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Identification of a new light-struck off-flavour in "light-stable" beers

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ABSTRACT

For decades, MBT (3-methyl-2-buten-1-thiol) is known as the compound responsible for the lightstruck off-flavour in beer. This leads many brewers to adapt the procedures by using reduced hop extracts. Unfortunately, other off-flavours including onion-defects often characterize these "light stable" beers. In the present work, a commercial lager beer which did not contain isohumulones (blend of dihydroisoalpha acids; bottled in clear glass) was submitted to various natural aging. Whereas no MBT-defect (skunky-like) was detected by sensorial analyses, a strong "onion-like" off-flavour was evidenced in the samples exposed to light. GC-PFPD and GC-O analyses of global (XAD) and thiol specific (*p*HMB) extracts allowed us to identify 2-sulphanyl-3-methylbutanol (2S3MBol) as the key-off-flavour (AEDA Flavour Dilution = 32–1024 for 2S3MBol while only 8–64 for MBT). 2S3MBol revealed to be synthesized from 3-methyl-2-buten-1-ol (MBOH) found in hop extracts. The involved radicalar mechanism is strongly enhanced by light. Although reduced hop extracts improve light stability regarding MBT, aroma-extracts give rise to strong onion-like off-flavours in presence of light. The concentration of the hop allylic precursor should be monitored in commercial hop extracts.

Introduction

3-Methyl-2-buten-1-thiol (MBT) is responsible for the famous lightstruck skunky off-flavour of beers exposed to light (Kuroiwa et al., 1963). The usual iso-alpha acids are degraded either by UV (280–320 nm), or by visible light in presence of riboflavin (350–500 nm), leading to reactions between enoyl radicals and cysteine-derived products (also riboflavin mediated) (Burns et al., 2001; Huvaere et al., 2004, 2006). Brown glasses, which cut off wavelengths under 500 nm, allow minimizing such defects (De Keukeleire et al., 1992), but a better strategy is to remove those unsafe iso-humulones from beer (Verzele and De Keukeleire, 1991; Burns et al., 2001). Nowadays dihydroisohumulones (rho), tetrahydroisohumulones and hexahydroisohumulones are extensively used by brewers around the world. Unfortunately, other strong off-flavours emerged in such new "light stable" beers after aging. The aim of the present work was to identify which compound was responsible for the onion-like off-flavour found in aged beers containing reduced hop extracts.

Materials and methods

Materials

Diethyl ether (99.9%), dodecane (99.9%), *p*-hydroxymercuribenzoic acid (*p*HMB), HCl 37%, 3-sulphanyl-3-methylbutan-1-ol (3S3MBol), 3-methyl-2-buten-1-ol (MBOH) and hydrogen sulphide were purchased from Sigma–Aldrich (Bornem, Belgium). 4-Methoxy-2-methylbutane-2-thiol (IST) and 3-methyl-2-buten-1-thiol (MBT) were obtained from Oxford Chemicals (Oxford, UK). Dichloromethane (99.9%) obtained from Romil (Cambridge, UK) was distilled before use. Milli-Q water was used (Millipore, Bedford, MA). NaOH and Na_2SO_4 99% were supplied by Janssen (Geel, Belgium). Amberlite XAD 2 resin (Supelco, Bellefonte, PA) (pore size, 9 nm; specific area, 330 m^2/g) was sequentially washed with methanol and diethyl ether (each for 4 h) in a Soxhlet and stored in methanol at 4 °C. A strongly basic Dowex resin 1 × 2, Cl-form (Sigma–Aldrich, Bornem, Belgium) was stored in hydrogen chloride (0.1 M). Anhydrous sodium sulphate was obtained from Merck (Darmstadt, Germany) and tris(hydroxymethyl)aminomethane (Tris) from USB (Cleveland, OH, USA).

