Journal Code J I B | Article ID 34 | Dispatch: 00.00.00 | No. of Pages: 7 | CE: | ME:

Research Article

Institute of Brewing & Distilling

Received: 8 June 2012, Revised: 24 July 2012, Accepted: 25 July 2012 Published online in Wiley Online Library

(wileyonlinelibrary.com) DOI 10.1002/jib.34

Occurrence of sotolon, abhexon and theaspirane-derived molecules in Gueuze beers. Chemical similarities with 'yellow wines'

Q1

Caroline Scholtes, Sabrina Nizet and Sonia Collin*

The typical Belgian Gueuze beers are produced with aged hop from a grist of malt and wheat, according to a very long oxidation process (extended boiling, cooling overnight in an open-air container, oak-barrel ageing). Two theaspirane-oxidation-derived products, dihydrodehydro-β-ionone and 4-hydroxy-7,8-dihydro-β-ionone, are evidenced here for the first time in Gueuze beers. Both compounds have been recently identified in oxidative wines such as Jura Flor-Sherry and Sauternes wines. Another analogy with Jura Flor-Sherry wines was the presence of the nutty/curry odorants sotolon and abhexon, although at lower concentrations. Copyright © 2012 The Institute of Brewing & Distilling

Keywords: Gueuze beer; sotolon; theaspirane; Jura wines; Sauternes

Introduction

Lambics are produced with barley, wheat (at least 30%), and a large quantity of aged hop (low bitterness, strong cheese-like aroma), according to a very long oxidation process (use of hot water to better extract dextrins from spent grain, extended boiling, cooling overnight in an open-air container to allow spontaneous inoculation, long maturation in a wood barrel) *(1)*. They are characterized by a typical acidic taste conferred by the endogenous flora, including *Enterobacteriaceae* members (production of 2,3-butanediol, acetic acid, phenols and dimethyl-sulphide), *Kloeckera apiculata*, *Saccharomyces cerevisiae* and *S. bayanus*, lactic bacteria (*Pediococcus cerevisiae*, production of lactic acid and ethyl lactate) and *Brettanomyces* yeasts (*B. bruxellensis*, *B. lambicus*, production of acetic acid, isovaleric acid, ethyl acetate, C8–C12 ethyl esters, 4-ethylguaiacol and 4-ethylphenol) *(2)*. For traditional Gueuzes beers ('Vieilles Gueuzes régulières' in the HORAL classification), lambics of about one year are blended with lambics of around 2–3 years before bottle refermentation by the endogenous flora, while filtered Gueuzes are artificially sweetened and saturated *(3)*.

The succession of growth phases of this mixed culture is responsible for ethanolic fermentation but also for levels of acetic acid (1000–3000 mg/L) *(1,3)*, lactic acid (2000–10,000mg/L) *(4,5)*, ethyl acetate (33–68 mg/L) *(4,6)* and ethyl lactate (107–490 mg/L) *(1,7)*, often above the sensorial thresholds (200, 400, 23 and 50 mg/L, respectively) *(1)*. Because of the *Brettanomyces* esterase activity, the 'banana-like' isoamyl acetate remains in lambic at a lower level (<0.5 mg/L required) than in top-fermentation beers. On the other hand, as ethyl caprate is almost absent in other beers, it might be considered as a typical aroma component *(8)*.

The long maturation of the inoculated wort in barrels also confers to lambics and Gueuze beers a wide variety of aromas, evoking in some cases Jura Flor-Sherry wines, made from Savagnin grapes *(9,10)*. The aroma profile of 'yellow wine' has recently been described by Collin *et al.* *(10)* Owing to long maturation over 6 years and 3 months in an old 228 L oak barrel, many oak-related and oxidation-derived compounds emerge as key odorants.

Among them is sotolon [3-hydroxy-4,5-dimethyl-(5H)-furan-2-one], ranging up to 500 μg/kg oxidation aromas of 'yellow wines', evoking spicy/nut notes *(10,11)*. Sulser *et al.* *(12)* first described sotolon as a degradation product of threonine, but the compound has since been extensively studied in various matrixes, including coffee *(13)*, aged sake *(14)*, Madeira wines *(15)*, old Port wines *(16)* and Sauternes wines *(5,17)*. In 'yellow wines', its formation pathway involves aldol condensation of acetaldehyde and 2-oxobutyric acid *(18,19)*. Under strong oxidative conditions, another mechanism based on acetaldehyde peroxidation probably explains the high sotolon level found in Madeira and Sherry wines *(20,21)*. Q3

Collin *et al.* *(10)* also demonstrated the ethyl analogue of sotolon, abhexon, in 'yellow wines' [ranging up to 75 μg/kg with a threshold evaluated at 4.5 μg/kg in a 12:88 ethanol–water (v/v) solution] *(4)*. Abhexon was previously identified in the well-known noble-rot Sauternes wines made from Sauvignon Blanc, Semillon or Muscadelle grapes as an indicator of long storage, reaching concentrations very close to its threshold value after 6 years of ageing *(4)*. It was also reported in coffee, where Blank *et al.* *(13)* investigated its chemical synthesis pathways from propionaldehyde and 2-oxobutyric acid.

