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Fate of Hop and Fermentation Odorants in Commercial Belgian Dry-hopped Beers Over Two Years of Bottle Storage: Key-role of Oxidation and Hop Esterases

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ABSTRACT

The aim of the present work was to compare levels of short chain fatty acids, esters, terpenoids and polyfunctional thiols in (mostly bottle-refermented) commercial Belgian dry-hopped beers before and after two years of storage at 20ºC (the usual best-before date in Belgium). Among the hop-derived volatiles, the terpenoids linalool and geraniol, the polyfunctional thiols 3SHol, 3SHA and 3S4MPol, and the esters ethyl isobutyrate, ethyl isovalerate and ethyl heptanoate (up to 499, 53, 0.2, 2, 3, 84, 63 and 19 µg/L, respectively) were found above their sensory thresholds in most fresh dry-hopped beers. The fermentation-derived esters reached concentrations similar to those previously reported for non-dry-hopped beers, with ethyl hexanoate and isoamyl acetate (up to 0.4 and 3.9 mg/L, respectively) often above their sensory thresholds.

Except ethyl isovalerate (more than 85% still present), most hop odorants and fermentation esters showed degradation over the two-year storage period: only 45-70% of linalool, geraniol, and ethyl hexanoate and even less than 40% for polyfunctional thiols, ethyl isobutyrate, and ethyl heptanoate initial concentrations were detected after storage. How the dry-hopping process affects this degradation was further investigated in model media. Fermentation esters proved to be more strongly impacted in dry-hopped than in non-dry-hopped beers because of hop esterase activity. In addition to being aware of the need to avoid hop esterases, craft brewers are here advised to use bottle refermentation for its ability to regenerate some flavors and consume packaged oxygen. No deleterious effect of yeast, such as short chain fatty acid excretion, was evidenced.

Key-words: dry hopping, staling, beer flavor, hop enzymes, SAFE, bottle refermentation

INTRODUCTION

Although a longstanding practice among English brewers, dry hopping (addition of hop at the cold stage of the brewing process [1]) was introduced worldwide only in recent decades, especially for craft-brewed beers. The emergence of dual-purpose hop varieties (containing high levels of both bitter acids and flavoring compounds) has made it possible to impart distinct aromas to such beers, never found in kettle- or late-hopped beers.

Most dry-hopped beer (DHB) flavors originate from hop essential oils [2–4]. Among them, the monoterpenic alcohols linalool, geraniol, and β-citronellol [5–9], together with the polyfunctional thiols 3-sulfanylhexanol (3SHol), 3-sulfanylpentanol (3SPol), 3-sulfanyl-4-methylpentanol (3S4MPol), and 4-sulfanyl-4-methyl-2-pentanone (4S4M2Pone) [10–13] impart their typical tropical/citrusy/blackcurrant notes to such beers. In addition, the hop-derived esters ethyl isobutyrate and ethyl isovalerate can impart a pleasant red fruit aroma, if found above their sensory thresholds of 6.3 and 2.0 µg/L, respectively [14,15].

Recent studies have shown that dry hopping is more than a cold extraction of volatile compounds. The extracted non-volatile fraction contains humulinones (responsible for the bitterness changes of DHBs [16–18]), glycosidic precursors of terpenols [5,8,19,20], cysteinyl and glutathionyl precursors of 3SHol, 3SPol, 3S4MPol, and 4S4M2Pone [10,21–23], and enzymes such as amylases and dextrinases which can convert beer dextrins to fermentable sugars [24–26]. Moreover, the plant material itself might act in the beer as an adsorbent for hydrophobic particles [16,27].

The increased popularity of DHBs has brought to light new challenges related to beer stability. Besides the usual stale flavors conferred by *trans*-2-nonenal (cardboard) [28,29], dimethyltrisulfide (onion soup) [30], methional (wort/stale) [31], β-damascenone (cooked

apple) [32], 4-vinylsyringol (tobacco) [33], guaiacol (burnt) [34], sotolon (madeira) [35], 3-methyl-2-buten-1-thiol (skunky) [36–38] and 3-sulfanyl-3-methylbutyl formate (ribes) [39], additional defects could arise through uncontrolled oxygen uptake or the higher temperature usually applied for DHB maturation. Barnette and Shellhammer [40] did evidence a decrease in the tropical, citrus, and hoppy characteristics of such beers after 14 days of storage at 30ºC, and this could not be totally explained by the masking effect of usual staling off-flavors. Tran *et al.* have reported strong degradation of the polyfunctional thiols 3SHol and 4S4M2Pone in non-dry-hopped beers after twelve months at 20°C [41].

Very few data are available in the literature about the utility of using bottle refermentation to improve DHB stability. In high-residual-extract beers, of course, the use of maltose-negative yeast is advisable in order to prevent delayed consumption of fermentable sugars released by hop amylases. Yet apart from the problem of managing carbon dioxide pressure, bottle refermentation could offer many benefits. First, by consuming oxygen, active yeast in the bottle could both protect the highly oxidizable polyfunctional thiols and avoid masking of their flavors by oxidation defects, i.e. madeira [35] and ribes [39] off-flavors. Bottled yeast can also metabolize *trans*-2-nonenal to odorless nonenol and nonanol [29]. Moreover, it may extend the release of expected odorants, either from its own metabolism or by transforming precursors from hop [22]. But what about the adsorption of some lipophilic aromas? As recently evidenced for bitter acids, yeast cells do retain hydrophobic compounds during storage [16]. Bottle refermentation even appears as an interesting way to avoid excessive amounts of *cis*-humulinones which, according to a recent report, are to be feared as much as *trans*-isohumulones as regards their effect on bitterness stability [42]. The occurrence of hop co-constituents in unfiltered DHBs might also negatively impact yeast shape, causing excessive short chain fatty acid excretion and autolysis. It thus remains necessary to weigh both the advantages and disadvantages of bottle refermentation for dry-hopped beers.

The first aim of the present work was to assess differences between DHBs as regards key flavors and their stability through aging. Commercial Belgian DHBs were investigated, both when fresh and after two years of storage (20ºC in the dark). The focus was on both hop-derived flavors (mostly terpenoids, esters, and polyfunctional thiols, here extracted by solvent assisted flavor evaporation/SAFE [43] or pHMB [13]) and yeast-derived flavors (mostly short chain fatty acids and esters, analyzed after n-hexanol [44] or SAFE extraction). The second aim was to better understand the fate of esters and terpenols through DHB aging by investigating the impact in model media of hop particles and enzymes.

EXPERIMENTAL

Chemicals

Dichloromethane, ethanol absolute (99%), anhydrous sodium sulfate, sodium hydroxide and hydrogen chloride 37% were purchased from VWR International (Leuven, Belgium). Acetic acid, sodium salt 99+% was purchased from Acros Organics (Geel, Belgium). L-cysteine hydrochloride monohydrate, Dowex resin 1x2, chloride form, *p*-hydroxymercuribenzoic acid (pHMB) and tris(hydroxymethyl)amino-methane (Tris) were purchased from Sigma-Aldrich (Bornen, Belgium). Standards of isovaleric acid, hexanoic acid, octanoic acid, nonanoic acid, decanoic acid, myrcene, linalool, α-terpineol, geraniol, geranyl acetate, dodecane, isobutyl isobutyrate, ethyl hexanoate, ethyl heptanoate, ethyl octanoate, ethyl decanoate, ethyl isovalerate, 2-phenylethyl acetate, isoamyl acetate, 2-acetylthiophene, 4-methoxy-2-methylbutane-2-thiol, 2-sulfanylethanol, 2-sulfanylethyl acetate, 3-sulfanylpropanol, 3-sulfanyl-3-methylbutanol, 3-sulfanylhexyl acetate, and 3-sulfanylhexanol were purchased from Sigma-Aldrich (Bornen, Belgium). Standard of methyl geranate was purchased from Alfa Aesar (Karlsruhe, Germany). Milli-Q water was used (Millipore, Bedford, USA).