Abbreviations: AEDA, Aroma Extract Dilution Analysis; FD, Flavour Dilution; GC, Gas-Chromatography; GC-O, GC-Olfactometry; GC–MS, GC–Mass Spectrometry; IST, Internal Standard; LS, Light-Stable; *p*HMB, *p*-hydroxymercuribenzoic acid; PFPD, Pulse Flame Photometric Detector; RI, Retention Index; SIM, Single Ion Monitoring; 2S3MBol, 2-sulphanyl-3-methylbutan-1-ol; 3S3MBol, 3-sulphanyl-3-methylbutan-1-ol; MBT, 3-methyl-2-buten-1-thiol; MBOH, 3-methyl-2-buten-1-ol.

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Reference compounds synthesized in our laboratory

2-Sulphanyl-3-methylbutan-1-ol (2S3MBol) has been synthesized, according to Vermeulen et al. (2006), from 3-methyl-2-buten-1-ol (MBOH) and hydrogen sulphide.

Beer and hop samples

A fresh "Light-stable" beer (LS), including a blend of reduced dihydroisohumulones (rho) and bottled in clear glass, was used. Nugget bred in the USA and Saaz bred in Czech Republic were provided by Hopsteiner (Mainburg, Hallertau, Germany). Tomahawk and Cascade bred in USA were provided by Yakima Chief (Louvain-la-Neuve, Belgium). Nelson Sauvin bred in New Zealand was provided by Hops Limited (Richmond, Nelson, New Zealand).

Aging procedures

A commercial LS beer, bottled in clear glass, was submitted to two storage conditions: natural day and night light alternance (LS-L), or in the dark (LS-D). Global (Amberlite XAD-2 resin) and thiol-selective (*p*HMB) extractions were conducted after 0, 3, 4 and 7 months of aging.

Sensorial analyses

An expert tasting panel (8 panellists) evidenced the organoleptic defects by completing a form with 21 attributes.

Global aroma extraction procedure (Lermusieau et al., 2001)

Odorants were extracted by the Amberlite XAD 2 resin. A total of 2 g of XAD 2 were thoroughly rinsed with Milli-Q water (100 mL) and poured into a 100 mL Schott flask containing 50 mL of beer. This mixture was shaken on a platform shaker at 200 rpm for 2 h at 20 °C. The content of the flask was then transferred into a glass column (60 cm × 1 cm inner diameter). The column was first rinsed with 4× 50 mL Milli-Q water to eliminate sugars and the water-soluble substances. Apolar aroma compounds were then eluted with 2× 20 mL diethyl ether at a flow rate of 0.75 mL/min. The extract was dried with anhydrous sodium sulphate; dodecane was added (EST; spiking with 0.5 mL of the 20 mg/L stock solution), and the mixture was concentrated to 0.5 mL in a Kuderna-Danish (concentration factor = 100, final EST concentration = 20 mg/L). The final extract was stored at −80 °C for further analyses. All extractions have been performed in duplicate, from two different bottles.

Specific extraction of polyfunctional thiols (Tominaga et al., 1998)

Beer (500 mL) was stirred with distilled CH_2Cl_2 (200 mL) for 30 min. 4-Methoxy-2-methylbutan-2-thiol was added as internal standard (IST, at 0.67 μg/L in beer). After decantation (±15 min), the lower phase and the interfacial emulsion were centrifuged for 20 min at 4000 rpm. The organic phase was then extracted by 2× 20 mL of a *p*HMB solution (360 mg of *p*HMB, 24.6 g of Tris in 1 L of Millipore water) for 5 and 10 min, respectively. The combined aqueous phases were loaded into a strongly basic anion exchanger column (Dowex 1WX2-100 resin from Aldrich Chemicals), washed beforehand by NaOH 2 M, HCl 2 M, and in between rinsed by ultrapure water. Then 50 mL of sodium acetate buffer (0.1 M, pH 6) was poured on the resin to remove impurities. Volatile thiols were released by percolating a purified cysteine solution (640 mg of hydrochloride L-cysteine monohydrated in 60 mL of Millipore water – this solution was washed with 2× 5 mL of distilled CH_2Cl_2 before use). The eluate containing the volatile thiols was collected and extracted by 4 and then 3 mL of distilled CH_2Cl_2 using magnetic stirring (5 min). The organic phases were pooled, dried on anhydrous Na_2SO_4, and finally concentrated in a Kuderna to 250 μL and to 70 μL in a Dufton column, to be stored at −80 °C.

