p 829 - 832

93

Occurrence of polyfunctional thiols in fresh and aged lager beers. New additional synthesis pathway for 3-methyl-2-buten-1-thiol (MBT)

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SUMMARY

Until now, beer thiols and especially polyfunctional thiols have been very few studied, although their huge organoleptic impact. In the present work, a specific analysis including trapping by pHMB (p-HydroxyMercuriBenzoic acid) has been applied on fresh and aged lager beers. GC-MS, GC-O and GC-PFPD analyses of the extracts revealed the presence of 11 potent odorants such as 3-mercaptohexanol (rhubarb), 4-mercapto-4-methyl-2-pentanone (blackcurrant, box tree), 3-mercaptopropyl acetate (roasted meat), 2-mercaptoethyl acetate (toasted, empyreumatic), 3-mercapto-3-methylbutanol (onion, vegetable), etc. Surprisingly, MBT, known as the skunky off-flavour in beers exposed to sunlight, was also detected in fresh light-protected beers. A new additional synthesis pathway is therefore proposed.

INTRODUCTION

Around 80 different thiols have been identified in foods and beverages (5). These sulfur compounds are in most cases only present as traces, highly reactive (oxidation, heat, light), and commercially unavailable. Therefore, their occurrence in beer is most probably underestimated.

Our first contribution to this field was the synthesis and the sensorial characterization of more than sixty thiols potentially present in foods: mercaptoaldehydes and mercaptoketones (coming from the reaction between H_2S or thioacetic acid and α,ß-unsaturated aldehydes or ketones) as well as mercaptoalcohol and mercaptoacetate analogues (resulting from reduction and esterification of the former). The combinatorial chemistry methodology hyphenated to the use of an equimolar SCD detector and/or mass spectrometer allowed us to build the library (6-9).

All those data were further used to identify new thiols in four lager beers pHMB extracts, which were analysed by GC-O, GC-PFPD and GC-MS. In order to find potential precursors, the same specific extraction was applied on wort and aged beer (5 days at 40 °C).

MATERIALS AND METHODS

Extraction of polyfunctional thiols by pHMB

The protocol is the same as the one proposed by Tominaga *et al.* (4) for wines except that only one liquid-liquid extraction was applied (500 ml of beer - 200 ml of CH_2Cl_2) and that two centrifugation steps were needed to break the emulsion. An external standard (thiazole) was added just before concentration under nitrogen.

Gas Chromatography hyphenated to an Olfactometric Detector (GC-O)

This analysis was performed using a Chrompack CP9001 gas chromatograph which was equipped with a splitless injector maintained at 250 °C and opened after 0.5 min. Sulfur compounds were analysed using a 50 m × 0.32 mm i.d., wall-coated open tubular (WCOT) apolar CP-Sil 5 CB capillary column (film thickness, 1.2 μm). An initial oven temperature of 40 °C was maintained for 4 min and then programmed to rise from 40 to 132 °C at 2 °C/min followed by 132 to 250 °C at 10 °C/min. The eluent was sent to a GC-odour port at 250 °C where it was diluted with a large volume of air (20 ml/min) previously humidified in an aqueous copper (II) sulfate solution to improve the transport of the effluent out of the funnel.

Gas Chromatography hyphenated to an Electronic Impact Mass Spectrometer (GC-MS)

Mass spectra (m/z = 40 to 380) were recorded at 70 eV on a ThermoFinnigan Trace MS mass spectrometer connected to a ThermoFinnigan Trace GC 2000 gas chromatograph equipped with a splitless injector. The separation was performed using the previously described column and oven temperature program. Spectral recording was automatic throughout elution using the Xcalibur software. The compounds were identified on the basis of their fragmentation patterns (combinatorial library).

Gas Chromatography hyphenated to an Pulsed Flame Photometric Detector (GC-PFPD)

The injections were carried out in the splitless mode at 250 °C, the split being turned on after 0.5 minute. At the detector, the following parameters were selected: 210 °C as temperature, 600 V as voltage, 18 ms as gate width, 6 ms as gate delay, 600 mV as trigger level and 3,45 Hz as pulse frequency. The flows of H2, Air1, and Air2 were respectively of 8.5 ml/min, 8.7 ml/min, and 10,8 ml/min. The chromatographic column was the same as the one used in GC-O and the temperature program was similar to the previous one (last rate was set on 5 °C/min).