Beer samples

Twenty-one Belgian DHB were investigated: Orval (A), Bastogne Pale Ale (B), La Trouffette Blonde (C), Vedett Extra Ordinary IPA (D), IV Saison (E), V Cense (F), Leffe Royale Cascade IPA (G), Leffe Royale Mapuche (H), Leffe Royale Mount Hood (I), Houblon Chouffe (J), Taras Boulba (K), St. Feuillien Saison (L) St. Feuillien Grand Cru (M), Guldenberg (N), Houppe (O), Duvel Triple Hop 1 – Amarillo (P), Duvel Triple Hop 2 – Citra (Q), Duvel Triple Hop 3 – Sorachi Ace (R), Duvel Triple Hop 4 – Mosaic (S) Duvel Triple Hop 5 - Equinox (T), and Duvel Triple Hop 6 – HBC291 (U). Beers were received from brewers or bought in the market by the time they were ready to be commercialized. Bottles used to fresh sample were directly analyzed while bottles intended to natural aging were stored at 20ºC in the dark for a period of two years. Each beer was analyzed in duplicate while fresh and after two years of storage.

Terpenoid and ester extraction by Solvent Assisted Flavor Evaporation

Most odorant compounds were extracted with the Solvent Assisted Flavor Evaporation (SAFE) system described by Engel *et al.* [43]. The SAFE analysis conditions were as follows: the water bath temperature was set at 40ºC, the pressure was kept below 1 x 10^{-4} mbar, and the temperature of the apparatus body (Glasblaeserei Bahr, Manching, Germany) was 30ºC. Beer samples (50 mL) degassed by sonication were mixed with 1.0 mL of a solution containing 2-acetylthiophene at 20 mg/L (IST) and introduced dropwise into the system. After 40 minutes of distillation, the distillate was recovered in a liquid-nitrogen-cooled flask and then extracted three times with 16 mL dichloromethane. The combined organic phases were washed three times with 16 mL milli-Q water, dried over anhydrous sodium sulfate, concentrated to 0.5 mL in a Kuderna-Danish apparatus at 45ºC, and stored at -80ºC prior to GC-MS analysis.

pHMB Extraction of free polyfunctional thiols

Polyfunctional thiols were extracted according to the procedure described by Gros *et al.* [13]: liquid/liquid extraction of 660 mL beer with 300 mL dichloromethane for 30 minutes, extraction of the organic phase with pHMB solution (360 mg pHMB and 24.6 g Tris in 1 L Milli-Q water), loading of the bound thiols onto a preconditioned strong anion exchanger resin (Dowex resin 1x2, chloride form), rinsing of impurities with 50 mL acetate buffer (pH 6, 0.1 M), release of free thiols with 60 mL washed L-cysteine solution at pH 7.0 (4 x 50 mL dichloromethane for washing 640 mg L-cysteine hydrochloride in 60 mL water), final extraction with 25 mL bidistilled dichloromethane, and concentration to 0.5 mL in a Danish-Kuderna apparatus and to 70 µL on a Dufton column at 45ºC. 4-Methoxy-2-methylbutane-2-thiol was added as internal standard (IST, at 1.4 µg/L in beer) and 2-acetylthiophene as external standard (EST, 1 mL at 200 µg/L added to 25 mL bidistilled dichloromethane extract before concentration).

n-Hexanol extraction of short chain fatty acids

Isovaleric, hexanoic, octanoic, and decanoic acids were extracted from beers according to Silva Ferreira *et al*. [44]. Ten milliliters of degassed beer was mixed with 100 µL internal standard (IST – 1000 mg/L nonanoic acid) in a 20-mL glass flask and shaken for 10 seconds. Then 300 µL n-hexanol was added and the flask shaken for 5 minutes. After centrifugation at 14000 rpm for 10 min, the n-hexanol layer was recovered and transferred to a GC vial and stored at 4ºC prior to analysis by GC-FID.

Gas chromatography - electronic impact mass spectrometry (GC-MS) for terpenoid and ester quantitation

Two microliters of each SAFE extract was analyzed with an Agilent 6890N gas chromatograph equipped with a splitless injector maintained at 250ºC. The split vent was opened after 0.5 min.

Compounds were separated with a WCOT capillary column (CP-Sil 5 CB, 50 m × 0.32 mm i.d., 1.2 µm film thickness). The carrier gas was helium and the pressure was set at 63 kPa. 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, finally to 250°C at 3°C/min, and then held at this temperature for 30 min. The column was connected to a single quadrupole mass spectrometer (Agilent 5973N MSD) operating in single ion monitoring (SIM) mode with electron ionization at 70 eV. The following *m/z* values were monitored: 43 (for isoamyl acetate), 69 (for geraniol and geranyl acetate), 71 (for dodecane, ethyl isobutyrate and isobutyl isobutyrate), 88 (for ethyl hexanoate, ethyl heptanoate, ethyl octanoate, ethyl decanoate, and ethyl isovalerate), 93 (for myrcene, linalool, and α-terpineol), 104 (for 2-phenylethyl acetate), 111 (for 2-acetylthiophene - IST), 114 (for methyl geranate). Chromatograms were recorded throughout elution. Agilent Chemstation software (version D.31.00.61) was used to process the resulting data. Calibration curves (with areas relative to IST) were constructed for each compound, and the following equation was used for quantitation of compound A: concentration of A (in µg/L) = IST concentration (in µg/L) × (A area/IST area) × (IST response coefficient/A response coefficient) × (IST recovery factor/A recovery factor). The IST-relative recovery factor was set at 1 for all compounds (experimental values from 0.7 to 1.1, determined beforehand by standard addition).

Gas chromatography - pulsed-flame photometric detector (GC-PFPD) for polyfunctional thiols quantitation

One microliter of each pHMB free thiol extract was analyzed with an Agilent 6890N gas chromatograph. The specifications relative to the splitless injection, the column and the oven temperature were identical to those described above for GC-MS analyses. Yet, the helium pressure was set here at 90 kPa. The column was connected to the OI Analytical PFPD detector (model 5380, combustor internal diameter = 2 mm) for which the following parameters were

selected: temperature, 220°C; voltage, 590 V; gate width, 18 ms; gate delay, 6 ms; trigger level, 400 mV; pulse frequency, 3.45 Hz. PFPD chromatograms were recorded throughout elution. Agilent Mass Hunter software (version 10.0) was used to process the resulting data. Identifications were done as previously described by Gros *et al.* [13]. The following general equation was used for compound B quantitation: concentration of B (in µg/L) = IST concentration (in µg/L) × (B molecular weight/IST molecular weight) × (B area/IST area) × (IST molar response coefficient/B molar response coefficient) × (IST recovery factor/B recovery factor). The IST-relative recovery factor was set at 1 for all compounds (experimental values from 0.8 to 1.2, previously determined by standard addition) except for 2-sulfanylethanol (bad recovery at the first dichloromethane extraction; approximate concentrations assessed by using a relative recovery factor to the IST of 0.1, previously assessed by standard addition). For commercially available thiols, calibration curves relative to the IST were used. For 3-sulfanylpropyl acetate and 3-sulfanyl-4-methylpentanol, the equimolarity of the PFPD detector enabled us to set the IST-relative molar response coefficients at 1.

Gas chromatography—flame ionization detector (GC-FID) for short chain fatty acid quantitation

One microliter of each n-hexanol extract was analyzed with an Agilent 6890N gas chromatograph equipped with a split injector (split ratio 73.6) maintained at 240ºC. Fatty acids were separated with a wall-coated open tubular (WCOT) 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 60 kPa. The oven temperature was programmed to start at 125ºC for 10 min, then to rise to 160 at 1.6ºC/min, finally to 250ºC at 15ºC/min, and held for 15 min. The column was connected to an FID detector (set at 250ºC with inlet of 21 mL/min H_2 and 210 mL/min air). Quantitation

was done by standard addition considering the relative response of each compound to IST. Results are expressed as the average of duplicates.

Assessment of terpenol and ester degradation in model media

A model medium containing 2 g/L Mandarina Bavaria was prepared (milled hop pellets in 5% ethanol v/v, pH 4.0). This medium was either used as such (and called NH medium) or pre-heated at 80°C for 10 min (and called H medium). Duplicate samples of both media were spiked (20 mg/L in the model medium) with a mixture of six selected flavors: ethyl hexanoate, ethyl octanoate, isoamyl acetate, 2-phenylethyl acetate, linalool, and α-terpineol. Duplicate samples of a blank (hop-free) medium (5% v/v alcohol, pH 4.0 – called F) were spiked in the same way. The samples were stored for one or ten days at 20ºC in the dark. They were spiked after storage with 20 mg/L internal standard (2-acetylthiophene) and aroma compounds were recovered from 10 mL model medium by two successive extractions with 5 mL dichloromethane. The resulting organic phases were combined, dried over anhydrous sodium sulfate, and analyzed by GC-MS.