3-Methyl-2-buten-1-ol (MBOH) extraction from hop pellets

Steam distillation-solvent extraction (Likens Nickerson method) was carried out in a microextractor (Alltech 8910) according to Bouseta and Collin (1995). Hop pellets (0.5 g) were milled and mixed with 50 mL deoxygenated ultra pure water and 1.5 mL of a carvone solution at 20 mg/L (IST) in flask A. Dichloromethane and ultra-pure, deoxygenated water (1.5 mL each) were introduced into the liquid/liquid extraction area. 1.5 mL of dichloromethane was introduced in the organic phase vessel (B). A few clean grains of carborundum were added into flasks A and B. Prior to the procedure, the entire system was purged with nitrogen (2–3 mL/min) for 5 min. Flask A was then heated in a 140 °C oil bath and flask B in a 70 °C water bath. The vapours were condensed by means of a cold finger maintained at −10 °C by a cryostat. The entire steam distillation-solvent extraction procedure was carried out under a 2 mL/min nitrogen flow. The steam distillation was stopped after 45 min. The dichloromethane extract was then concentrated to 0.5 mL at 45 °C with a Kuderna-Danish concentrator.

Gas Chromatography hyphenated to olfactometric detection (GC-O) or to a flame ionization detector (GC-FID)

1 μL of the *p*HMB (thiol-specific), XAD-2 (global) or Likens-Nickerson hop extracts was analysed with a Chrompack CP9001 gas chromatograph equipped with a splitless injector maintained at 250 °C; the split vent was opened 0.5 min post-injection. Compounds were analysed with a wall-coated open tubular (WCOT) apolar CP-Sil 5 CB (50 m × 0.32 mm i.d., 1.2 μm film thickness) and a polar FFAP (25 m × 0.32 mm i.d., 0.3 μm film thickness) capillary column. The carrier gas was nitrogen and the pressure was set at 50 kPa (CP-Sil 5 CB) or 30 kPa (FFAP). The oven temperature was programmed to rise from 36 °C to 85 °C at 20 °C/min, then to 145 °C at 1 °C/min, and finally to 250 °C at 3 °C/min and held for 30 min. The FID detector was set at 250 °C. In order to assess the olfactory potential of the extract, the column was connected to a GC-O port (Chrompack) maintained at 250 °C. The effluent was diluted with a large volume of air (20 mL/min) pre-humidified with an aqueous copper (II) sulphate solution. All extracts were analysed immediately after extraction by 2 trained panellists. Complete Aroma Extract Dilution Analysis (AEDA) (Grosch, 1994) was performed on *p*HMB extracts and LNEs by one operator, with the CP-Sil 5 CB column. The extracts were diluted stepwise with dichloromethane (1 + 1 by volume). The Flavour Dilution (FD) is defined as the highest dilution at which the compound could still be detected (FD = 2^n with $n + 1$ = number of dilutions applied on the extract until no odor was perceived). The precision of this AEDA analysis is $n \pm 1$ (factor 2 between FD values).

Gas Chromatography hyphenated to an electronic impact mass spectrometer (GC–MS)

Mass spectra (m/z = 40–380) were recorded at 70 eV on a Thermo Finnigan Trace MS mass spectrometer connected to a Thermo Finnigan Trace GC 2000 gas chromatograph equipped with a splitless injector and an apolar CP-Sil-5-CB MS capillary column (50 m × 0.32 mm i.d., 1.2 μm film thickness). The carrier gas was helium and the pressure was set at 100 kPa. The oven temperature program was the same as that described for GC-O. Spectral recording was automatic throughout elution; Xcalibur software was used.