Two other strong odorants directly derived from theaspirane oxidation were identified by GC-HRMS in 'yellow wines' *(10)*. One of these, 4-hydroxy-7,8-dihydro-β-ionone, exhaled a good grenadine aroma while the other, dihydrodehydro-β-ionone,

* Correspondence to: Sonia Collin, Unité de Brasserie et des Industries Alimentaires, Earth and Life Institute, Université Catholique de Louvain, Croix du Sud, 2 box L7.05.07, B-1348 Louvain-la-Neuve, Belgium. E-mail: sonia.collin@uclouvain.be

Unité de Brasserie et des Industries Alimentaires, Earth and Life Institute, Université Catholique de Louvain, Croix du Sud, 2 box L7.05.07, B-1348 Louvain-la-Neuve, Belgium

Table 1. Major sensorial attributes *(9)* and chemical data for the 14 selected beers

	Filtered Gueuzes				Traditional Gueuzes		
	GB-1	GB-2	GB-3	GB-4	GB-5	GB-6	GB-7
	Madeira, apricot, honey, acidic taste (low)	Mushroom, sweet, cherry, apricot, acidic taste (strong)	Wood, beans, apricot, medicine, vinegar	Wort, apricot, peach, cereals, acidic taste (low)	Madeira, oak, nutty, lemon, vinegar	Spicy, sulphur, horse, fruity, vinegar (strong)	Apricot, mandarin, cheese, acidic taste (strong)
Alcohol (%vol)	5.5	3.2	5.4	4.1	6.9	7.4	5.9
Original extract (g/100 g)	12.7	21.5	12.9	12.7	14.2	15.1	12.2
Real extract (g/100 g)	4.4	17.1	4.7	6.5	3.8	3.9	3.3
pH	3.5	3.3	3.3	3.3	3.2	3.3	3.4
Bitterness (BU)	9	9	11	11	8	19	22
Colour (EBC)	27	45	29	19	16	15	17
Haze (EBC)	2.9	2.5	0.5	2.6	0.5	1.7	5.9
	Top-fermentation beers						
	SB-1	SB-2	SB-3	SB-4	SB-5	SB-6	SB-7
	Butter, apple, hop, and green	Butter, sulphur, hop	Alcohol, banana, cheese, phenols	Orange, pineapple, spicy, phenols	Lemon, banana, apple, spicy, phenols	Malt, sulphur, green	Hop, phenols, citrus, cheese
Alcohol (%vol)	6.5	7.9	10.5	7.4	8.1	9.4	6.6
Original extract (g/100 g)	14.4	16.8	20.8	14.9	16.3	19.8	13.3
Real extract (g/100 g)	4.6	5.1	5.5	3.7	4.2	6.2	3.2
pH	4.2	4.5	4.4	4.5	4.4	4.7	4.1
Bitterness (BU)	15	21	18	24	29	16	29
Colour (EBC)	12.5	16.5	11	15.5	14.5	11	26
Haze (EBC)	3.7	1.7	1.1	2.1	0.8	1.1	11.9

