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Flavour stability of lager beer: identification of a new key staling compound

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SUMMARY

In the last decade, many papers have underlined the key-role on beer staling of *trans*-2-nonenal, dimethyltrisulfide, ß-damascenone, and 3-(methylthio)-proprionaldehyde. Recent data have indicated, however, that a phenolic compound could be as important. The aim of this work was to identify this compound. The flavour profiles of various beers were determined by the AEDA methodology. Different techniques were used in order to identify the unknown: odour description at the sniffing port, injection on two capillary columns, GC-MS/EI and CI, chemical synthesis, specific extraction techniques, and cold trapping. 4-Vinylsyringol has been identified as one of the most important key-flavour in aged lager beers.

INTRODUCTION

Beer staling has been for a long time of prime concern for most brewers (1). Through storage, flavour appears to deteriorate greatly with time at a rate depending on beer composition [pH (2,4,6,10,28), oxygen (20,22), antioxidants (7,15), precursor concentrations (3,6,8,16,17,28)] and storage conditions [packaging (18), temperature (27), light (9)]. Improvement of beer stability requires a better knowledge of all chemicals involved.

In the past decade, many papers have underlined the importance of a few number of aged beer off-flavours. More especially, attention has been paid to *trans*-2-nonenal which is released from protein adducts by acidic hydrolysis through ageing (16,21). GC-olfactometry (6) and sensorial analysis (10) allowed to confirm the key-role of this cardboard flavouring in most beer brands. For some beer productions, however, other defects can be sometimes more pronounced. It can be the case of dimethyltrisulfide with its typical onion-odour or 3-methyl-2-butene-1-thiol, the well-known "light-skunky" off-flavour. More seldom ß-damascenone (3,6,25), 3-

(methylthio)propionaldehyde (8,23,29), and 2-furfuryl ethyl ether (11,27,28) can be responsible of the consumer disappointment.
Recently, Gijs *et al.* (2002) mentioned the presence of a strong "dentist-smoked" off-flavour at RI $_{\text{CP-SIL 5 CB}}$ = 1532 in a dichloromethane extract issued from an accelerated aged lager beer (5 days at 40 °C). It revealed to be as important as *trans*-2-nonenal. Lermusieau *et al.* (2001) also mentioned its presence in a fresh beer hopped with Saaz pellets but did not detect it in unhopped beer (14).
The aim of the present work was to check the role of this stale flavour in beers aged in more natural conditions. GC-olfactometry was applied on extracts of two lager beers stored for 3 and 6 months at 20 °C. Identification trials were further conducted on an over-aged beer extract (10 days at 40 °C).

MATERIALS AND METHODS

Beer treatments and extraction procedures

Commercial beers were aged in a dark room for 5 days at 40 °C (accelerated ageing) or 10 days at 40 °C (forced accelerated ageing), and for 3 or 6 months at 20 °C (natural ageing). Extractions were carried out under red light. The extracts before concentration to 0.5 ml were in both case dried with anhydrous sodium sulfate and 20 ppm dodecane added (external standard). The final extracts were stored at 81 °C and analysed by GC-FID, GC-MS, and GC-O.

Fresh and aged beers representative flavour extract

Two grams of Amberlite XAD-2 resin was thoroughly rinsed with Milli-Q water (100 ml) and poured into a 100 ml Schott flask (Vel, Leuven, Belgium) containing 50 ml 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 to a liquid chromatography glass column (60 x 1 cm i.d.) ending with a coarse glass ball. The lower part was also filled with glass ball beads (3 g with a diameter of 3.5-4.5 mm and 1 g with a diameter of 0.8-1.2 mm) in order to retain the resin. The column was first rinsed with 4 x 25 ml Milli-Q water in order to eliminate sugars and other water-soluble substances. Aroma apolar compounds were then eluted with 2 x 20 ml diethyl ether at a flow rate of 0.75 ml/min.

