}^1\tau_0{}^1k_q$, where $^1\tau_0$ is the singlet-excited riboflavin lifetime reported to be 5.75 ns in methanol (*18*) and ^1k_q the second-order quenching constant.

Phenols were found to quench the emission of singlet-excited riboflavin with a rate constant, ^1k_q, of $\approx 10^9$ M^{-1} s^{-1} as may be seen in **Table 2**. An exception is seen for 4-hydroxystilbene, which shows a strong interaction with singlet-excited riboflavin with a rate constant as high as 1.1×10^{10} M^{-1} s^{-1}.

Electrospray Ionization Mass Spectrometry. The mass spectra in positive and negative ion mode for unexposed and exposed solution of riboflavin and ferulic acid are shown in **Figure 2A**. The main ion in the mass spectrum in positive ion mode for the control sample at *m/z* 377 belongs to riboflavin $(M + H)^+$, and the ion at *m/z* 195 is ferulic acid $(M + H)^+$. The ion at *m/z* 177 originates from ferulic acid after the loss of H_2O, and the ion at *m/z* 399 is a sodium adduct of riboflavin $(M + Na)^+$ (one H atom of riboflavin replaced by a Na atom). The mass spectrum of a control sample in the negative ion mode shows an ion at *m/z* 193 corresponding to ferulic acid $(M - H)^-$ and at *m/z* 569, indicating a riboflavin−ferulic acid adduct $(M_{\text{ferulic acid}} + M_{\text{riboflavin}} - H)^-$, which was found to be formed in the ion source. **Figure 2A** also illustrates the negative and positive mass spectra obtained for the sample exposed at 436 nm with an intensity of 9.6×10^{16} photons during a period of 30 min in a 10 mm × 10 mm quartz cell. The ions observed by mass spectrometry are characterized in **Table 3**.

Figure 2B shows the mass spectra in positive and negative ion modes for the unexposed and exposed samples containing

