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REAL TIME ANALYSIS OF THE PHOTOOXIDATION OF BEER BITTERING PRINCIPLES BY QUADRUPOLE TIME-OF-FLIGHT MASS SPECTROMETRY COMBINED WITH HEADSPACE GAS CHROMATOGRAPHY MASS SPECTROMETRY

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Introduction

When beer is exposed to light, significant taste and flavour changes are observed. Formation of 3-methylbut-2-ene-1-thiol (MBT) is responsible for the so-called lightstruck flavour (LSF). Few ng per liter can make beers unpalatable. Isohumulones, the main bittering principles in beer, are essential in the pathway to LSF formation. Photooxidation of isohumulones as well as their reduced forms, by excited beer flavins, resulted in particular degradation products.

Materials and methods

Electrospray ionization-mass spectrometry (ESI-MS)

Reaction mixtures were irradiated simultaneously with a continuous flow injection in the electrospray ionization source:

- Mass Spectrometer: quadrupole time-of-flight (Q-TOF) hybrid mass spectrometer (Waters, Manchester, UK).
- Ion Source: orthogonal nanospray source (Z-spray®) in negative ion mode
- Infusion: Harvard syringe pump, equipped with a 0.25 mL Hamilton glass syringe, infusion rate of 5 µL/min
- Source and desolvation temperatures: 80°C and 120°C, respectively
- capillary voltage: 2900 V; cone voltage: 33 V.
- acquisition range: 50 amu to 800 amu.

Gas chromatography-mass spectrometry (GC-MS)

Photoreaction mixtures were analyzed with GC-MS using a headspace sampler and a CIS-4 Cooled Injection System (Gerstel, Mülheim-an-der-Ruhr, Germany), the injector liner being cooled with liquid nitrogen.

- Agilent 6890 series GC system, connected to a HP5973 mass-selective detector (Agilent, Palo Alto, CA, USA).
- Chrompack fused-silica CP-Select 624 (6% cyanopropylphenyl-dimethylsilicone) capillary column (41 m x 0.25 mm I.D.; 2.1 µm film thickness)
- temperature program: 10 min at 38°C, raised to 170°C at 10°C/min, and 10 min at 170°C
- Reaction products were identified by comparing retention times and mass spectra with reference compounds. Experimental spectra were compared with the library mass spectra on a NIST Mass Spectral Database v. 2.0

Irradiations

Aqueous solutions (2 mL), containing isohumulones (4.2 mM) or derivatives and flavin mononucleotide (FMN) (1.1 mM) were prepared in glass Gerstel headspace 5 mL-vials. Samples were degassed by flushing with nitrogen and were protected from light after FMN was added. Reactions were carried out in a photoreactor equipped with Philips Cool White lamps (8 x 9 W), immediately followed by GC-MS analysis.

For analyses by ESI-MS, solutions were prepared in the dark as described above for the GC-MS analysis, followed by dilution (1/10) in acetonitrile/water (v/v, 1:1). Final concentrations of FMN and five-membered-ring hop derivatives were 0.11 mM and 0.42 mM, respectively. Time-dependent formation of reaction products was monitored by direct irradiation of the infusion syringe with a commercially available 50 W incandescent lamp.

Results and discussion

Loading the reaction mixtures into transparant syringes and exposing them to visible light at the same time as they were injected into the ionisation source of a quadrupole time-of-flight hybrid mass spectrometer, gave us a real-time analysis technique. Irradiation combined with continuous flow injection in the electrospray ionisation source resulted in the elucidation of the non-volatile reaction products. Volatile reaction products were analysed by headspace gas chromatography mass spectrometry.

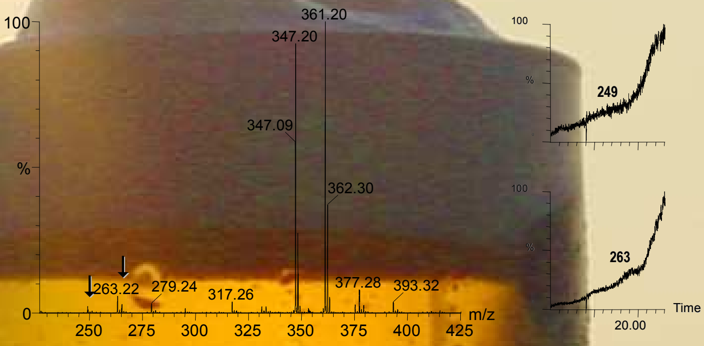

Figure 1. Mass spectrum after visible-light exposure (25 min) of a solution containing isohumulones and flavin mononucleotide (left panel; arrows indicate reaction products) and extracted ion chromatograms of ions observed on negative ESI-MS) (right panel).

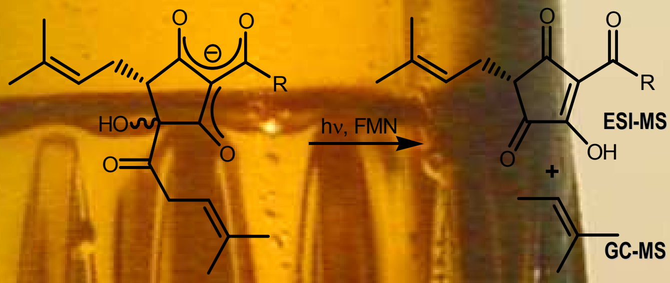

Figure 2. Formation of non-volatile (top) and volatile (bottom) reaction products from the degradation of isohumulones under irradiation of visible light in the presence of FMN.

Conclusion

Visible-light irradiations of isohumulones and their reduced foms in the presence of flavin mononucleotide (FMN), readily induced decomposition. From identification of reaction products thus formed, feasible degradation mechanisms could be proposed. Degradation of isohumulones results in a 3-methylbuyt-2-enyl radical, which is the key precursor on the route to the development of MBT. Moreover, reduced isohumulones e.g. dihydroisohumulones, used in the brewing of light-stable beers, also showed radicaloid decomposition when exposed to visible light in the presence of FMN. This result contradicts the commonly held belief that these compounds withstand photoinduced degradation.

References

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*Chem. Sci., 2004, **1**, C58*

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