Statistical analyses

All analytical measurements were carried out in duplicate. Multiple comparisons of means were performed with the Student-Newman-Keuls test. Values not sharing any common letter in the same row of a table (or bar in a graph) are significantly different ($p < 0.05$).

RESULTS AND DISCUSSION

Twenty-one Belgian DHBs (A-U) were first analyzed for various flavor compounds, either hop-derived (mostly terpenoids, esters, and polyfunctional thiols) or yeast-derived (mostly short chain fatty acids and esters). A representative set of 12 beers (A, D, G, H, J, M, P-U) were further analyzed after two years of storage at 20ºC in the dark (two years is the usual best-before

date of Belgian special beers). As the pHMB specific thiol extraction entails a heavy workload, only six beers (P-U, single beers dry-hopped with either Amarillo, Citra, Sorachi Ace, Mosaic, Equinox, or HBC291) were selected to assess the fate of polyfunctional thiols. Lastly, in order to better understand the fate of esters and terpenols through DHB aging, the impact of hop particles and enzymes was determined in model media.

Hop-derived terpenols (and terpenes) in fresh and aged samples

Among the analyzed terpenols, linalool, α-terpineol, and geraniol were detected in most beers (Table 1a). Besides direct solubilization from hops, biotransformation from other terpenoids (linalool synthesis from geraniol or geranyl acetate, α-terpineol synthesis from nerol or linalool [45,46]) and release from glycosidic precursors (previously evidenced by Kollmannsberger *et al.* [19] and Kankolongo Cibaka *et al.*[5]) might explain the high levels measured.

Linalool emerged as the most abundant terpenoid, with concentrations ranging from 60 (beer M) to 499 (beer T) µg/L, far above its sensory threshold of 8 µg/L [47]. The literature suggests that, as in hop, the R form of this chiral compound should be predominant in DHBs (distribution ratio close to 80:20 [48]).

The less odorant α-terpineol (sensory threshold: 330 µg/L [49]), whose concentration varied from 14 (beer G) to 113 µg/L (beer T), was the second most abundant terpenoid. Interestingly, the linalool and α-terpineol concentrations showed partial correlation ($R^2 = 0.74$, Figure 1A) regardless of parameters such as hop variety, dosage or dry hopping process.

Geraniol was detected at much lower concentrations than linalool, but still above its sensory threshold of 4 µg/L [6] in all beers except A, B, L, M and N (Table 1a). The highest level occurred in beer T, with 53 µg/L. It was recently shown that its concentration in the Cascade cultivar allowed to predict aroma quality and intensity of the correspondent DHBs [50]. Besides the low amount of geraniol supplied by some hop cultivars [3,51,52], biotransformations

[9,15,46,53] can still further reduce its concentration. Yet even at such low levels, geraniol is reported to enhance the perception of linalool (citrus character) in beer [9,52,54,55].

Myrcene was the only terpene identified here (Table 1a). Despite its abundance in hop oil, its hydrophobicity (poor extraction rate, 0.05-2.2% [56,57]) and possible uptake by yeast [58] led to very low levels. In fact, it was detected above its sensory threshold of 9 µg/L only in three beer (E, O, and T; 9-17 µg/L).

In the 12 DHBs sampled after two years of storage at 20°C, the average concentration of linalool was only 57% and that of geraniol, only 45%, compared to fresh samples (Figure 1B). Among other mechanisms, we can suspect electrophilic additions to occur slowly on double bonds, yielding inodorous less volatile analogs. Considerable variations were measured between beers (Figure 1C), indicating how strong an impact process parameters like pH (3.8 to 4.5), alcohol content (4.9 to 9.6 %v/v) or apparent extract (-0.18 to 3.94 ºP) can have. Yet linalool remained above its sensory threshold in all samples (lowest level: 30 µg/L in beer M), at least if racemization to (S)-linalool [59] (80 times less odorant [48]) did not occur too much during storage. α-Terpineol, on the other hand, exhibited much higher stability, even showing an average increase of 19% compared to fresh samples (Figures 1B and 1C, degradation observed only in beers Q and T). These results are in accordance with those of Gahr *et al*. [60], who linked the apparent stability of α-terpineol to linalool degradation. Hydrolysis of glycosidic forms, as reported by Kankolongo Cibaka *et al*. [5], could be an additional mechanism of its synthesis. Myrcene was no longer detected after aging, except in beers T (12 µg/L) and, surprisingly, J (7 µg/L) (Table 1a). This suggests that traces initially adsorbed by bottled yeast [58] could be slowly released through storage (yet without reaching its sensory threshold).

Hop-derived esters in fresh and aged samples

Geranyl acetate and methyl geranate were detected in most samples, at concentrations up to 5 (beer T) and 64 µg/L (beer D), respectively (Table 1b). Similar low concentrations of geranyl acetate have been reported for American DHBs [49]. Methyl geranate (here found above 15 µg/L in only seven beers) has been reported as a discrimination marker of hop varieties, issued from geranic acid [61,62].

Hop-derived ethyl isobutyrate, ethyl isovalerate, isobutyl isobutyrate, and ethyl heptanoate were also detected in most beers, at concentrations up to 84 (beer A), 63 (beer A), 114 (beer T), and 19 µg/L (beer T), respectively (Table 1b). They most probably impact DHB flavor because of their much lower thresholds, compared to fermentation esters (respectively 6.3 and 2 µg/L in beer for ethyl isobutyrate and ethyl isovalerate [6], 30 and 2 µg/L in water for isobutyl isobutyrate and ethyl heptanoate [63,64]). Although mainly issued directly from late and dry hopping [65], ethyl isobutyrate, ethyl isovalerate, and ethyl heptanoate could also be produced, in the presence of ethanol, from isobutyric, isovaleric, and heptanoic acids (or from hop methyl heptanoate by transesterification) [14,53,66]. As expected, beer A (matured and refermented in the presence of *Brettanomyces*) showed the highest concentrations of both ethyl isobutyrate and isovalerate (Table 1b).

After two years of aging, terpenoid esters appeared severely degraded: only 48% of the geranyl acetate and 31% of the methyl geranate, on the average, were still found (Figures 2A and 2B). Likewise, less than 40% of the ethyl heptanoate subsisted (except in beers A, P, S, and U, Figure 2A). On the other hand, ethyl isovalerate (except in beer T) remained more stable (residual concentrations higher than 85%; it even appeared slightly produced, probably by bottled yeast, in beers A, P, R, S, and U, Figure 2A). This contrasts surprisingly with the fate of ethyl isobutyrate, found after two years to have completely disappeared (Table 1b).

Hop-derived isovaleric acid in fresh and aged samples

Isovaleric (or 3-methylbutanoic) acid, known for its unpleasant rancid/cheese odor when above 1-1.5 mg/L [44,67], was detected in all samples (from 1.0 (beer M) to 2.6 mg/L (beer N), Table 1c). Yet by comparison with late-hopped beers (0.6-4.8 mg/L [44]), dry hopping does not significantly impact the isovaleric acid level of fresh beer. Besides its production through oxidative cleavage of the acyl side chain of hop alpha and beta acids, observed during hop storage [2,66,68], isovaleric acid can also be produced by yeast from leucine [69,70].

As previously depicted for late-hopped beers, the level of isovaleric acid remained stable in DHBs through aging (Table 1c). There was only one exception to this rule, beer A, where *Brettanomyces* strains most probably use this acid as a substrate (only 0.2 mg/L recovered after two years-from the 2.2 mg/L initial concentration).