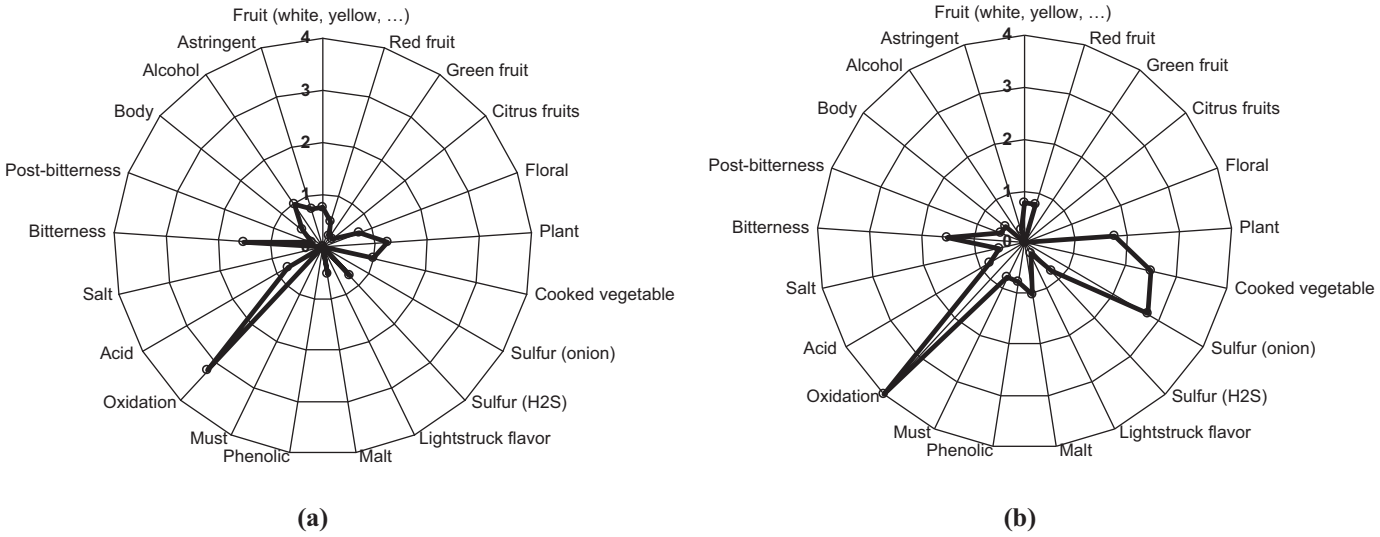

Fig. 1. Flavour profile of aged "light stable" beer stored for 3 months in the dark (LS-D; (a)) or exposed to natural night and day light cycle (LS-L; (b)).

Gas Chromatography hyphenated to a pulsed-flame photometric detector (PFPD)

2 μL of the *p*HMB extracts were analysed on a Thermo Finnigan Trace GC 2000 gas chromatograph equipped with a splitless injector maintained at 250 °C and connected to the O.I. Analytical PFPD, model 5380. The injections were carried out in the splitless mode at 250 °C, the split being turned on after 0.5 min. The carrier gas was helium at a pressure of 90 kPa. At the detector, the following parameters were selected: 250 °C as the temperature, 600 V as the voltage, 18 ms as the gate width, 6 ms as the gate delay, 580 mV as the trigger level and 3.70 Hz as the pulse frequency. The oven temperature program and the column were the same as that described for GC-O.

