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Characterization of flavour compounds in fresh and aged beer by purge and trap-gas chromatography-mass spectrometry

Flavour constituents of fresh and aged beer were characterized using Purge and Trap-Gas Chromatography-Mass Spectrometry (P&T-GC-MS). This technique allowed the identification of 104 volatile compounds in beer. Identification was performed based on the mass spectrum, the Kovats' retention index and by using reference compounds. Various classes of staling related compounds could be characterized and some compounds were for the first time detected in beer.

Descriptors: Beer, staling, aging, flavours, chromatography

1 Introduction

Gas chromatography (GC) is the ideal tool in the study of flavour compounds present in beer. Since the 1960s, its application in food chemistry has enormously increased the knowledge about flavour active entities in food products. It has been used to investigate changes in volatile beer constituents during beer aging in order to identify the molecules responsible for flavour changes. A real breakthrough came after the development of systems in which GC was coupled with mass spectrometry (MS) (1;2). Besides quantification, combined GC-MS systems allow as well a rapid identification of unknown volatile compounds, making it a powerful analytical tool. Since then, continued efforts have resulted in more effective gas chromatography with capillary columns, capable of resolving flavour extracts in hundreds of components. There have likewise been improvements in the instrumentation of mass spectrometry for the production, separation and detection of charged molecules and their fragments.

The application of GC to beer analysis requires sample preparation steps consisting of separating the volatiles from the beer matrix and concentrating them to detectable levels. Standard sample preparation techniques include direct injection, solvent extraction, distillation and static headspace techniques. Although each technique serves some specific analytical purpose, they all suffer inherent drawbacks such as interference with non-volatiles, thermal decomposition of analytes, artifact formation, poor sensitivity and reproducibility (3). In recent years, advanced sample preparation techniques such as dynamic headspace (4;5) and solid-phase microextraction (SPME) (6-8) have become applicable for beer analysis. These rapid solvent free extraction techniques often show improved reproducibility and sensitivity and are interesting for research on staling compounds.

SPME is a sample preparation technique based on adsorption on a fiber, which is useful for extraction and concentration of analytes either by submersion in beer or by exposure to its gas phase. Following exposure of the fiber to the sample, absorbed analytes can be thermally desorbed in a conventional GC injection port.

In the dynamic headspace technique, the sample is purged with an incondensable inert gas that sweeps the volatiles in the headspace onto a trapping adsorbent. The trapped and enriched volatiles are subsequently recovered by thermal desorption before being introduced to the analytical system. This sampling process, often called "purge and trap" (P&T) technique, has been used extensively for environmental study, such as of organic pollutants in water.

In this study, the characterization of the flavour constituents in fresh and aged beer using a P&T-GC-MS system is discussed. This characterization consists in the identification of detected volatile molecules and in the comparison of fresh and aged beer chromatograms.

2 Materials and Methods

2.1 Chemicals

Reference substances, used in the identification of beer volatiles as indicated in Table 1, had a purity of at least 95%. They were supplied by Sigma Aldrich Chemie GmbH (Munich, Germany). 2-Furfuryl ethyl ether with a purity of 95% was purchased from Narchem Corporation (Chicago, IL, USA). A retention index standard consisting of a mixture of aliphatic hydrocarbons ranging C8 through C32 was obtained from Sigma Aldrich Chemie GmbH (Munich, Germany).

2.2 Beer aging conditions

A fresh pale top-fermented beer (alcohol: 7.5% v/v, colour: 6.6 EBC, bitterness: 31 EBU) was obtained from a Belgian brewery and subjected to aging. Initially, the dissolved oxygen concentration was less than 0.25 mg/L and the headspace contained less than 0.1 mg oxygen. The beer was aged in bottles containing 250 ± 1 mL beer and 10 ± 0.5 mL of headspace volume, filled with air. Air was brought into the headspace by opening the bottles, flushing the headspace briefly with air and recapping it. Beer samples were stored at 40°C for 40 days.