Hop-derived polyfunctional thiols in fresh and aged samples

3-Sulfanylhexanol (3SHol, threshold: 0.055 µg/L [6]) was found at similar concentrations (0.1-0.2 µg/L, Table 2) in all beers. This was to be expected, since this compound is ubiquitous in most hop varieties [3,11,13] and even in malt [12]. Its derived ester 3-sulfanylhexyl acetate (3SHA, threshold: 0.005 µg/L [12]) was found only in two fresh samples (P and Q). The grapefruit/rhubarb-like 3-sulfanyl-4-methylpentanol (3S4MPol, threshold: 0.07 µg/L [71]) and the onion-like 3-sulfanyl-3-methylbutanol (3S3MBol, threshold: 1.5 µg/L [41]) are known to be more hop-variety-dependent. Among the six DHBs investigated here, beers S (Mosaic), T (Equinox), P (Amarillo) and Q (Citra) contained 3S4MPol at 3.9-1.1 µg/L, far above its threshold, while 3S3MBol reached its threshold in beer Q only (Table 2). Dry hopping with Sorachi Ace (beer R) or HBC291 (beer U) proved not to bring significant amounts of odorant polyfunctional thiols (Table 2). These results are in accordance with those of Kankolongo

Cibaka *et al*. [3] and Takazumi *et al.* [11], who report 3S4MPol at 24-49 µg/kg in the Citra and Mosaic varieties but very low levels of free thiols in Sorachi Ace hop.

After two years of storage and despite severe degradation (only 17% still detected, on the average), 3S4MPol concentration remained above its threshold in beers P, Q, S, and T (Table 2 and Figure 3). 3SHol seemed slightly less degraded than other thiols (29% of the initial amount was still present, on the average), as previously evidenced in Sauternes wines [72]. Residual concentrations still reached its sensory threshold in beers Q, S, and T. 3SHA was not detected in any of the aged samples.

Fermentation-derived short chain fatty acids in fresh and aged samples

Although hop might also bring small amounts of short chain fatty acids [66], yeast remains the main producer of hexanoic, octanoic, and decanoic acids in DHBs (biosynthesis from acetyl-CoA [73]). Unsurprisingly, good correlations were observed between levels of hexanoic and octanoic acid (R^2=0.79) and between levels of octanoic and decanoic acid (R^2=0.63, Figure 4A). As depicted in Table 3a, these concentrations are in exactly the same range as recently published for Belgian non-dry-hopped beers [44,67,74]. The sensory thresholds of these fatty acids (8, 13, and 10 mg/L, respectively [75]) were never reached, even in beers L, M, and N, which exhibited the highest concentrations (up to 4.7 mg/L hexanoic, 7.9 mg/L octanoic, and 2.9 mg/L decanoic acid in beer N).

After two years of storage at 20ºC in the dark, hexanoic acid and octanoic acid showed good stability (Table 3a). Only the most hydrophobic, decanoic acid, was found to have decreased to levels below 0.1 mg/L (except in the non-refermented beers G and H, where 0.3 mg/L subsisted after two years). In any case, no significant release of fatty acids appears to have occurred through autolysis of bottled yeast during storage of the DHBs.

Fermentation-derived esters in fresh and aged samples

The range of ester concentrations evidenced here was similar to that observed for Belgian non-dry-hopped beers [44,74] (often produced with the same yeast in Belgian family breweries). Among the ethyl esters, only ethyl hexanoate was detected in most beers above its sensory threshold of 200 µg/L [67] (Table 3b). Up to 403-426 µg/L was found in beers L-N, which also emerged as the most concentrated in hexanoic acid. Ethyl octanoate and ethyl decanoate concentrations ranged, respectively, from 69 (beer H) to 557 (beer L) µg/L and from 7 (H) to 136 µg/L (N), far below their sensory thresholds of 900 and 1500 µg/L [67]. Levels of these esters were correlated in the 21 DHBs (R^2 = 0.61 between ethyl hexanoate and ethyl octanoate, and R^2 = 0.79 between ethyl octanoate and ethyl decanoate, Figure 4B).

How efficiently yeasts produce acetate esters is known to be strain-dependent [76]. Therefore, a correlation was also evidenced between isoamyl acetate and 2-phenylethyl acetate levels (R^2 = 0.79, Figure 4C). Only isoamyl acetate, however, was detected above its threshold of 510 µg/L [77], except in beers A, B, E, and F.

The specific pHMB extraction method enabled us to investigate two additional acetates in beers P-U (all fermented with the same yeast). 2-Sulfanylethyl acetate (2SEA) and 3-sulfanylpropyl acetate (3SPrA) are issued from Ehrlich degradation of cysteine and homocysteine (Table 2), but might also be found as traces in some hop varieties [3,4]. Compared to other published data [13,41,78], relatively high concentrations of 2SEA (4-7 µg/L) and 3SPrA (1-2 µg/L) characterized all six beers, already richer in isoamyl acetate and 2-phenylethyl acetate. Yet the sensory threshold (40 µg/L [78]) was not reached in any of these beers.

On the average, ethyl hexanoate, ethyl octanoate, isoamyl acetate, and 2-phenylethyl acetate were still detected at 55, 58, 66, and 80%, respectively, of their initial concentrations after two years of storage at 20ºC (Figure 5A). In each beer, similar losses (shown as percentages, Figure

352 5B) were observed for ethyl hexanoate and ethyl octanoate (slope = 1, R^2 = 0.88), while
353 degradation was systematically slightly higher for isoamyl acetate than for 2-phenylethyl
354 acetate (slope = 0.9, R^2=0.68, Figure 5B). This overall degradation of esters was higher in the
355 here-investigated DHBs than in Belgian non-dry-hopped beers, where losses were always under
356 30% after one year at 20ºC [44,79]. One possible explanation for this additional loss of esters
357 in DHBs could be the hop esterase activity, recently evidenced by Werrie [26].

358 Among the fermentation-derived polyfunctional thiols, 2SEA and 3SPrA were also severely
359 degraded during storage. As compared to initial concentrations, only 4% of the former and 9%
360 of the latter were detected, on the average, in the aged samples (Table 2 and Figure 3). In
361 contrast, 3SProl proved relatively stable, even showing an increase in samples P, R, S, and T,
362 probably due to hydrolysis of 3SPrA.

363 **Fate of terpenols and esters in DHB-model media**

364 To determine if hop enzymes could be responsible of the instability of some DHB flavors, a
365 DHB model medium (Mandarina Bavaria hop at 2 g/L in 5% alcohol v/v, pH 4) was spiked
366 with terpenols (linalool and α-terpineol) and esters (ethyl hexanoate, ethyl octanoate, isoamyl
367 acetate and 2-phenylethyl acetate), and stored for one or ten days at 20ºC. This one (called NH)
368 was compared to a second DHB model medium for which hop enzymes were inactivated (called
369 H, heated for 10 min at 80ºC prior aroma spiking) and to a hop-free blank (called F).

370 Slight adsorption of esters to the plant material occurred already during the first day of storage,
371 causing similar losses in H and NH (Figure 6A, the highest loss for the most lipophilic
372 compound, ethyl octanoate). The presence of unheated hop was found, over the 10-day aging
373 period, to increase ester degradation significantly (as compared to heated hop), without
374 impacting the fate of terpenols (Figure 6A). Also worth stressing is the emergence of four new
375 chromatographic peaks at the respective retention indices of hexanoic acid, octanoic acid,

isoamyl alcohol, and 2-phenylethanol (Figure 6B, structures checked by electron impact mass spectrometry), confirming the activity of hop esterases in NH medium (impacts slightly stronger on acetates than on ethyl esters). The data thus show that both hop enzymatic activity and adsorption to hop particles should be taken into account in modeling the fate of DHB esters through storage.

CONCLUSION

The results of various complementary aroma analyses highlight both hop-derived compounds (linalool, geraniol, ethyl isovalerate, ethyl isobutyrate, 3SHol, 3SHA and 3S4MPol) and fermentation esters (isoamyl acetate and ethyl hexanoate) as key flavors of Belgian DHBs. Yet except ethyl isovalerate, most of these are unstable after two years at 20ºC (only 45-70% of linalool, geraniol and ethyl hexanoate, and even less than 40% for polyfunctional thiols, ethyl isobutyrate, and ethyl heptanoate were detected after storage). As for other special beers, limiting oxidation of polyfunctional thiols by applying bottle refermentation could be recommended, especially with selected yeast strains able to release free thiols from cysteine and glutathione precursors [22]. No harmful effects linked to yeast autolysis were observed in the 19 bottle-refermented beers investigated here. While terpenols are most probably subject to water electrophilic addition through aging, enzymatic hydrolysis emerges as the biggest threat for esters, explaining why they appear less stable in DHBs. Complementary researches are now needed to determine how to inactivate hop enzymes without losing aromas.