Identifications

MS identifications were done by comparing the mass spectra obtained from each sample with those obtained with pure or synthesized compounds injected under the same conditions and

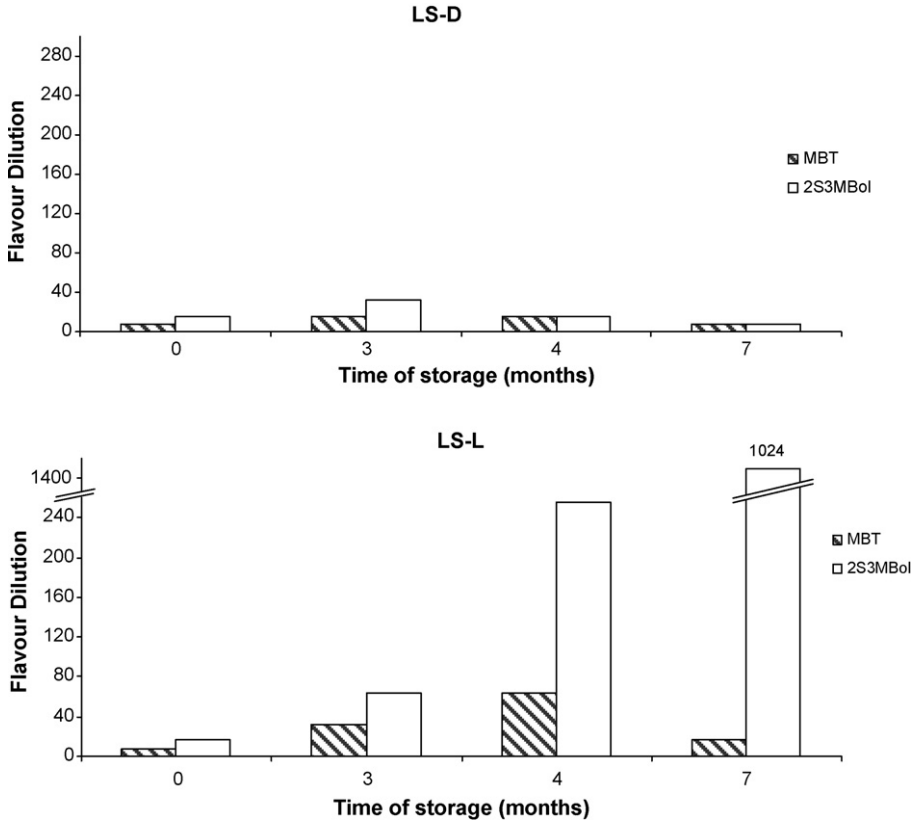

Fig. 2. Evolution of Flavour Dilution (AEDA method) of MBT (RI CP-Sil 5 CB = 808, odour: skunky, coffee) and 2-sulphanyl-3-methylbutanol (2S3MBol RI CP-Sil 5 CB = 964, odour: onion, sulphur) during aging of the LS beer under light exposure or in the dark.