RESULTS

As depicted in table 1, eleven polyfunctional thiols have been identified in lager beers. Most of them have been found in the four commercial samples. 2-Mercapto-3-methylbutanol seemed to be more specific of one brand probably characterized by a lower antioxidant power. Among all those compounds, only 2-methyl-3-furanthiol (1), 3-methyl-2-buten-1-thiol (3), 3-mercaptohexanol (10), 2-mercapto-3-

methylbutanol (2), and 4-mercapto-4-methyl-2-pentanone (10) were previously described as beer natural constituents in the literature.
None of these compounds were found in the pHMB wort extract, suggesting a key role of fermentation. On the other hand, thiol concentration revealed to be still higher in beer I after artificial aging (5 days at 40 °C).

Table 1: Retention indices, odour, and structure of polyfunctional thiols detected in fresh and aged beers.

Thiol Retention Indices Odour	Detected by GC-	Present in the pHMB extract of
2-Methyl-3-furanthiol $Ir_{CP\text{-}Sil\,5\,CB} = 847$, $Ir_{FFAP} = 1306$ Broth, meaty	O/PFPD/MS	Fresh lager beers I,II,III,IV Aged lager beer I
2-Mercaptoethanol $Ir_{CP\text{-}Sil\,5\,CB} = 717$, $Ir_{FFAP} = 1501$ Roasted, garbage	O/PFPD	Fresh lager beers I,II,III,IV Aged lager beer I
3-Mercaptopropanol $Ir_{CP\text{-}Sil\,5\,CB} = 840$, $Ir_{FFAP} = 1665$ Roasted, broth, potato	O/PFPD	Fresh lager beers I,II,III,IV Aged lager beer I
3-Mercapto-3-methylbutanol $Ir_{CP\text{-}Sil\,5\,CB} = 944$, $Ir_{FFAP} = 1671$ Broth, onion, sweat, vegetable	O/PFPD/MS	Fresh lager beers I,II,III,IV Aged lager beer I
2-Mercapto-3-methylbutanol $Ir_{CP\text{-}Sil\,5\,CB} = 964$, $Ir_{FFAP} = 1656$ Onion	O/PFPD/MS	Fresh lager beers IV
1-Mercapto-3-pentanol $Ir_{CP\text{-}Sil\,5\,CB} = 981$, $Ir_{FFAP} = 1698$ Nettle, burned	O/PFPD/MS	Fresh lager beers I,II,III,IV Aged lager beer I
3-Mercaptohexanol $Ir_{CP\text{-}Sil\,5\,CB} = 1095$, $Ir_{FFAP} = 1853$ Rhubarb, fruity	O	Fresh lager beers I,II,III,IV Aged lager beer I
3-Methyl-2-buten-1-thiol $Ir_{CP\text{-}Sil\,5\,CB} = 808$, $Ir_{FFAP} = 1112$ Plastic, old beer, pungent, skunky	O/MS	Fresh lager beers I,II,III,IV Aged lager beer I
4-Mercapto-4-methyl-2-pentanone $Ir_{CP\text{-}Sil\,5\,CB} = 915$, $Ir_{FFAP} = 1382$ Catty, blackcurrant, box tree	O	Fresh lager beers I,II,III,IV Aged lager beer I
2-Mercaptoethyl acetate $Ir_{CP\text{-}Sil\,5\,CB} = 887$, $Ir_{FFAP} = 1444$ Toasted, burned	O/PFPD/MS	Fresh lager beers I,II,III,IV Aged lager beer I
3-Mercaptopropyl acetate $Ir_{CP\text{-}Sil\,5\,CB} = 992$, $Ir_{FFAP} = 1565$ Roasted meat, burned	O/PFPD/MS	Fresh lager beers I,II,III,IV Aged lager beer I

CONCLUSION

The pHMB extraction technique hyphenated to GC-O/PFPD/MS analyses allowed us to identify 6 new polyfunctional thiols in fresh lager beers. Hydrogen sulfide excreted by yeast during fermentation revealed to be able:

- to substitute hop allylic alcohols (synthesis of 3-methyl-2-buten-1-thiol);

- to achieve a 1,4 addition to wort α,ß-unsaturated aldehydes or ketones (synthesis of 1-mercapto-3-pentanol, 3-mercaptohexanol, 4-mercapto-4-methyl-2-pentanone);
- to carry out a radical anti-Markovnikov addition on a double bonds (synthesis of 2-mercapto-3-methylbutanol, especially in beers with low antioxidant activity) or an electrophilic Markovnikov addition (synthesis of 3-mercapto-3-methylbutanol);
- to interact with Maillard reaction intermediates (synthesis of 2-methyl-3-furanthiol).

Other beer thiols were issued from amino acid Ehrlich degradation followed by a reduction and an esterification (synthesis of 2-mercaptoethanol, 3-mercaptopropanol and their corresponding acetates).

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