Specific extraction for phenols

Fifty milliliter of beer, eugenol (5 ppm), 1 ml of 37 % (v/v) hydrochloric acid, and 6.45 g sodium chloride (for increasing salting out) were mixed together. After the entire dissolution, 150 ml of chloroform/methanol (3:1) were added and stirred for 10 min at 1500 rpm. The lower organic solvent layer was retained while the aqueous phase was extracted a second-time in the same manner. The 300 ml organic phase was then shaken with 50 ml of 10 % potassium hydroxide solution for 10 min at 1500 rpm. The upper aqueous phase (pH 13) was recovered and the lower organic phase extracted a second time as described before. The pH of the aqueous phase was then adjusted to 9.0 with hydrochloric acid and extracted two times with 25 ml dichloromethane after 10 min stirring at 1500 rpm. The combined organic phases were further analysed.

Cold trapping

In order to isolate part of the GC effluent, the CP-SIL 5 CB GC column was connected to a T-junction splitting the effluent between the cold trap and a throw gap

(ratio 1:1, figure 1). The trap consisted in an uncoated inactivated tube (51 cm x 0.53 mm i.d.) wrapped in a copper sleeve with two coiled ends immersed in Dewar vessels (Leuven, Belgium). The GC side was cooled with ice while the other one was filled with liquid nitrogen, generating by this way a temperature gradient inside the tube. After each 2-µl injection (30 injections required), an electronic switch allowed collecting our unknown between 77 and 81 minutes.

Gas Chromatographic analyses hyphenated to FID (GC-FID) or olfactometric detection (GC-O)

1 µl of beer extract was analysed on a Chrompack CP9001 gas chromatograph equipped with a splitless injector maintained at 250 °C; split vent was opened 0.5 min post-injection. The carrier gas was nitrogen at a flow rate of 1 ml/min. Compounds were analysed using a wall-coated open tubular (WCOT) apolar CP-Sil 5 CB capillary column (50 m × 0.32 mm i.d., film thickness, 1.2 µm), and/or a polar FFAP CB capillary column (WCOT, 25 m × 0.32 mm i.d., 0.3 µm film thickness). In each case, 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 to 250 °C at 3 °C/min. This temperature was further maintained for 30 min. To assess the olfactory potential of the extract, the column was connected to a GC-O port maintained at 250 °C. The effluent was diluted with a large volume of air (20 ml/min) pre-humidified with an aqueous copper (II) sulfate solution. The column was in some cases directly connected to an FID detector equipped with a Shimadzu CR6-A integrator.

Gas Chromatographic analyses hyphenated to Mass Spectrometry (GC-MS)

The electronic impact (EI) mass spectra were recorded at 70 eV (full scan with mass range from 40 to 380 m/z) on a ThermoFinnigan Trace MS simple quadrupole mass spectrometer connected to a ThermoFinnigan Trace GC 2000 gas chromatograph equipped with a splitless injector. The chemical ionization (CI) mass spectra were recorded in positive mode with a CH_4-N_2O (75/25, v/v) gas mixture on a TSQ 7000 Finnigan Material. In both cases, the carrier gas was helium at a flow rate of 1 ml/min. Spectral recording was automatic throughout separation using the Xcalibur software. Identification was tentatively achieved with the NIST data base.

RESULTS AND DISCUSSION

Key-flavours in natural aged beers

In order to determine new key-flavours responsible of beer staling, a natural storage of 3 or 6 months at 20 °C has been applied on two commercial lager beers of the same brand (I and II; see table 1). Prior to GC-O analyses, beers were extracted with Amberlite XAD-2 resin. The so-obtained very representative samples (14) were further analysed by using the AEDA (Aroma Extract Dilution Analysis) methodology initially proposed by Ullrich and Grosch (26). All FD values were compared to that obtained for isoamyl acetate (FD = 27). This ester usually occurs in lager beers at a concentration close to its aroma threshold value (threshold = 1.60 ppm (19) and concentration in beer I = 1.90 ppm). Therefore, we can suspect that all FD values above 27 most probably indicate organoleptically active compounds in beer (table 1).

Table 1: Compounds in lager beers I and II with FD values >27 either in fresh or in aged (3 or 6 months at 20 °C or 5 days at 40 °C) beer extracts.