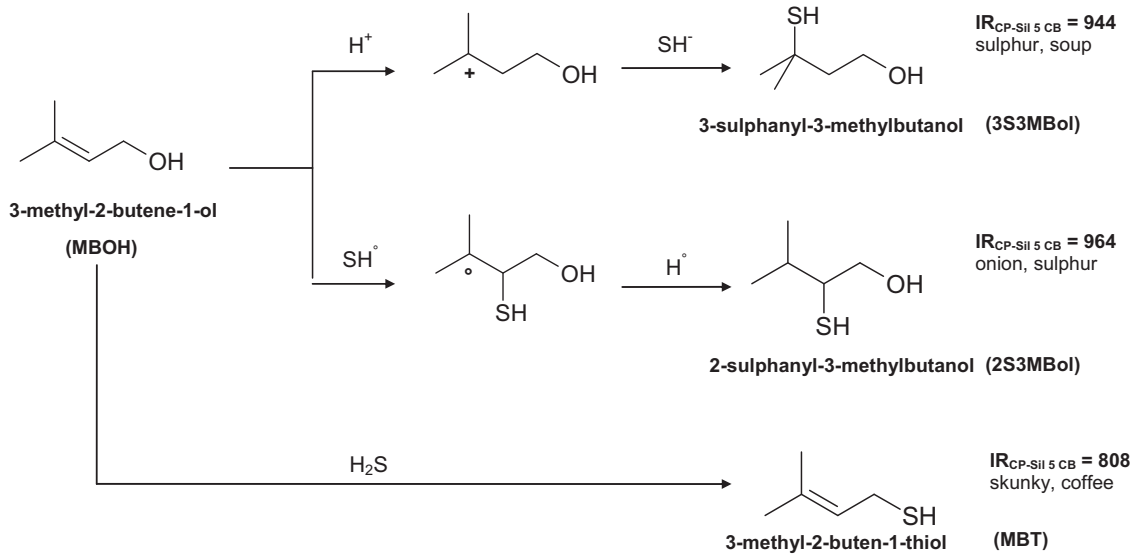

Fig. 3. Formation pathway of MBT, 3S2MBol and 3S3MBol from MBOH and H_2S (Vermeulen et al., 2006; Gros et al., 2009).

present in the NIST library. The retention indices were determined by injection onto two capillary columns (CP-Sil 5 CB and FFAP-CB) connected to the FID or the olfactometric detector (identification checked by co-injection). In the case of PFPD detections (interesting for traces giving no GC–MS peak), injection of thioesters (synthesis of Khan et al., 1999) allowed translation into the alkane-related decimal numeral system.

Quantification

For MBOH, full scan MS calibration curve (area relative to carvone) was used.

Results and discussion

The commercial "Light-Stable" (LS) beer bottled in clear glass was submitted for 7 months to two storage conditions: natural day/night light alternance (LS-L) or protection from light (LS-D). Whereas no "MBT" defect was detected by sensorial analyses in any samples, a strong "onion-like" off flavour was evidenced, only in LS-L (Fig. 1).

The beer flavours were first extracted by an Amberlite XAD-2 resin (according to Lermusieau et al., 2001) after 3 months of aging. The extracts were analysed by GC-Olfactometry-AEDA. In the XAD-2 extracts of the LS-L, a powerful "onion-like" odorant zone (IR = 964) was evidenced by GC-O. Both the kind of descriptor and the absence of a resolved peak by GC–MS lead us to suspect the presence of a polyfunctional thiol. Therefore, a thiol-selective extraction procedure (*p*HMB) was conducted on all samples through aging. The PFPD detector allowed us to confirm the presence of a sulphur compound at RI = 964. By comparison with recently synthesized polyfunctional thiols (Gros et al., 2009), the onion-like defect was identified to be 2-sulphanyl-3-methylbutanol (2S3MBol).

As depicted in Fig. 2, the Flavour Dilution (FD, AEDA procedure) of 2S3MBol increased through aging under light exposure (LS-L), reaching values up to 1024 after 7 months. In the meantime, 2S3MBol remained very slightly odorant (FD = 8–32) in the beers stored in the dark (LS-D). By comparison, MBT was detected at FD = 16 and 8 in LS-L and LS-D beers, respectively (FD from 204 to 1458 for usual lager beers, Lermusieau et al., 2001; Gijs et al., 2002).

In fresh conventional beers tainted with strong onion-like defects, the well known hop aglycone 3-methyl-2-buten-1-ol (MBOH) (Goldstein et al., 1999; Kollmannsberger et al., 2006) has been recently identified as the main precursor of 2S3MBol (Gros et al., 2009). As shown in Fig. 3, MBOH can generate three odorant thiols in presence of hydrogen sulphide: 3-sulphanyl-3-methylbutan-1-ol (3S3MBol, by electrophilic addition), 2S3MBol (by radicalar addition), and MBT (by nucleophilic substitution). As issued from the radicalar pathway (Fig. 3), it is not surprising that light revealed in our experiments to be an enhancer of 2S3MBol.