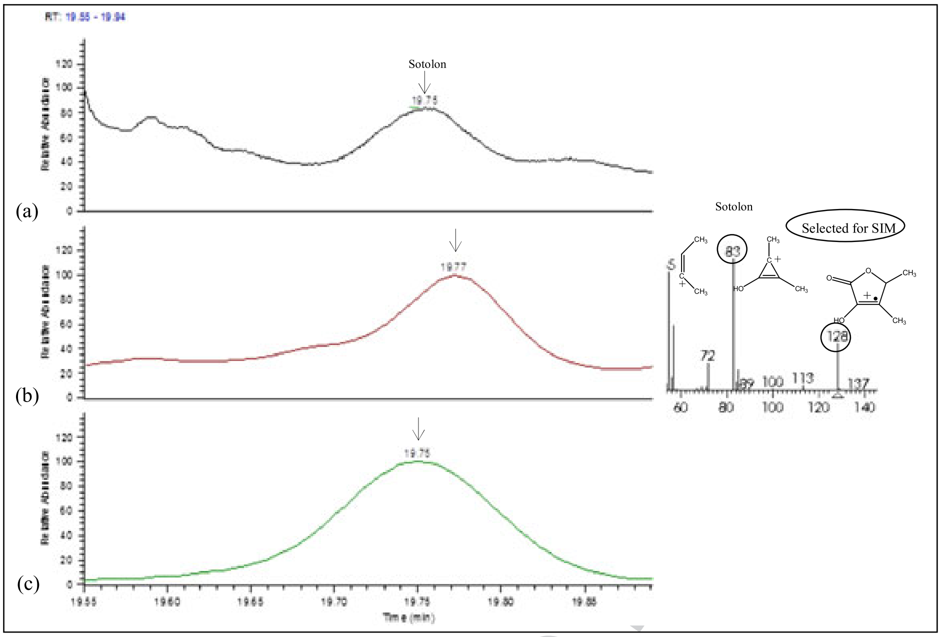


Figure 1. Sotolon (SIM data) in GB-1 (a) and GB-6 (b). Comparison with the commercial standard (c) and complete mass spectrum. Q4

was described as dried fruit/Sauternes-like. Surprisingly, theaspirane, dihydrodehydro-β-ionone and 4-hydroxy-7,8-dihydro-β-ionone were revealed also to coexist in Sauternes wines, famous for their dried fruit and honey-like aromas *(5,10)*.

The aim of the present work was to show chemical similarities between Gueuze beers and 'yellow wines'. Two different extraction procedures (XAD 2 to investigate the theaspirane-derived compounds and specific extraction for sotolon and abhexon) were applied in duplicate to seven Gueuze beers. Gas chromatography–olfactometry (GC-O; AEDA method) and GC–mass spectrometry (GC-MS) were used to compare them with three previously investigated 'yellow wines' *(10)*, and seven top-fermentation special beers, freshly commercialized.

Experimental

Beer samples

Four 'filtered Gueuze' beers (GB-1 to GB-4) and three 'traditional Gueuze' beers (GB-5 to GB-7) were bought at a local supermarket. Seven fresh commercial Belgian special beers were also investigated (SB-1 to SB-7). Six of these were fermented only by top-fermentation yeasts, while the seventh (dry-hopping process) additionally contained *Brettanomyces* yeasts, as is the case for Gueuzes.

Chemicals

Diethyl ether (99.9%), dodecane (99,9%), hydrogen peroxide (30%), iron sulphide, eicosane (99%), sotolon (99%), abhexon (99%) and theaspirane (97%) were purchased from Sigma-Aldrich (Bornem, Belgium). Methanol (99.9%), acetone and absolute ethanol were obtained from Analar Normapur (Fontenay-sous-bois, France). Dichloromethane (99.9%) was obtained from Romil (Gent, Belgium). Amberlite XAD 2 resin came from Supelco, Bellefonte, PA. Anhydrous sodium sulphate (99%) was obtained from Merck (Darmstadt, Germany); chlorohydric acid (36%) was purchased from Fisher Scientific (Tournai, Belgium). Sodium hydroxide was purchased from VWR international. 4-Hydroxy-7,8-dihydro-β-ionone and dihydrodehydro-β-ionone were synthesized from theaspirane in the presence of hydrogen peroxide and iron sulphide, as described by Collin *et al. (10)*

Sensorial analyses *(9)*

All samples were presented to 10 trained panellists in 500 mL 'Breughel' glasses (Durobor, Belgium) covered with a glass top and containing 20 mL of beer per glass. Samples were assessed at room temperature in individual booths. Descriptors were scored on a 1–5 scale.

Beer traditional analyses

Alcohol, original extract and real extract were measured with a Density Meter Anton Paar DMA 4500M (5 mL introduced in the system). Bitterness *(6)*, pH *(22)*, colouration *(23)* and haze *(24)* were determined according to the EBC analytical methods.

Extraction procedure for theaspirane and its derived molecules (global extract) *(25)*

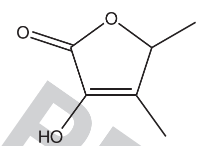
Beer samples were extracted in duplicate with XAD 2 resin, as described by Lermusieau *et al. (25)*. A 2 g XAD 2 aliquot was thoroughly rinsed with Milli-Q water (100 mL) and poured into a Schott flask containing 50 mL wine. This mixture was shaken on a platform shaker at 200 rpm for 2 h at 20°C. The content of the flask was then transferred into a glass column (60 × 1 cm i.d.). The column was first rinsed with 4 × 50 mL Milli-Q water in order to eliminate sugars and other 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 and 0.5 mL of dodecane (20 mg/L) was added as external standard (EST). The mixture was concentrated to 0.5 mL in a Kuderna–Danish at 39°C (total concentration factor = 100, final EST concentration = 20 mg/L). The final extract was stored at −80°C for further analysis.