RI [a]	Individual odours	FD [b] of beer I				FD [b] of beer II				Compound
		Fresh	Natural ageing (months)		5 Days at 40°C	Fresh	Natural ageing (months)		5 Days at 40°C	
			3	6			3	6		
774	fruity	9	27	27	81	3	9	9	27	ethyl butyrate [c,d,f]
807	hop	2187	2187	2187	2187	2187	2187	2187	6561	isopentenylmercaptan [d,e,f]
843	sweet, candy	27	27	27	27	27	27	27	27	isoamyl acetate [c,d,f]
846	nutty	729	2187	243	2187	9	9	9	3	2-methyl 3-furanethiol [d,e,f]
863	potato	27	27	27	81	9	9	9	27	3-(methylthio)-propionaldehyde [c,d,e,f]
957	genarium	81	243	729	243	27	81	243	243	dimethyltrisulfide [d,f]
1058	curry	27	27	243	9	9	1	3	3	sotolone [d,f]
1079	floral	243	243	243	243	243	243	243	243	ß-phenylethanol [c,d,e,f]
1147	cardboard	27	81	243	81	27	81	81	243	*trans*-2-nonenal [c,d,e,f]
1262	honey	81	243	81	243	27	81	81	81	2'-aminoacetophenone [c,d,f]
1289	smoky	81	81	81	729	27	81	81	81	4-vinylguaiacol [c,d,e,f]
1369	red fruits	27	81	81	243	27	81	81	243	ß-damascenone [c,d,e,f]
1532	dentist, smoky, tobacco, old beer	27	243	243	243	27	81	81	243	unknown

[a] RI, retention index on CP-SIL 5 CB
[b] FD = Dilution factor (AEDA) = 3^{n-1} with n, the number of dilutions required for no odour to be perceived
[c] Confirmation by GC-MS
[d] Confirmation by the comparison of the RI on CP-SIL 5 CB column
[e] Confirmation by the comparison of the RI on FFAP column
[f] Confirmation by the odour of standard

Already before ageing, isopentenylmercaptan (FD = 2187), 2-methyl 3-furanethiol (FD = 729 for beer I), and ß-phenylethanol (FD = 243) were perceived at dilutions much higher than isoamylacetate. At low level, the former, issued from hop (14), imparts a pleasant hoppy flavour while at higher concentrations, it is responsible of the lightstruck off-flavour of lager beers.

As expected (6), in addition to *trans*-2-nonenal (cardboard off-flavour, FD = 81-243), dimethyltrisulfide (onion flavour, FD = 81-729), and ß-damascenone (red fruit odour, FD = 81) emerged relevant to the sensory profile of the natural aged beers (3 or 6 months at 20 °C). On the other hand, in comparison to the accelerated ageing (5 days at 40 °C), 3-(methylthio)-proprionaldehyde and ethyl butyrate revealed released in very few amounts at 20 °C.

Also to be emphasized was the presence in beer I of sotolone with an FD up to 243 after 6 months at 20 °C. Surprisingly, this curry odour was completely underestimated after the accelerated ageing. Very few amounts also characterized beer II, whatever the ageing procedure applied.

The unknown at RI $_{CP\text{-}SIL\ 5\ CB}$= 1532 appeared strongly relevant after storage for our two beers (FD = 81-243). Its smoky, tobacco, old beer descriptors reinforced our desire to chemically identify it despite the absence of a nice mass spectrum.

Identification of the unknown at RI$_{CP\text{-}SIL\ 5\ CB}$ = 1532

In order to artificially increase the level of the unknown in a beer XAD 2 extract, an accelerated ageing of 10 days at 40 °C was first applied. The compound was still further concentrated by using the GC method proposed by Gallois (5). From RI = 1509 to RI = 1563, the eluent was deviated to a cold trap (figure 1).

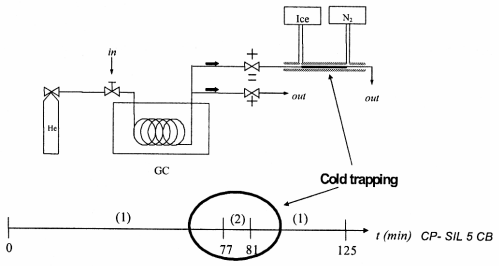

Figure 1: Cold trap scheme.