ABBREVIATIONS

SAFE: Solvent assisted flavor evaporation, pHMB: 4-(hydroxymercuri)benzoic acid sodium salt, DHB: dry-hopped beer, EST: external standard, IST: internal standard.

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641

642

FIGURE CAPTIONS

643 **Figure 1**. Correlation between linalool and α-terpineol concentrations in fresh Belgian DHBs (A),
644 change in concentration (% of the initial amount) of terpenols after two years at 20°C, bars with different
645 letters are significantly different ($p<0.05$) according to the Student-Newman-Keuls test, error bars
646 represent the standard deviation among the samples (B), and concentrations, in µg/L, of terpenols in
647 fresh (black bars) and two years aged (gray bars) samples (C),

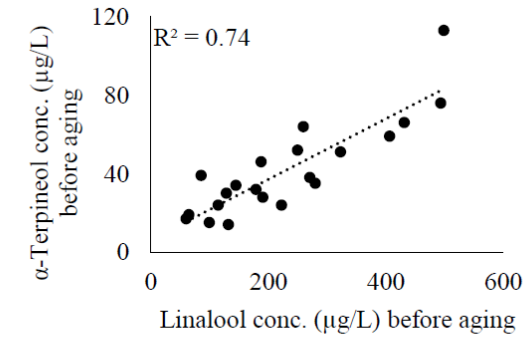
648 **Figure 2.** Concentrations, in µg/L, of hop-derived esters in fresh (black bars) and two years aged (gray
649 bars) Belgian DHBs (A), change in concentration (in % of the initial amount) of hop-derived esters after
650 two years at 20°C, bars with different letters are significantly different ($p<0.05$) according to the
651 Student-Newman-Keuls test, error bars represent the standard deviation among the samples (B).

652 **Figure 3.** PFPD chromatograms of beer T before (left) and after (right) aging.

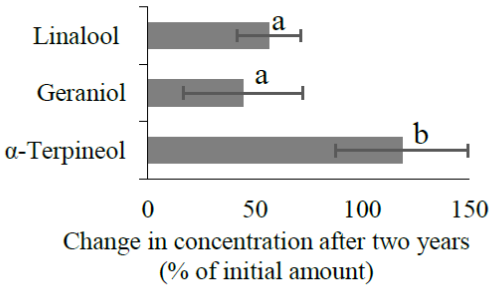
653 **Figure 4**. Correlations between the concentrations in fresh DHBs of: hexanoic and octanoic acids, or
654 octanoic and decanoic acids (A); ethyl hexanoate and ethyl octanoate, or ethyl octanoate and ethyl
655 decanoate (B); isoamyl acetate and 2-phenylethyl acetate (C).

656 **Figure 5**. Change in concentrations (% of the initial amount) of fermentation-derived esters after two
657 years at 20°C, bars with different letters are significantly different ($p<0.05$) according to the Student-
658 Newman-Keuls test, error bars represent the standard deviation among the samples (A), and correlations
659 between the change in concentration (% of the initial amount) after two years of storage at 20ºC of ethyl
660 hexanoate and ethyl octanoate or isoamyl acetate and 2-phenylethyl acetate (B).

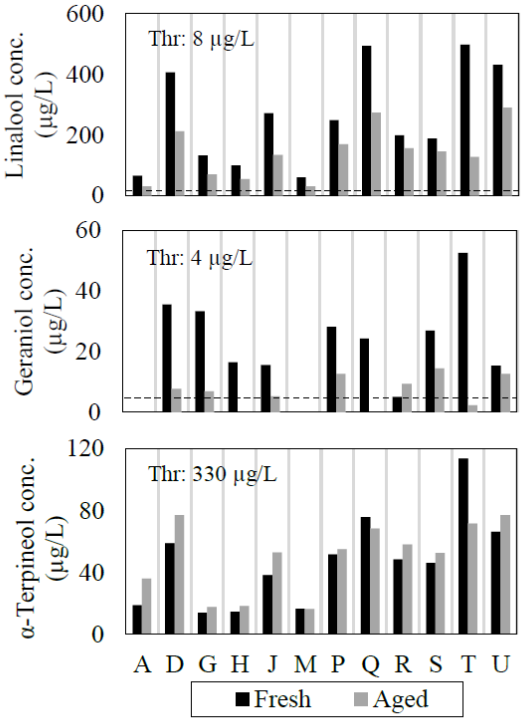
661 **Figure 6.** Concentrations (in IST equivalents) of ethyl hexanoate, ethyl octanoate, 2-phenylethyl acetate,
662 isoamyl acetate, α-terpineol, and linalool in pH 4 hydroalcoholic model media (F: without hop addition,
663 H: 2g/L of heat-treated hops, and NH: 2g/L of not heated hops) after one or ten days of storage at 20ºC
664 in the dark (A), and production of hexanoic acid, octanoic acid, isoamyl alcohol and 2-phenylethanol,
665 after ten days of storage at 20ºC (B). Points and error bars in the graphs represent the average result of
666 two analyzed flasks and the associated standard deviation, respectively.


R² = 0.74
α-Terpineol conc. (µg/L) before aging
Linalool conc. (µg/L) before aging
0
40
80
120
0
200
400
600

(A)


Linalool
Geraniol
α-Terpineol
a
a
b
0
50
100
150
Change in concentration after two years
(% of initial amount)

(B)


Linalool conc. (µg/L)
Thr: 8 µg/L
Geraniol conc. (µg/L)
Thr: 4 µg/L
α-Terpineol conc. (µg/L)
Thr: 330 µg/L
A D G H J M P Q R S T U
Fresh
Aged

(C)