Extraction procedure for sotolon and abhexon (hydrophilic extraction) *(10)*

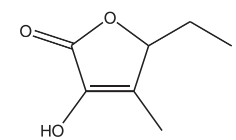
The procedure, derived from Blank *et al. (13)* and Bailly *et al. (5)*, was recently described by Collin *et al. (10)*. Before the beer was placed in contact (2 h, 200 rpm) with the resin, its pH was brought to 11.5 with sodium hydroxide. The eluate from the XAD 2 resin and the first 50 mL of resin washing water were mixed before adjusting the pH to 3 with chlorhydric acid. This aqueous phase was extracted three times with 40 mL dichloromethane (10 min, 1000 rpm). The 40 mL fractions thus

Table 2. Oxidation-derived flavours in Gueuzes and top-fermentation beers. Data from GC-O (AEDA) and GC-MS (SIM mode)

RI CPSIL5	RI FFAP	Compound	Filtered Gueuzes								Traditional Gueuzes					
			GB-1		GB-2		GB-3		GB-4		GB-5		GB-6		GB-7	
			FD	µg/L	FD	µg/L	FD	µg/L	FD	µg/L	FD	µg/L	FD	µg/L	FD	µg/L
1068	2213	Sotolon	16	6^{b}	16	8^{a}	16	9^{a}	16	6^{b}	16	6^{b}	32	9^{a}	8	1^{c}
1150	2304	Abhexon	0	0.3^{a}	1	0.5^{a}	1	0.4^{a}	1	ud^{a}	0	0.8^{a}	0	ud^{a}	1	0.3^{a}
			Top-fermentation beers													
			SB-1		SB-2		SB-3		SB-4		SB-5		SB-6		SB-7	
			FD	µg/L	FD	µg/L	FD	µg/L	FD	µg/L	FD	µg/L	FD	µg/L	FD	µg/L
1068	2213	Sotolon	1	nq^{d}	1	nq^{d}	1	nq^{d}	1	nq^{d}	1	nq^{d}	1	nq^{d}	1	nq^{d}
1150	2304	Abhexon	0	ud^{a}	0	ud^{a}	0	ud^{a}	0	ud^{a}	0	ud^{a}	0	ud^{a}	0	ud^{a}
			'Yellow wines'[a]													
				FD			µg/kg		Odour							
1068	2213	Sotolon		256–1024			112–387		Curry							
1150	2304	Abhexon		64–256			31–74		Curry							



Sotolon



Abhexon

All samples that do not share a common letter are significantly different ($p < 0.05$) according to Tukey's test.
ud, Undetected, means of duplicates; nq, detected but not quantifiable.
[a]Comparison with 'yellow wines' *(10)*. Dilution factors (FD) are given for a total concentration factor of 100 between the samples and the undiluted extracts.

obtained were centrifuged for 10 min at 2500 rpm. Extracts were then dried with anhydrous sodium sulphate and 0.5 mL of eicosane (10 mg/L) was added as EST before concentration until 0.5 mL in a Kuderna–Danish at 45°C (total concentration factor = 100, final EST concentration = 10 mg/L).

Gas chromatography–mass spectrometry

Electronic impact (EI) mass spectra were recorded at 70 eV on a ThermoFinnigan Trace MS simple quadrupole mass spectrometer connected to a ThermoFinnigan Trace GC 2000 gas chromatograph equipped with a splitless injector (splitless time = 0.5 min) and an apolar CP-Sil 5 CB MS column (50 m × 0.32 mm i.d., 1.2 μm film thickness) or a polar FFAP-CB column (Varian, CP7485, 25 m × 0.32 mm i.d., 0,3 μm film thickness). The carrier gas was helium and the pressure was set at 100 kPa (50 for FFAP column). The injection volume was 1 μL. The oven temperature was programmed to rise from 36 to 85°C at 20°C/min, then to 145°C at 1°C/min, and finally to 250°C at 3°C/min. Compounds were quantified by standard addition, as recently described by Collin *et al.* *(10)* The SIM mode was used, with *m/z* = 83/128, 97/142, 138/179, 126/154 and 119/134 for sotolon, abhexon, theaspirane, 4-hydroxy-7,8-dihydro-*β*-ionone and dihydrodehydro-*β*-ionone, respectively. Spectral recording was automatic throughout separation (Xcalibur software was used, NIST databank).