2.3 Analysis of volatile compounds with P&T-GC-MS

Prior to analysis, beer was degassed by kieselguhr filtration. Then, 200 µL internal standard solution (250 mg/L 2-heptanol) and 200 µL of a 10% antifoam solution Sigma Aldrich Chemie GmbH (Munich, Germany) were added to 50 mL of degassed beer. Then five millilitre were then transferred into the Tekmar Dohrman 3000 (Emerson, Mason, USA) purge and trap concentrator unit with a Vocarb 3000 trap (Supelco, Bellefonte, PA, USA) in the following conditions; helium was the carrier gas, 10 min purge

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at 140°C, 8 min dry purge at 140°C, 6 min desorption at 250°C, 10 min bake at 260°C. The indicated temperatures are those of the adsorbing trap, the beer sample temperature was kept at 20°C during purging. Before entering the GC, volatiles were focused using a cold trap with MFA 815 control unit (ThermoFinnigan, San Jose, CA, USA) in the following conditions: initial temperature: –70°C, final temperature: 200°C. GC was performed using a Fisons GC 8000 gas chromatograph equipped with a Chrompack CP-WAX-52-CB column (length 50 m, internal diameter 0.32 mm, film thickness 1.2 µm; Varian, Palo Alto, CA, USA). The temperature program was: 1 min at 50°C / 4°C min-1 to 120°C / 2.5°C min-1 to 165°C / 15°C min-1 to 240°C and 5 min at 240°C. Total ion mass chromatograms were obtained in the Fisons MD 800 quadripole mass spectrometer (ionization energy: 70eV; source temperature: 250°C) and analysed using the Masslab software program for identification and quantification of volatiles.

3 Results and Discussion

3.1 Analysis of chromatograms from fresh and aged beer

Total ion chromatograms (TIC) of fresh and aged beers were obtained with the presented analysis technique. The purge and trap concentrator allowed the extraction of volatiles at ambient temperature without sample modifications. Standard trap temperatures during sample purging, as e.g. used in the analysis of drinking water, are generally below 40°C. However, at such conditions, even a short purge time of a few minutes resulted in a large ethanol peak obscuring most other peaks and causing very bad chromatographic separation. The abundance and volatility of ethanol in beer quickly saturated the adsorbent resin during purging. At relatively high trap temperatures (>130°C) and using long dry purge times (>10 min) saturation could be avoided. Increasing the trap temperature led to a reduced adsorption of preferentially low-boiling components such as ethanol. The elevated trap temperature reduced far less the sensitivity of interesting aging compounds because they are generally higher boiling. Furthermore, the reduced sensitivity for these components could be compensated by longer purge times. Following desorption from the trap, volatiles were first focused in a cryofocusing unit and then separated on a capillary column, which resulted in more than 120 distinguishable peaks.

Three methods were used for the identification of compounds responsible for peaks in the chromatogram. A first step consisted in statistically comparing the mass spectral data with the 62234 mass spectra in a NIST (National Institute of Standards and Technology) MS-library. Although the computerized library search often provides strong evidence ("best match") for a particular compound, it is never completely conclusive. Therefore, additional confirmation for GC peak identity was obtained on the basis of the Kovats' retention index system (9). The system relates the retention of substances during isothermal GC to the retention of a series of n-alkanes analysed under identical conditions. The Kovats' retention index (RI) is calculated from equation 1 and can be used to compare peaks eluting in different chromatographic systems.

$$RI_x = 100N + 100\frac{(\log t_x - \log t_N)}{(\log t_{N+1} - \log t_N)} \quad (1)$$

Where X is the compound of interest, N and N+1 are the adjacent n-alkanes (which differ only by one methylene group) and log t is the logarithm of the retention time. The great variety of volatiles in beer limited the use of isothermal conditions and temperature programming was thus necessary to obtain sufficient gas chromatographic separation. While the precision of temperature-programmed retention indices is less than that of those measured isothermally, it is generally considered to be adequate. Following mass spectral identification, the obtained Kovats' retention indices were compared with cited indices values in literature. Retention indices depend on the stationary phase of the GC column and comparison with literature values was only possible when a similar type of stationary phase (polyethylene glycol polymers) is employed in the reference. A retention index was not found in literature for all compounds. Final confirmation for a GC peak identity was obtained using reference compounds and comparing their mass spectrum and retention index with those of the peaks under investigation. However, this requires that compounds are commercially available as pure substances, which was not the case for all products.

The given identification approach was applied on the GC peaks of the chromatograms and the results are summarized in Table 1. For most peaks, this resulted in a convincing identification, but problems arose for peaks 8, 16, 22, 33, 34, 47, 48, 57 and 103 because the NIST library could not recognize their mass spectrum. The structure of these molecules was derived by manual interpretation of the mass spectrum.