Diethylether elution of this cold trap after thirty gas chromatographic injections allowed us to recover an extract with a very strong "phenolic-tobacco-old beer" odour. A retention index of 2809 was determined for this compound on the polar FFAP column. The unknown was recovered by applying a specific extraction for phenolic compounds, indicating that its pKa value was in the 9-13 range. The suspected phenolic structure was further confirmed by GC-MS analyses on the cold trapped extract.

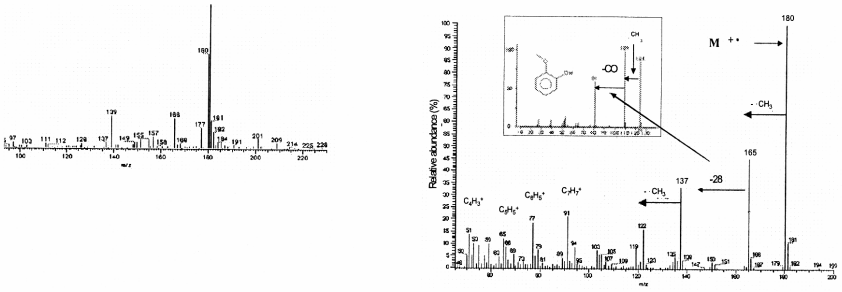

Figure 2: Mass spectrum of the unknown (XAD-2 beer I extract after cold trapping) obtained by GC-MS-chemical ionisation mode (a) and GC-MS-electronic impact mode (b).

By using chemical ionisation with methane-N_2O (75/25, v/v), a pseudo-molecular ion of 181 (Mw = 180) was obtained (figure 2a). As depicted in figure 2b, the electronic impact ionisation lead to the following fragmentation (unfortunately not listed in the NIST MS library): 180 (100), 165 (48), 137(35), 91 (24), 77 (22). The stable molecular ion (m/z = 180) was consistent with an aromatic ring. The highly favourable loss of 43 units (m/z = 137) indicated the release of the CO and methyl moieties, as observed in many other phenols with an m/z = 180. However, our unknown was also characterized by the loss of a methyl radical (m/z = 165), as it is the case in guaiacol but not in phenolic methyl ketones.

All those data completed by literature on tobacco lead us to suspect 4-vinylsyringol (13). Decarboxylation of commercial sinapic acid by heat treatment allowed us to confirm this identification. Its mass fragmentation pattern is tentatively explained in figure 3.

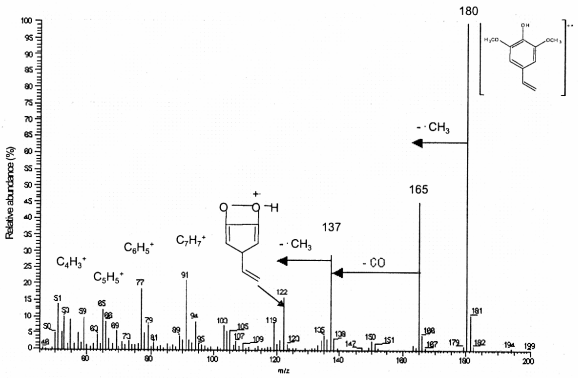

Figure 3: Proposed fragmentation pattern of 4-vinylsyringol synthesised by decarboxylation of the corresponding acid. Mass spectrum obtained by GC-MS-electronic impact mode.

Although already mentioned in some fresh beers (12,24,30), nobody previously underlined the strong "smoky-old beer" odour that 4-vinylsyringol can impart to aged beers.

4-Vinylsyringol which is characterized by "phenolic-tobacco-old beer" descriptors revealed to be perceived in aged beer extracts with FD values as high as trans-2-nonenal. In fresh beer, it could be issued from malt and hop sinapic acid decarboxylation. Its release through ageing seems to indicate the presence of glycosides which could be acid hydrolyzed as previously demonstrated for ß-damascenone (3). Further investigations are now needed to sensorially characterize its impact in beer and to determine how the brewing process could modulate its final concentration.

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