Gas chromatography–olfactometry

A 1 μL sample of extract (global or hydrophilic) 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 an apolar CP-Sil5-CB column (50 m × 0.32 mm i.d., 1.2 μm film thickness) and on a polar FFAP capillary column (Varian, CP7485, 25 m × 0.32 mm i.d., 0.3 μm film thickness). The carrier gas was nitrogen and the pressure was set at 60 kPa (CP-Sil5-CB) or 30 kPa (FFAP). The oven temperature program was the same as described for GC-MS. 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.

Complete AEDA was performed by two trained panellists. The extracts were diluted stepwise with diethyl ether or dichloromethane (1 + 1 by volume, initial EST concentration checked to be 20 or 10 mg/L). The dilution factor (FD) is defined as the highest dilution at which the compound can still be detected ($FD = 2^n$ with $n + 1$ = the number of dilutions applied to the extract until no detection by GC-O).

Statistical analyses

Analyses were carried out in duplicate and comparison of means were performed by means of Turkey's test with SAS software version 9.2 (SAS Institute, INC., Cary, NC, USA) Values that do not share a common letter are significantly different ($p < 0.05$).

Results and discussion

Seven commercial Gueuze beers, qualified by the trained panellists for their strong sensorial analogies to 'yellow wines' (Madeira, nutty, spicy descriptors) and Sauternes wines (apricot, dried fruit, honey descriptors) *(9)*, were compared with seven Belgian top fermentation beers. The analytical data depicted in Table 1 confirm the high acidity of all Gueuze samples (pH 3.2–3.5), their low bitterness (although artefacts can occur with humulone degradation products issued from aged hop), and a lower real extract for traditional Gueuzes and SB-7 (3.2–3.9%, use of dextrins by *Brettanomyces*).

The specific extraction dedicated to hydrophilic compounds enabled us to show, for the first time, sotolon in Gueuze beers (Fig. 1). Its concentrations and dilution factors (1–9 μg/L, FD = 8–32; Table 2) were found to be much lower than those found in 'yellow wines' (up to 387 μg/kg), but in some cases were close to the threshold (15 μg/kg in wine) *(4,10)*. On the other hand, only unquantifiable traces were found in the seven investigated fresh top-fermentation Belgian beers.