3.2 Interpretation of unknown mass spectra

The mass spectrometer was of the electron impact type and molecules entering it are bombarded with high-energy electrons (70eV). The energy is sufficiently high to bring about the loss of an electron from the molecule and the cleavage of certain chemical bonds. As a consequence, charged moieties (ions) are produced. The fragment ions are separated based on their mass to charge ratio (m/z) by a quadrupole and their intensity is recorded. Plotting the intensity as a function of the m/z ratio creates mass spectra. The mass spectra were used for the structural elucidation of unknown compounds.

3.2.1 Identification of furanic ethers

Most important fragment ions in the mass spectrum of chromatogram peak 48 (Figure 1) are those with m/z 81 and 82. These mass spectral peaks suggest the compound is most likely a 2-substituted furan derivate with a side chain of n-propyl or longer. Indeed, for these kinds of molecule, β-cleavage is a dominant fragmentation process producing a resonance-stabilized carbocation with m/z=81. McLafferty rearrangement also easily takes place leading to an ion with m/z=82. Probably, γ–scission is responsible for the ion with m/z 97. Furan derivates often show relatively intense peaks of the molecular ion due to their stability and therefore the molecular weight of the molecule equals most likely 126. These findings suggested the spectrum was that of furfuryl ethyl ether (FEE). This was confirmed by comparing the retention index and the mass spectrum with those obtained from a reference solution of FEE (Figure 1B).

Interpretation of the mass spectrum of chromatogram peak 16 (Figure 2) was less straightforward. From the spectrum it seems the molecule has a molecular mass of 114. Most important fragment ions have an m/z of 99 and 71. The spectrum was linked with the ether 2-ethoxy-2,5-dihydro-furan. Homolytic side chain cleavage of this molecule at the α, β and γ positions indeed produces fragment ions with m/z of respectively 69, 85 and 99. In the formation of the molecular ion, the double bond of 2-ethoxy-2,5-dihydro-furan is also a preferential site for an electron abstraction in addition to the two oxygen atoms. Starting from such a molecular ion, ring opening followed by heterolytic bond cleavage is a plausible mechanism in the formation of a m/z 71 fragment ion. No reference compound or literature citation of this molecule was available and therefore it was not possible to further confirm the identification. Nonetheless, kinetic behaviour during beer aging also suggests this compound arises from an etherification reaction (10-12). 2-ethoxy-2,5-dihydro-furan was for the first time detected in beer.

Based on the mass spectrum (Figure 3), the compound responsible for chromatogram peak 103 has most likely a molecular mass of 154. Major mass spectral peaks arose at m/z 125, 109 and 97. Interpretation of the mass spectrum suggested this mass spectrum belongs to 5-(ethoxymethyl)-2-furfural. Indeed, cleavage at the β-position and γ-position of this molecule results in fragment ions with m/z of respectively 109 and 125. The formation of an ion with m/z 97 is somewhat unclear. Possibly, it proceeds through a ring opening mechanism. The identification of the compound was confirmed by comparison of its Kovats' retention index (RI = 1932) with that cited by Cutzach et al. (13) obtained for a reference solution of 5-(ethoxymethyl)-2-furfural (RI = 1940). The mass spectrum also corresponds with the mass spectral data cited by Simpson (14) for this compound. This furanic ether was also for the first time detected in beer.

3.2.2 Identification of dioxolanes

Chromatogram peaks 8, 22, 33 and 34 all had a major mass spectral peak at m/z 101. This indicated that these compounds all were dimethyl-dioxolanes. Abstraction of an electron in this kind of molecules (Figure 4) preferentially occurs at an oxygen atom producing an unstable molecular ion. Hence, the molecular ion peak is very weak in the mass spectrum. Stable fragment ions arise by cleavage of a hydrogen radical or an alkyl radical from a carbon atom adjacent to the radical oxygen atom. This produces ions with an m/z equalling the molecular weight minus one and an m/z of 101, respectively. Consequently, the mass spectrum in Figure 4 is that of 2,4,5-trimethyl-1,3-dioxolane. The identification of these dioxolanes was confirmed by comparison of the mass spectra with the mass spectral data cited by Peppard and Halsey (15) for reference solutions of dioxolanes.

3.2.3 Identification of ethyl esters

Ethyl esters with an acid fragment of C3 or longer can easily be recognized by their characteristic mass spectral peak at m/z 88. The fragment ion with m/z 88 is formed in a McLafferty rearrangement (Figure 5), which consists in a transfer of a γ hydrogen atom followed by a heterolytic cleavage (16). It is a very prominent degradation mechanism for these esters. Chromatogram peaks 48 and 57 have a m/z 88 base peak and are therefore most likely ethyl esters.