Figure 1

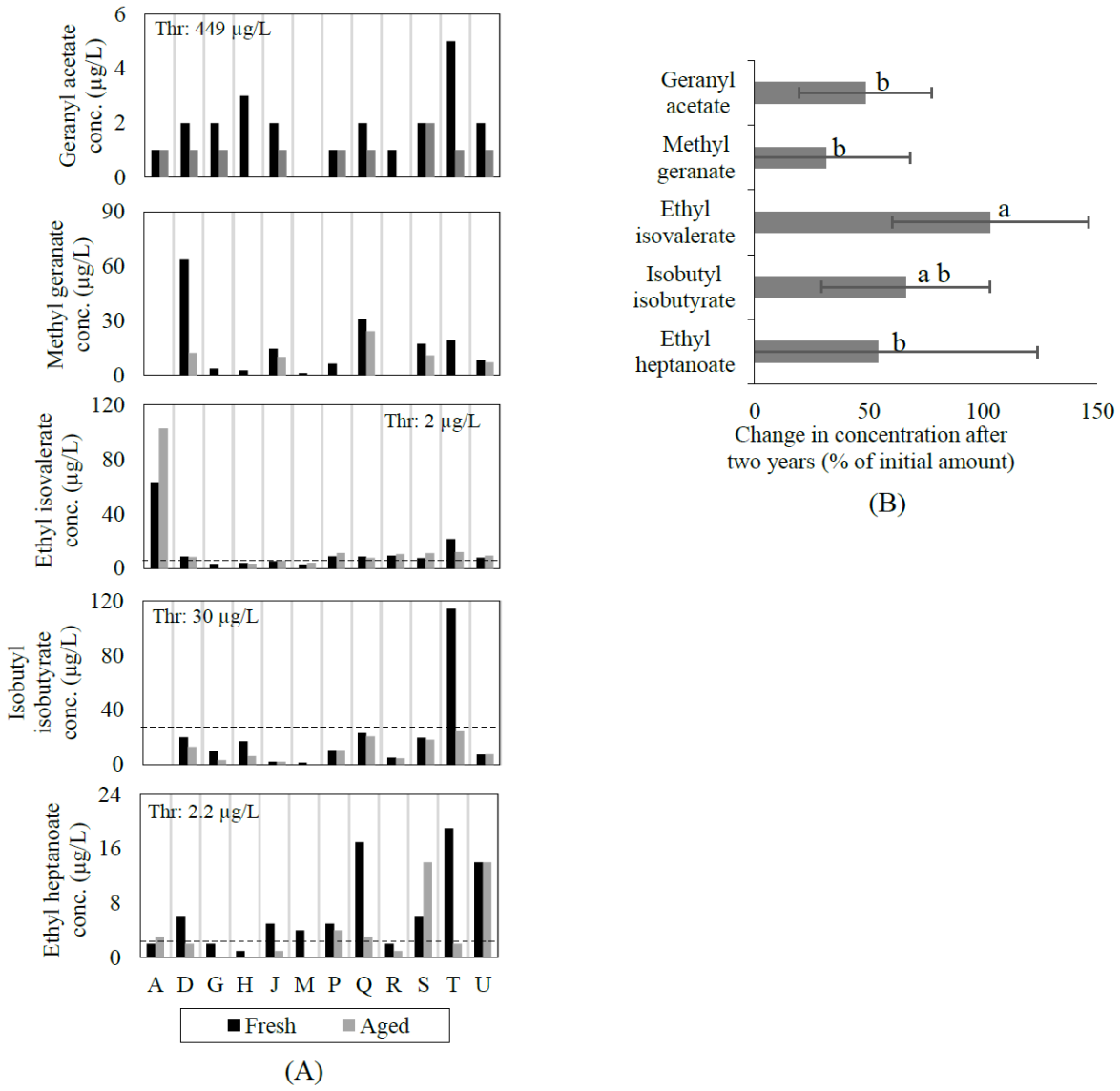

Geranyl acetate conc. (µg/L)
Thr: 449 µg/L
Methyl geranate conc. (µg/L)
Ethyl isovalerate conc. (µg/L)
Thr: 2 µg/L
Isobutyl isobutyrate conc. (µg/L)
Thr: 30 µg/L
Ethyl heptanoate conc. (µg/L)
Thr: 2.2 µg/L
A D G H J M P Q R S T U
Fresh
Aged
(A)
Geranyl acetate
Methyl geranate
Ethyl isovalerate
Isobutyl isobutyrate
Ethyl heptanoate
b
b
a
a b
b
Change in concentration after two years (% of initial amount)
(B)

Figure 2

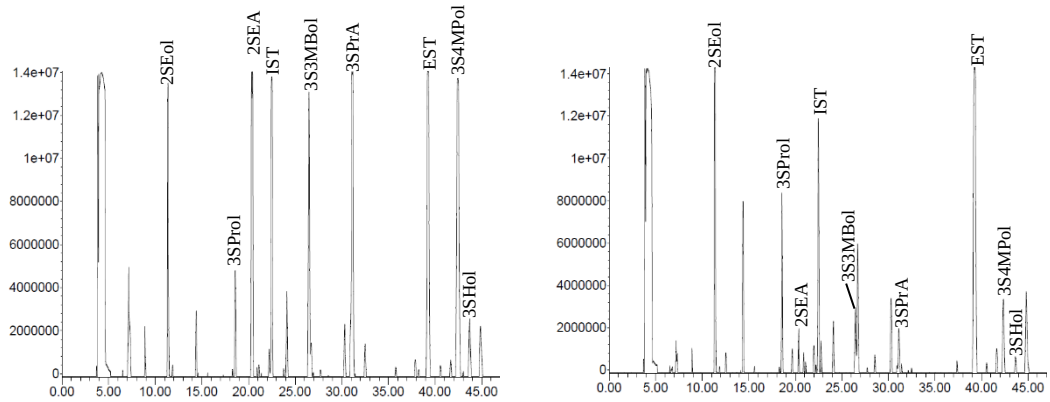

1.4e+07
1.2e+07
1e+07
8000000
6000000
4000000
2000000
0
0.00
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
45.00
2SEol
3SProl
2SEA
IST
3S3MBol
3SPrA
EST
3S4MPol
3SHol

Figure 3

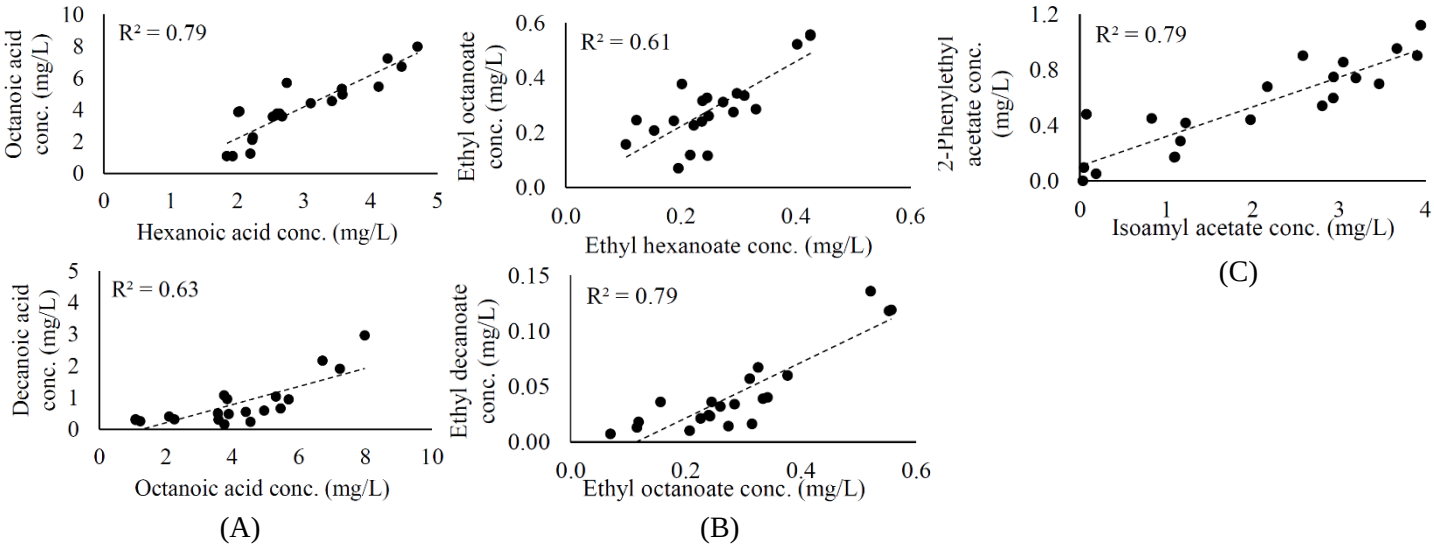

Octanoic acid conc. (mg/L)
R² = 0.79
Hexanoic acid conc. (mg/L)
Ethyl octanoate conc. (mg/L)
R² = 0.61
Ethyl hexanoate conc. (mg/L)
2-Phenylethyl acetate conc. (mg/L)
R² = 0.79
Isoamyl acetate conc. (mg/L)
(C)
Decanoic acid conc. (mg/L)
R² = 0.63
Octanoic acid conc. (mg/L)
Ethyl decanoate conc. (mg/L)
R² = 0.79
Ethyl octanoate conc. (mg/L)
(A)
(B)