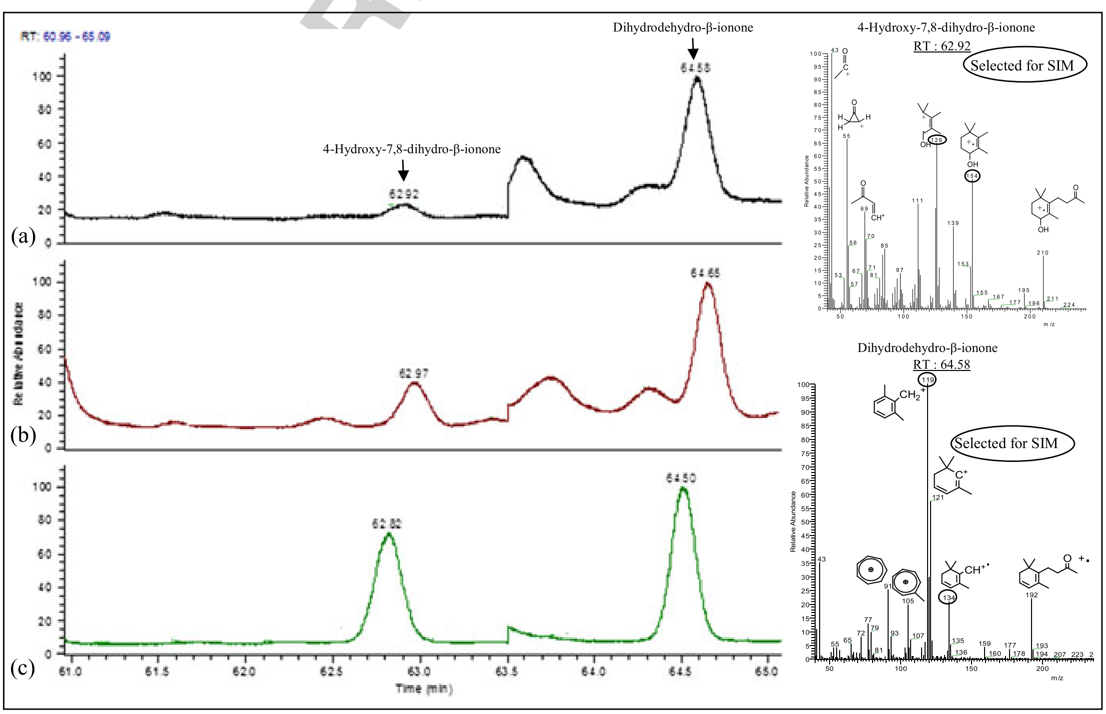


Figure 2. Theaspirane-derived compounds (SIM data) in GB-1 (a) and GB-6 (b). Comparison with the synthetic medium (c). Complete mass spectrum of both compounds.

Table 3. Theaspirane-derived compounds in Gueuzes and top-fermentation beers. Data from GC-O (AEDA) and GC-MS (SIM mode), applied to global XAD 2 extracts

RI CPSIL5	RI FFAP	Compound	Filtered Gueuzes								Traditional Gueuzes					
			GB-1		GB-2		GB-3		GB-4		GB-5		GB-6		GB-7	
			FD	µg/L	FD	µg/L	FD	µg/L	FD	µg/L	FD	µg/L	FD	µg/L	FD	µg/L
1305	1498	Theaspirane	16	ud^{a}	4	ud^{a}	4	ud^{a}	8	ud^{a}	8	ud^{a}	8	ud^{a}	8	ud^{a}
1373	1698	4-Hydroxy-7,8-dihydro-β-ionone	256	7^{b}	64	2.2^{d}	8	0.2^{e}	8	0.1^{e}	1024	10^{a}	128	2.8^{c}	16	0.2^{e}
1419	1783	Dihydrodehydro-β-ionone	4	0.2^{d}	32	4.8^{a}	8	1.5^{c}	4	0.5^{d}	64	4^{a}	8	3.2^{b}	2	ud^{d}
			Top-fermentation beers													
			SB-1		SB-2		SB-3		SB-4		SB-5		SB-6		SB-7	
			FD	µg/L	FD	µg/L	FD	µg/L	FD	µg/L	FD	µg/L	FD	µg/L	FD	µg/L
1305	1498	Theaspirane	2	ud^{a}	9	ud^{a}	ud	ud^{a}	64	ud^{a}	8	ud^{a}	2	ud^{a}	32	ud^{a}
1373	1698	4-Hydroxy-7,8-dihydro-β-ionone	0	ud^{e}	0	ud^{e}	0	ud^{e}	0	ud^{e}	0	ud^{e}	0	ud^{e}	0	ud^{e}
1419	1783	Dihydrodehydro-β-ionone	2	nq^{d}	8	nq^{d}	ud	nq^{d}	2	nq^{d}	2	nq^{d}	2	nq^{d}	16	nq^{d}

RI CPSIL5	RI FFAP	Compound	'Yellow wines'[a]		
			FD	µg/kg	Odour
1305	1498	Theaspirane	4–16	nq	Honey
1373	1698	4-Hydroxy-7,8-dihydro-β-ionone	64–1024	0.6-8	Grenadine
1419	1783	Dihydrodehydro-β-ionone	1–4	0.5-2	Dried fruit, Sauternes

All samples that do not share a common letter are significantly different ($p < 0.05$) according to Tukey's test.
Means of duplicates. ud, Undetected; nq, detected but not quantifiable.
[a]Comparison with 'yellow wines' *(10)*. Dilution factors (FD) are given for a total concentration factor of 100 between the samples and the undiluted extracts.

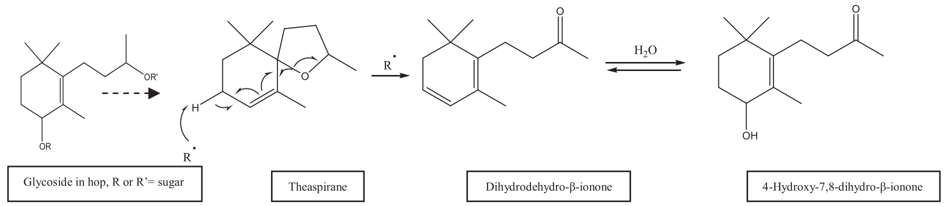


Figure 3. Hypothetical oxidative degradation of theaspirane leading to dihydrodehydro-β-ionone and 4-hydroxy-7,8-dihydro-β-ionone in Gueuzes.

Abhexon was also evidenced for the first time in Gueuze beers, but at very low concentrations (≤0.8 µg/L). No trace was found in the seven top-fermentation beers (Table 2).