In "light stable" beers, MBOH most probably arise from aroma extracts added to the beer. In order to assess how the hop variety could influence the MBOH content of beer, five cultivars from two following crops were analysed. The Likens Nickerson extracts

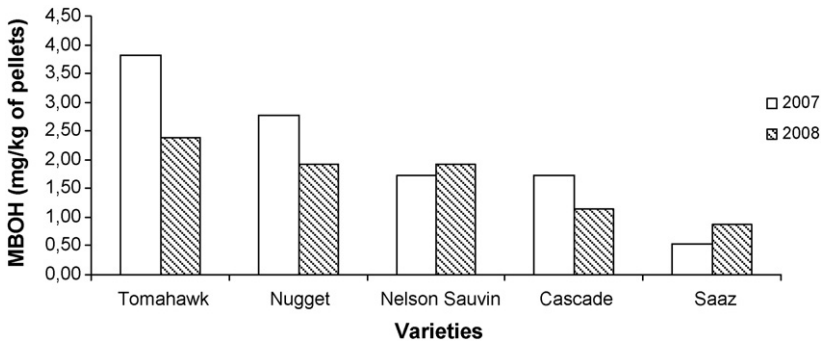

Fig. 4. Amount of 3-methyl-2-buten-1-ol (MBOH) in hop pellets (five varieties) within two following crops (GC–MS analysis, SIM Mode).

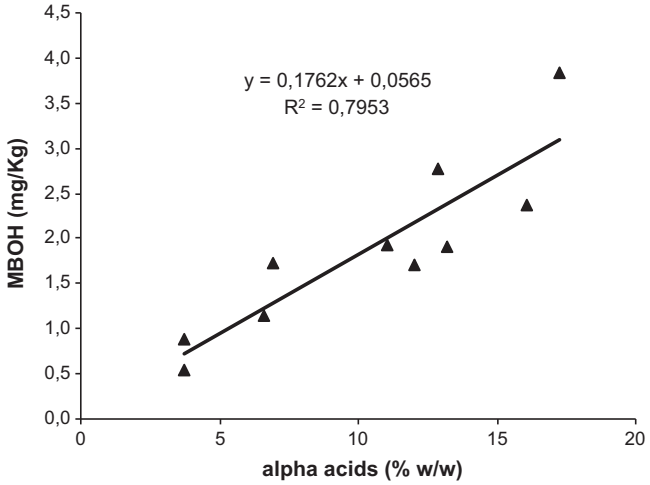

Fig. 5. First order linearization for *alpha* acid contents (%, w/w) and 3-methyl-2-buten-1-ol (MBOH, mg/kg) in hop pellets within the five varieties within two following crops.

evidenced strong differences in MBOH concentrations between the Super Alpha Tomahawk (up to 3.7 mg/kg) and the low bitter Saaz variety (>1 mg/kg) (Fig. 4). The bitter varieties Nugget and Nelson Sauvin gave intermediate values. Big differences also occurred between successive harvests. As issued from the isohumulone lateral chain, MBOH revealed logically well correlated with the hop bitterness (Fig. 5). Nevertheless, by *beta*-glucosidase treatment, Kollmannsberger et al. (2006) revealed more glycosylated MBOH in the low-bitter Saaz hop variety than in Hallertauer Mittelfrüh variety, richer in *alpha*-acids. This contradiction remains to be clarified.

In conclusion, in order to improve the stability of "light-stable" beers, a low MBOH content should be required in the hop aroma extracts. MBOH being also potentially present under glycosylated forms, beta glucosidase activity and hydrogen sulphide potential of yeast (Daenen et al., 2008; Lejeune et al., 2007) could also impact the relative stability of such beers.

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References

Bouseta, A., Collin, S., 1995. Optimized Likens-Nickerson methodology for quantifying honey flavours. Journal of Agricultural and Food Chemistry 43, 1890–1897.

Burns, C.S., Heyerick, A., De Keukeleire, D., Forbes, M.D.E., 2001. Mechanism for formation of the lightstruck flavor in beer revealed by time-resolved electron paramagnetic resonance. Chemistry European Journal 21, 4553–4561.