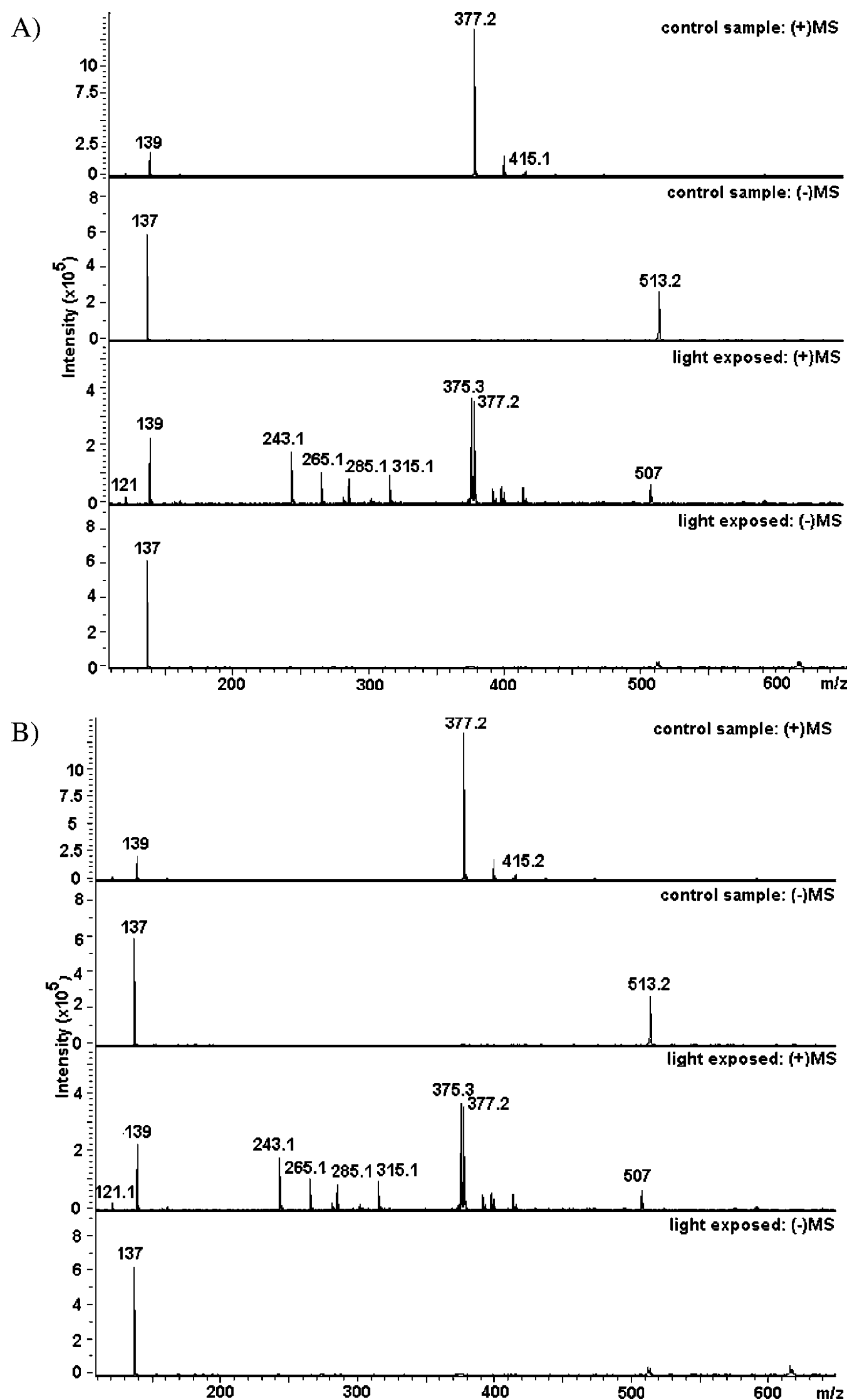


Figure 2. (**A**) Positive and negative ESI-MS spectra for the unexposed and exposed solution containing 110 μM riboflavin and 900 μM ferulic acid in anaerobic acetonitrile/citrate (pH 4.6, 10 mM, 1:1 v/v). (**B**) Positive and negative ESI-MS spectra for the unexposed and exposed solution containing 108 μM riboflavin and 890 μM 4-hydroxybenzoic acid in anaerobic acetonitrile/citrate (pH 4.6, 10 mM, 1:1 v/v).

riboflavin and 4-hydroxybenzoic acid. The mass spectrum in positive ion mode for the control sample exhibits the ions at *m/z* 377.2 and 139.6, corresponding to riboflavin $[M + H]^+$ and 4-hydroxybenzoic acid $[M + H]^+$, respectively, and a sodium adduct for riboflavin is also observed. The mass spectrum in negative ion mode for the control sample shows the presence of two ions, at *m/z* 137and 513, corresponding to 4-hydroxybenzoic acid $[M - H]^-$ and the riboflavin−(4-hydroxybenzoic acid) adduct, $[M_{riboflavin} + M_{4-hydroxybenzoic\ acid} - H]^-$, respectively, formed in the ion source. In the same way as for ferulic acid, the negative and positive ions in the mass spectra for the light-exposed sample show the riboflavin degradation products as it can be seen in **Table 3**. The degradation product for 4-hydroxybenzoic acid and ferulic acid were not observed under the present conditions.

DISCUSSION

The electron transfer from isohumulones and their reduced and oxidized derivatives to triplet-excited riboflavin was recently identified as the main mechanism for light-struck flavor formation in beer. Isohumulones, dihydroisohumulones, and tetrahydroisohumulones were reported to interact with triplet-excited riboflavin with bimolecular rate constants ranging from

1.6 to 2.3 10^8 M^{-1} s^{-1} in acetronitrile/aqueous buffer (pH 4.6, 1:1 v/v) (*8*). However, triplet-excited riboflavin is also known to be quenched by amino acids, peptides, proteins, purine and guanidine bases, polyphenols, and simple phenols (*12*, *14*−*16*, *19*). A detailed description of the interaction between phenols, terpenes, and excited riboflavin should allow a better understanding of the reaction pathway leading to the formation of light-struck flavor compounds in beer.