Figure 4

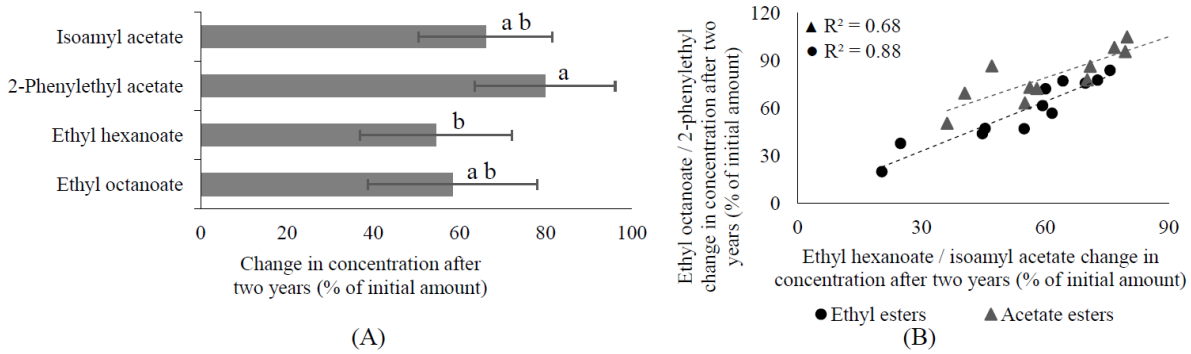
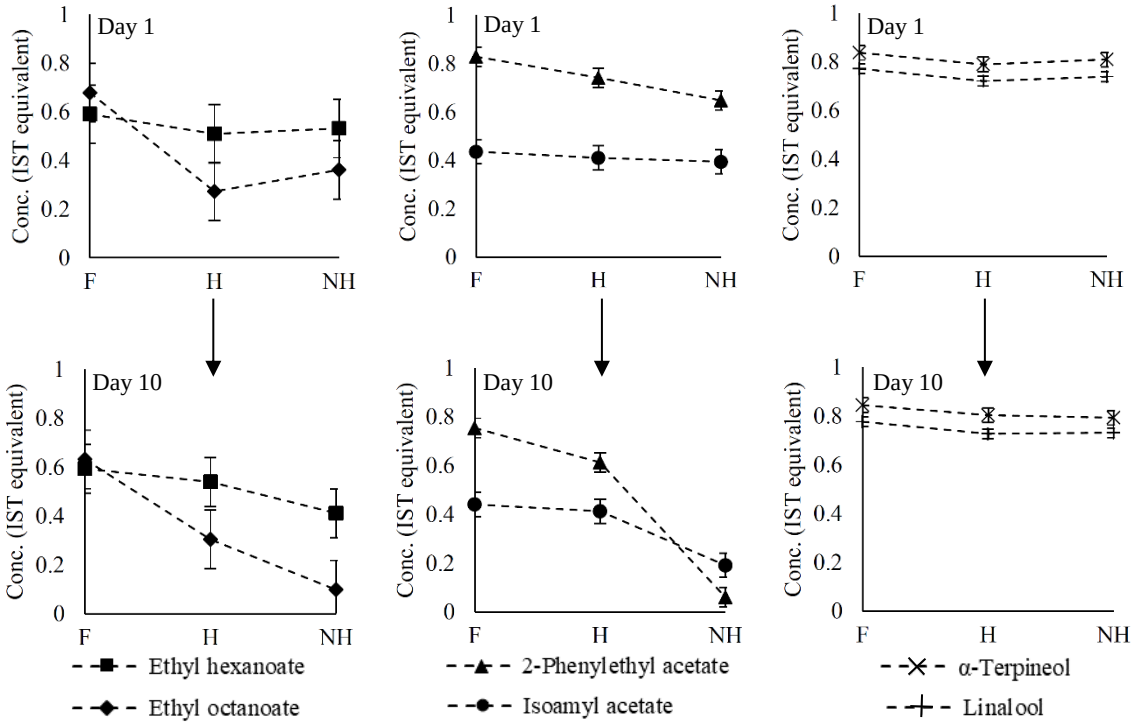
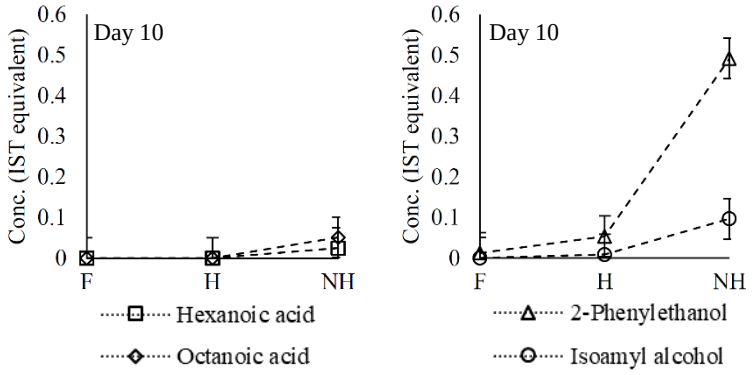

Isoamyl acetate
2-Phenylethyl acetate
Ethyl hexanoate
Ethyl octanoate
a b
a
b
a b
0
20
40
60
80
100
Change in concentration after two years (% of initial amount)
(A)
120
90
60
30
0
0
30
60
90
R² = 0.68
R² = 0.88
Ethyl octanoate / 2-phenylethyl change in concentration after two years (% of initial amount)
Ethyl hexanoate / isoamyl acetate change in concentration after two years (% of initial amount)
Ethyl esters
Acetate esters
(B)

Figure 5


Day 1
Day 10
Conc. (IST equivalent)
F
H
NH
Ethyl hexanoate
Ethyl octanoate
2-Phenylethyl acetate
Isoamyl acetate
α-Terpineol
Linalool

(A)


Day 10
Conc. (IST equivalent)
F
H
NH
Hexanoic acid
Octanoic acid
2-Phenylethanol
Isoamyl alcohol

(B)

Figure 6

Table 1. Concentrations, in µg/L, of terpenols and terpenes (1.a), hop-derived esters (1.b) and isovaleric acid (1.c) in 21 fresh samples; mean of duplicates; coefficient of variation inferior to 10% for all compounds. Residual concentrations (in % of the initial values, between parentheses) in 12 beers after two years at 20ºC in the dark.

1.a – Hop-derived terpenols and terpenes (in µg/L)

Compound (odor)	RI (CPSil-5)	Beer																					Odor threshold (µg/L)
		A	**B**	**C**	**D**	**E**	**F**	**G***	**H***	**I***	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**	
Myrcene (Hop, herbs)	985	nd (nd)	nd	6	3 (nd)	17	2	nd (nd)	6 (nd)	nd	2 (366)	nd	nd	nd (nd)	nd	9	nd (nd)	nd (nd)	nd (nd)	nd (nd)	17 (70)	nd (nd)	9.5 [80]
Linalool (Citrus, coriander)	1089	65 (47)	191	179	407 (52)	145	223	132 (53)	100 (54)	323	271 (50)	115	86	60 (51)	129	280	250 (68)	494 (56)	260 (79)	188 (77)	499 (26)	432 (67)	8 [47]
α-Terpineol (Moist orange)	1183	19 (192)	28	32	59 (131)	34	24	14 (127)	15 (126)	51	38 (139)	24	39	17 (99)	30	35	52 (107)	76 (91)	64 (120)	46 (114)	113 (63)	66 (116)	330 [49]
Geraniol (Flower)	1241	nd (nd)	2	19	36 (22)	20	13	33 (21)	16 (nd)	20	16 (33)	7	nd	nd (nd)	1	19	28 (45)	24 (nd)	5 (185)	27 (54)	53 (4)	15 (82)	4 [6]

1.b – Hop-derived esters (in µg/L)

Compound (odor)	RI (CPSil-5)	Beer																					Odor threshold (µg/L)
		A	**B**	**C**	**D**	**E**	**F**	**G***	**H***	**I***	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**	
Ethyl isobutyrate^{IST} (pineapple, apple)	741	84 (nd)	8	13	63 (nd)	39	26	7 (nd)	7 (nd)	7	15 (nd)	5	3	5 (nd)	19	20	19 (nd)	28 (nd)	7 (nd)	nd (nd)	80 (nd)	4 (nd)	6.3 [6]
Ethyl isovalerate (apple)	831	63 (162)	5	4	9 (98)	5	5	3 (nd)	4 (87)	5	5 (100)	4	2	3 (134)	7	6	9 (128)	9 (90)	9 (111)	8 (146)	21 (55)	8 (117)	2 [6]
Isobutyl isobutyrate (apple, apricot)	901	nd (nd)	3	5	20 (65)	6	1	10 (32)	17 (36)	8	2 (100)	4	1	1 (nd)	8	10	11 (100)	23 (89)	5 (90)	20 (92)	114 (22)	7 (100)	30^{water} [63]
Ethyl heptanoate (Fruity)	1080	2 (100)	4	10	6 (24)	3	4	2 (nd)	1 (nd)	2	5 (12)	5	5	4 (nd)	6	7	5 (94)	17 (17)	2 (39)	6 (230)	19 (12)	14 (100)	2.2^{water} [64]
Methyl geranate (Grass)	1309	nd (nd)	2	18	64 (19)	11	16	4 (nd)	3 (nd)	5	15 (68)	3	3	1 (nd)	2	42	6 (nd)	31 (78)	nd (nd)	17 (64)	19 (nd)	8 (84)	
Geranyl acetate (Flower)	1380	1 (100)	nd	1	2 (29)	1	1	2 (47)	2 (nd)	nd	2 (24)	1	1	nd (nd)	nd	1	1 (71)	2 (47)	1 (40)	2 (83)	5 (18)	2 (69)	449 [49]