Daenen, L., Saison, D., Sterckx, F., Delvaux, F., Verachtert, H., Derdelinckx, G., 2008. Screening and evaluation of the glucoside hydrolase activity in *Saccharomyces* and *Brettanomyces* brewing yeasts. Journal of Applied Microbiology 104, 478–488.

De Keukeleire, D., Vindevogel, J., Sziics, R., Pat, S., 1992. The history and analytical chemistry of beer bitter acids. Trends in Analytical Chemistry 77, 275–280.

Gijs, L., Chevance, F., Jerkovic, V., Collin, S., 2002. How low pH can intensify β-damascenone and dimethyl trisulfide production through beer aging. Journal of Agricultural and Food Chemistry 50, 5612–5616.

Goldstein, H., Ting, P., Navarro, A., Ryder, D.,1999. Water-soluble hop flavour precursors and their role in beer flavour. In: Proceedings of European Brewery Convention Congress, vol. 27. Fachverlag Hans Carl, Nürnberg, Germany, pp. 53–66.

Gros, J., Nizet, S., Collin, S., 2009. Hop allylic alcohols are precursors of sulfur-containing odorants in fresh beer. Acta Horticulturae 848, 273–278.

Grosch, W., 1994. Determination of potent odorants in foods by aroma extract dilution analysis (AEDA) and calculation of odor activity values (OAVs). Flavour Fragrance Journal 9, 147–158.

Huvaere, K., Andersen, M.L., Olsen, K., Skibsted, L.H., Heyerick, A., De Keukeleire, D., 2004. Riboflavin-sensitized photooxidation of isohumolones and derivatives. Photochemical and Photobiological Sciences 3, 337–340.

Huvaere, K., Andersen, M.L., Storme, M., Van Bocxlaer, J., Skibsted, L.H., De Keukeleire, D., 2006. Flavin-induced photodecomposition of sulfur-containing amino acids is decisive in the formation of beer lightstruck flavour. Photochemical and Photobiological Sciences 5, 961–969.

Khan, J.A., Gijs, L., Berger, C., Martin, N., Piraprez, G., Spinnler, H.E., Vulfson, E.N., Collin, S., 1999. Combinatorial approach to flavour analysis.1. Preparation and characterization of a S-methyl thioester library. Journal of Agricultural and Food Chemistry 47, 3269–3273.

Kollmannsberger, H., Biendl, M., Nitz, S., 2006. Occurrence of glycosidically bound flavour compounds in hops, hop products and beer. Monatsschrift Fur Brauwissenschaft 5/6, 83–89.

Kuroiwa, Y., Hashimoto, N., Hashimoto, H., Kokubo, E., Nakagawa, K., 1963. Factors essential for the evolution of sunstruck flavour. Proceedings of American Society of Brewing Chemists 21, 181–193.

Lejeune, I., Mélotte, L., Lopez del Castillo, M., Collin, S., 2007. A New Assay for the Screening of Brewing Yeasts in their Ability to Excrete Hydrogen Sulfide. In: Proceedings of European Brewery Convention Congress, vol. 40, Curran Associates, Red Hook, USA, pp. 380–386.

Lermusieau, G., Bulens, M., Collin, S., 2001. Use of GC-Olfactometry to identify the hop aromatic compounds in beer. Journal of Agricultural and Food Chemistry 49, 3867–3874.

Tominaga, T., Murat, M.L., Dubourdieu, D., 1998. Development of a method for analysing the volatile thiols involved in the characteristic aroma of wines made from *Vitis vinifera* L. cv. Sauvignon blanc. Journal of Agricultural and Food Chemistry 46, 1044–1048.

Vermeulen, C., Lejeune, I., Tran, T.T.H., Collin, S., 2006. Occurrence of polyfunctional thiols in fresh lager beers. Journal of Agricultural and Food Chemistry 54, 5061–5068.

Verzele, M., De Keukeleire, D., 1991. Chemistry and Analysis of Hop and Bitter Acids. Elsevier, pp. 88–126.