Another analogy between Gueuze beers and 'yellow wines' was the co-existence of both theaspirane-derived compounds, 4-hydroxy-7,8-dihydro-β-ionone (grenadine descriptor) and dihydrodehydro-β-ionone (dried fruit/Sauternes descriptor; Fig. 2 and Table 3) *(10)*. Except for GB-3, GB-4 and GB-7 (0.1–0.2 µg/L), the concentration of 4-hydroxy-7,8-dihydro-β-ionone (3.2–10 µg/L) was very similar to that found in 'yellow wines' (0.6–8 µg/L). For dihydrodehydro-β-ionone, levels were also equivalent and even higher in GB-2 and GB-5 (up to 4.8 µg/L). As depicted in Fig. 3, theaspirane or its glycosylated precursors are suspected of being oxidized and hydrolysed during hop storage, wort boiling and lambic oak-ageing, allowing the occurrence of both compounds in Gueuzes. Theaspirane A and B glycosides were described for the first time in hop by Daenen *et al. (26)* While high FD values (up to 1024) were obtained for most Gueuze beer extracts, 4-hydroxy-7,8-dihydro-β-ionone was not detected at all in the unaged top-fermentation beers, which were made with only fresh hops. In SB-7, an odour that was a little more persistent was detected at the retention time of dihydrodehydro-β-ionone (FD = 16), probably owing to the dry hopping process, which favours theaspirane glycoside dissolution *(27)*.

In conclusion, sotolon, abhexon, dihydrodehydro-β-ionone and 4-hydroxy-7,8-dihydro-β-ionone coexist in Gueuze beers and 'yellow wines', thus justifying some analogies mentioned by sensorial panellists. Strong protection against oxidation all along the process allows the avoidance of all four compounds in most fresh top-fermentation beers. Yet their occurrence should now be investigated through ageing. Complementary investigations are also required to determine the limiting factors for 4-hydroxy-7,8-dihydro-β-ionone production in Gueuze beers.