Our results demonstrate that terpenes (carveol, carvone, 1,8-cineole, limonene, and menthol) do not quench triplet-excited riboflavin, suggesting that the protective effect on light-induced off-flavor formation in beer sometimes assigned to these compounds may originate from their capacity to act as radical scavengers during the subsequent step in the photooxidative process. On the other hand, phenolic compounds as expected (*16*, *18*) quench both the singlet- and triplet-excited states of riboflavin. Although the interaction among phenols and singlet-excited riboflavin occurs with a rate constant close to the diffusion limit, a content of phenolic compounds of >0.3 M (**Table 2**) is necessary to quench 90% of the riboflavin in the singlet-excited state and consequently to hinder triplet-excited riboflavin generation. High phenol content is needed to hinder triplet-excited-state generation ($\Phi \approx 0.7$ in aqueous phosphate buffer, pH 7). From the rate constants in **Table 1**, the calculated quencher concentration needed to deactivate 90% of the triplet-excited state of riboflavin is 3.6×10^{-4} M, 3 orders of magnitude lower than that necessary to quench the singlet-excited state.

Phenols are present in beer at ≈450 ppm, and the content of iso-α-acids ranges from 15 to 100 ppm in extremely bitter beers, with an average of ≈15 ppm for larger beers (*11*, *13*). Assuming an iso-α-acids concentration of 15 ppm (41 μM) and a content of phenolic substances in beer of 450 ppm (2.39 mM of gallic acid equivalent), the competing rate constants for deactivation of triplet-excited riboflavin are 8×10^3 and 4.59×10^6 s^{-1} for iso-α-acids and beer phenols, respectively, indicating that the reaction between triplet-excited riboflavin with phenolic compounds is ≈550 times faster compared to the deactivation by iso-α-acids. An attack on the isohumulones (≈1.4 V vs NHE) (*10*) by radicals formed from the phenols is accordingly more likely as a key step for oxidation of isohumulones leading to the formation of 4-methylpent-3-enoyl radical in beer, which subsequently is trapped by a thiyl radical to give MBT.

Functional groups in the phenol ring inductively or by resonance effects alter the electron density surrounding the molecule and affect its behavior in electrophilic reactions. The effect of the substitute groups in the aromatic ring was tentatively evaluated through the Hammett relationship and also by the redox potential. However, the reactivity of triplet-excited riboflavin toward simple phenols shows little or no correlation with the electron-donating character of the substitute group and/or with the redox potential of the phenol compound, suggesting that electron transfer was not the sole determinant for the measured rate of reaction between phenols and triplet riboflavin. It is also important to note that all of the phenolic compounds studied interact with triplet-excited riboflavin by a common mechanism as may be seen by the observed compensation effect.

The photoproduct studies did not yield any substantial information concerning the oxidation products of the phenolic compounds. The main photoproducts identified originated from the riboflavin deactivation and triplet-excited riboflavin intramolecular photooxidation. From the photoproduct studies it is noted that the intramolecular attack at the carbon C4′ in the triplet-excited riboflavin as well as the reaction between triplet-excited riboflavin and ground-state riboflavin should be taken into account and further studied.

In conclusion, phenolic compounds present in beer are efficient quenchers of triplet-excited riboflavin otherwise responsible for the light-struck flavor development. The deactivation of triplet-excited riboflavin by phenolic compounds competes with isohumulones deactivation and has a kinetic advance of ≈550 times in comparison with the isohumulones. Decomposition of isohumulones in beer by radicals formed after triplet-excited riboflavin deactivation may be the major mechanism behind the light-struck flavor formation, and further studies should include the reactivity of phenoxyl radicals toward isohumulones together with the role of riboflavin intramolecular photooxidation.

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