1.c – Isovaleric acid (in mg/L)

Compound (odor)	RI (CPSil-5)	Beer																					Odor threshold (mg/L)
		A	**B**	**C**	**D**	**E**	**F**	**G***	**H***	**I***	**J**	**K**	**L**	**M**	**N**	**O**	**P**	**Q**	**R**	**S**	**T**	**U**	
Isovaleric acid (Racid, cheese)	817	2.22 (9)	2.44	1.40	1.40 (174)	1.75	1.70	1.61 (56)	1.24 (38)	1.62	1.52 (90)	2.10	1.14	1.02 (11)	2.61	1.39	1.16 (74)	1.37 (100)	1.55 (92)	1.64 (86)	1.51 (37)	1.64 (68)	1.0-1.5 [44,67]

*. without bottle refermentation
nd. not detected.
IST. quantitation done in IST equivalents.

Table 2. Concentrations, in µg/L (IST equivalents for compounds carrying the IST superscript), of polyfunctional thiols in fresh and two years aged beers (in italic); mean of duplicates, and residual concentrations (in % of the initial values, between parentheses). Fresh beer values within a row with different letters are significantly different ($p<0.05$) according to the Student-Newman-Keuls test.

Compound	**Acronym**	**Retention index (CPSil-5)**	**Odor** [3]	**Beer**						**Sensory threshold (µg/L)**
				P	**Q**	**R**	**S**	**T**	**U**	
Hop-derived thiols										
3-Sulfanyl-3-methylbutan-1-ol	3S3MBol	938	sulfur, soup	0.65^{bc} *d*	1.84^{a} *d*	0.33^{c} *d*	0.75^{bc} *0.22* (33)	1.21^{b} *0.21* (17)	0.58^{bc} *0.11* (18)	1.5 [41]
3-Sulfanyl-4-methylpentan-1-ol^{IST}	3S4MPol	1084	grapefruit	$1,71^{b}$ *0.20* (12)	$1,12^{b}$ *0.22* (20)	0.03^{c} *nd* -	3.19^{a} *0.83* (26)	2.97^{a} *0.31* (10)	nd^{c} *nd* -	0.07 [71]
3-Sulfanylhexan-1-ol	3SHol	1094	grapefruit/rhubarb	0.15^{abc} *0.04* (26)	0.25^{a} *0.07* (28)	0.11^{bc} *0.03* (27)	0.16^{ab} *0.06* (35)	0.21^{ab} *0.06* (28)	0.07^{c} *nd* -	0.055 [6]
3-Sulfanylhexyl acetate	3SHA	1215	passion fruit, grapefruit	0.19^{b} *nd* -	2.10^{a} *nd* -	nd^{c} *nd* -	nd^{c} *nd* -	nd^{c} *nd* -	nd^{c} *nd* -	0.005 [12]
Fermentation-derived thiols										
2-Sulfanylethan-1-ol	2SEol	722	grilled	26.31^{a} *13.79* (52)	40.97^{a} *11.56* (28)	25.97^{a} *14.53* (56)	33.63^{a} *17.77* (53)	37.25^{a} *12.14* (33)	38.72^{a} *10.46* (27)	2000 [78]
3-Sulfanylpropan-1-ol	3SProl	849	pop corn	0.23^{a} *0.26* (113)	0.65^{a} *0.62* (95)	0.23^{a} *0.32* (139)	0.23^{a} *0.44* (192)	0.38^{a} *0.48* (126)	0.41^{a} *0.30* (74)	40 [78]
2-Sulfanylethyl acetate	2SEA	880	toasted, grilled	4.26^{a} *0.09* (2)	5.87^{a} *0.42* (7)	4.44^{a} *0.15* (3)	6.64^{a} *0.32* (5)	6.36^{a} *0.17* (3)	7.35^{a} *0.30* (4)	400 [78]
3-Sulfanylpropyl acetate^{IST}	3SPrA	1000	grilled	1.20^{b} *0.09* (8)	2.31^{a} *0.27* (12)	1.87^{a} *0.13* (7)	1.78^{a} *0.20* (11)	2.07^{a} *0.15* (7)	2.08^{a} *0.14* (7)	40 [78]

nd. not detected.

d. detected but not quantifiable

Table 3. Concentrations of fermentation-derived short chain fatty acids (3.a, in mg/L) and esters (3.b, in µg/L) in 21 fresh samples; mean of duplicates; coefficient of variation inferior to 10% for all compounds. Residual concentrations (in %, between parentheses) in 12 beers after two years at 20ºC in the dark.

3.a – Fermentation-derived short-chain fatty acids (in mg/L)

Compound (odor)	RI (CPSil-5)	Beer																				Odor threshold (mg/L)
		A	B	C	D	E	F	G*	H*	I*	J	K	L	M	N	O	P	Q	R	S	T	U	
Hexanoic acid (Sweat)	963	2.53 (122)	2.64	3.42	3.58 (163)	2.04	2.75	3.10 (131)	2.68 (107)	4.12	3.57 (95)	2.02	4.26	4.47 (98)	4.71	2.60	2.23 (93)	1.85 (80)	2.20 (91)	1.93 (91)	2.24 (59)	1.93 (82)	8 [67]
Octanoic acid (Sweat)	1164	3.57 (59)	3.78	4.56	4.97 (91)	3.90	5.70	4.42 (133)	3.60 (128)	5.46	5.32 (93)	3.86	7.25	6.72 (70)	7.99	3.77	2.11 (76)	1.10 (195)	1.24 (151)	1.10 (128)	2.27 (31)	1.10 (131)	13 [67]
Decanoic acid (Sweat)	1370	0.49 (nd)	0.15	0.23	0.59 (nd)	0.47	0.94	0.54 (59)	0.30 (100)	0.65	1.03 (nd)	0.95	1.91	2.17 (nd)	2.96	1.07	0.39 (nd)	0.29 (nd)	0.26 (nd)	0.31 (nd)	0.32 (nd)	0.31 (nd)	10 [67]

3.b – Fermentation-derived esters (in µg/L)

Compound (odor)	RI (CPSil-5)	Beer																				Odor threshold (µg/L)
		A	B	C	D	E	F	G*	H*	I*	J	K	L	M	N	O	P	Q	R	S	T	U	
Isoamyl acetate (Banana)	856	34 (122)	74	832	1164 (55)	187	43	3047 (47)	2581 (40)	2934	2170 (58)	1221	1098	1092 (56)	2803	1974	3670 (70)	3463 (71)	3903 (80)	3193 (80)	3944 (36)	2933 (77)	510 [77]
Ethyl hexanoate (Pineapple)	980	274 (64)	154	238	188 (60)	123	202	217 (25)	196 (20)	247	331 (46)	105	426	426 (45)	403	246	298 (62)	237 (52)	292 (70)	223 (76)	311 (55)	249 (73)	210 [67]
Ethyl octanoate (Pineapple)	1181	311 (77)	207	315	242 (72)	245	377	118 (38)	69 (20)	115	285 (47)	156	557	553 (44)	521	326	342 (57)	240 (62)	274 (76)	226 (84)	334 (47)	260 (78)	900 [67]
2-Phenylethyl acetate (Rose)	1236	nd (**)	480	450	286 (63)	51	95	855 (87)	902 (69)	748	679 (72)	418	172	169 (73)	540	440	954 (80)	698 (86)	901 (105)	740 (96)	1121 (50)	597 (98)	3800 [67]
Ethyl decanoate (Apple, pineapple)	1384	57 (32)	10	16	23 (13)	36	60	18 (33)	7 (0)	13	34 (25)	36	119	118 (16)	136	67	40 (0)	24 (0)	14 (70)	21 (31)	39 (7)	32 (27)	1500 [67]

*. without bottle refermentation

nd. not detected.

**. although not detected in the fresh sample, 3.8 µg/L were detected after two years at 20ºC in the dark