References

1. Verachtert, H. and Derdelinckx, G. (2005) Acidic beers: Enjoyable reminiscences of the past. *Cerevisia*, *30*, 38–47.
2. Van Oevelen, D., De l'Escaille, F., and Verachtert, H. (1976) Synthesis of aroma components during the spontaneous fermentation of lambic and gueuze. *J. Inst. Brew.*, *82*, 322–326.
3. Daenen, L., Saison, D., and De Schutter, D. P. (2008) *Beer in Health and Disease Prevention* (Preedy, V. Ed.) pp. 33–49, Academic Press, London.
4. Bailly, S., Jerkovic, V., Meurée, A., Timmermans, A., and Collin, S. (2009) Fate of key odorants in Sauternes wines through aging. *J. Agric. Food Chem.*, *57*, 8557–8563.
5. Bailly, S., Jerkovic, V., Marchand-Brynaert, J., and Collin, S. (2006) Aroma extraction dilution analysis of Sauternes wines. Key role of polyfunctional thiols. *J. Agric. Food Chem. 54*, 7227–7234.
6. Bitterness of beer (IM) (2006) Analytica-EBC, Fachverlag Hans Carl, Nurnberg, Section 7, Method 9.8.
7. Spaepen, M., Van Oevelen, D., and Verachtert, H. (1978) Fatty acids and esters produced during the spontaneous fermentation of lambic and gueuze. *J. Inst. Brew.*, *84*, 278–282.
8. Spaenen, M. and Verachtert, H. (1982) Esterase activity in the genus *Brettanomyces*. *J. Inst. Brew.*, *88*, 11–17.
9. Test Achats (2011) *Bières Belges*, Dereume: Drogenbos, 206–210.
10. Collin, S., Nizet, S., Claeys-Bouuaert, T., and Despatures, P. M. (2012) Relative contributions of varietal, fermentation, oak-barrel and oxidation-derived odorants in Jura Flor-Sherry wines. *J. Agric. Food Chem.*, *60*, 380–387.
11. Martin, B., Etiévant, P., Le Quéré, J. L., and Schlich, P. (1992) More clues about sensory impact of sotolon in flor sherry wines. *J. Agric. Food Chem.*, *40*, 475–478.
12. Sulser, H., De Pizzol, J., and Büchi, W. (1967) A probable flavouring principle in vegetable-protein hydrolysates. *J. Food Sci.*, *32*, 611–615.
13. Blank, I., Sen, A., and Grosch, W. (1992) Potent odorants of the roasted powder and brew of arabica coffee. *Z. Lebensm. Unters. Forsch.*, *195*, 239–245.
14. Kobayashi, A. (1989) *Flavor Chemistry: Trends and Developments* (Teranishi, R. Ed.) pp. 49–59, American Chemical Society: Washington, DC.
15. Camara, J. S., Marques, J. C., Alves, M. A., and Silva Ferreira, A. C. (2004) 3-Hydroxy-4,5-dimethyl-2(5H)-furanone levels in fortified Madeira wines: Relationship to sugar content. *J. Agric. Food Chem.*, *52*, 6765–6769.
16. Silva Ferreira, A.C.S., Barbe, J.C., and Bertrand, A. (2003) 3-Hydroxy-4, 5-dimethyl-2(5H)-furanone: a key odorant of the typical aroma of oxidative aged Port wine. *J. Agric. Food Chem.*, *51*, 4356–4363.
17. Masuda, M., Okawa, E., Nishimura, K., and Yunome, H. (1984) Identification of 4,5-dimethyl-3-hydroxy-2(5H)-furanone (sotolone) and ethyl 9-hydroxynonanoate in botrytised wine and evaluation of the roles of compounds characteristic of it. *Agric. Biol. Chem.*, *48*, 2707–2710.
18. Charpentier, C., Pham, T. T., Guichard, E., and Arbault, B. (1995) Production de sotolon par les levures isolées des 'Vins Jaunes' du Jura. *Proceedings for the 5ème Symposium d'oenologie*, 179–182.
19. Pham, T. T., Guichard, E., Schlich, P., and Charpentier, C. (1995) Optimal conditions for the formation of sotolon from alpha-ketobutyric acid in the French 'Vin Jaune'. *J. Agric. Food Chem.*, *43*, 2616–2619.
20. Cutzach, I., Chatonnet, P., and Dubourdieu, D. (1999) Study of the formation mechanisms of some volatile compounds during the aging of sweet fortified wines. *J. Agric. Food Chem.*, *47*, 2837–2846.
21. Pisarnitsky, A. K., Bezzubov, A., and Egorov, I.A. (1987) Nonenzymatic formation of 4,5-dimethyl-3-hydroxy-2(5H)-furanone in foodstuffs. *Prikl. Biokhim. Microbiol.*, 642–646.
22. pH of beer (2006) Analytica-EBC, Fachverlag Hans Carl, Nurnberg, Section 7, Method 9.35.
23. Colour of beer (2006) Analytica-EBC, Fachverlag Hans Carl, Nurnberg, Section 7, Method 9.6.
24. Haze in beer (2006) Analytica-EBC, Fachverlag Hans Carl, Nurnberg, Section 7, Method 9.29.
25. Lermusieau, G., Bulens, M., and Collin, S. (2001) Use of GC-olfactometry to identify the hop aromatic compounds in beer. *J. Agric. Food Chem.*, *49*, 3867–3874.
26. Daenen, L., Saison, D., Cooman, L., Derdelinckx, G., Verachtert, H., and Delvaux, F. R. (2007) Flavour enhancement in beer: hydrolysis of hop glycosides by yeast *beta*-glucosidase. *Cerevisia*, *32*, 24–36.
27. Collin, S., Nizet, S., and Gros, J. (2011) Le houblonnage à cru des bières spéciales belges est bien plus qu'une simple dissolution des composés aromatiques du houblon. *Cerevisia*, *36*, 119–124.

Author Query Form

Journal: Journal of the Institute of Brewing

Article: jib_34

Dear Author,

During the copyediting of your paper, the following queries arose. Please respond to these by annotating your proofs with the necessary changes/additions.

- If you intend to annotate your proof electronically, please refer to the E-annotation guidelines.
- If you intend to annotate your proof by means of hard-copy mark-up, please refer to the proof mark-up symbols guidelines. If manually writing corrections on your proof and returning it by fax, do not write too close to the edge of the paper. Please remember that illegible mark-ups may delay publication.

Whether you opt for hard-copy or electronic annotation of your proofs, we recommend that you provide additional clarification of answers to queries by entering your answers on the query sheet, in addition to the text mark-up.

Query No.	Query	Remark
Q1	AUTHOR: This paper has been edited for English and JIB format/style of writing – please check your page proofs carefully to make sure meanings were not changed in the process.	
Q2	AUTHOR: A running head short title was not supplied; please check if this one is suitable and, if not, please supply a short title of up to 60 characters that can be used instead.	
Q3	AUTHOR: Please check the sense of the sentence beginning 'Among them is sotolon'.	
Q4	AUTHOR: Please provide clearer copies of figures.	