3.3 Chromatographic profiles of fresh versus aged beer

In total 104 compounds were identified from the chromatograms of fresh and aged beer. They belonged to following chemical classes: acids (6), alcohols (17), carbonyl compounds (20), dioxolanes (4), ethyl esters (25), acetate esters (10), S-esters (2), methyl esters (1), ethers (5), heterocyclic compounds (10), terpenes (3) and alkenes (1). Most prominent peaks (Figure 6) were those of ethyl acetate, ethanol, ethyl butanoate, propanol, isobutanol, 2-methyl-butyl acetate, 3-methyl-butyl acetate, 2-methyl-butanol, 3-methyl-butanol, ethyl hexanoate, ethyl octanoate, 2-phenylethyl acetate and 2-phenylethanol. Overall, the analysis technique had best sensitivity for relatively apolar compounds like ethyl esters, carbonyl compounds, terpenes and ethers. The sensitivity seemed largely determined by the extraction efficiency of the volatiles from the beer matrix in the purge and trap concentrator. More polar compounds as alcohols and acids were retained more in the beer matrix, which resulted in reduced sensitivity towards these compounds.

Comparison of the chromatographic profiles of fresh versus aged beer revealed some marked differences. The largest part of the compounds identified, was present in different concentrations in aged and fresh beer. A concentration increase was observed for certain ethyl esters, aldehydes, ethers, acids and heterocyclic compounds. In particular, some esters were present in lower concentrations in aged beer. On the other hand, most higher alcohols and some linear aldehydes and organic acids did not show a significant concentration difference between the two beers.

4 Conclusion

Purge and Trap-Gas Chromatography-Mass Spectrometry was very suitable in the analysis of staling compounds in beer. Data obtained from the analysis of a top-fermented beer successfully allowed identification of 104 compounds of which the largest part changed in concentration during storage. The evolution of several classes of volatiles could be followed simultaneously in beer, which is very beneficial in evaluating the occurrence of different processes during aging. Some furanic ethers were for the first time detected in beer and were present in higher concentrations in the aged beer

For most compounds, the analysis method had good reproducibility and was sensitive in µg/L range. Compared to analysis techniques based on solvent extraction and derivatisation procedures, the P&T-GC-MS technique is relatively rapid (1h / sample) and requires little manual sample preparation. Furthermore, volatiles were extracted in the purge and trap unit directly from the beer matrix without sample modification, avoiding artifact formation or thermal decomposition.

The high ethanol concentration of beer initially gave some difficulties due to saturation of trap and GC-column with it. Increasing the trap temperature effectively resolved the problem, but also compromised sensitivity and reproducibility of compounds such acetaldehyde and ethyl acetate, which eluted before ethanol. Moreover, the technique was also not suited for compounds very abundant in beer such as isoamyl acetate and isoamyl alcohol, since their peak intensity reached the upper sensitivity limited of the MS detector. A static headspace technique is more appropriate for their measurement in beer.

With the characterization of volatile compounds present in beer and the optimisation of the P&T-GC-MS system, the behaviour of volatiles during beer aging can be monitored. This can give substantial information about the aging processes taking place under different storage conditions.

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Appendix

Table 1 **Identification of compounds in fresh and aged beer together with their behaviour during beer aging and their flavour properties.**

Peek No.	RI[a]	Molecule	Identification[b]			Beer Aging[c]	Flavour description	Ref.[d]
			LIB	RI	RC			
1	879	2-methyl-furan	+	+	-	+		
2	895	ethyl acetate	+	+	+	-	light, estery, fruity, solvent-like	I
3	913	isoamyl vinyl ether	+	-	-	+		
4	920	amyl vinyl ether	+	-	-	+		
5	928	2-methyl-butanal	+	+	+	+	green grass, fruity, sour, iodoform	I
6	933	3-methyl-butanal	+	+	+	+	unripe banana, apple, cherry, cheese	I
7	941	ethanol	+	+	+	0	alcoholic, strong	I
8	958	2,4,5-trimethyl-1,3-dioxolane	-	-	-	+	phenolic, astrigent	III
9	970	2-ethyl furan	+	+	-	+	powerful, sweet, ethereal, burnt	V
10	971	ethyl propionate	+	+	+	+		
11	978	ethyl isobutyrate	+	+	+	+	apple, sweet	I
12	985	ethyl acrolein	+	-	-	0		
13	990	propyl acetate	+	+	+	-	solvent-like, sweet, perfume	I
14	992	diacetyl	+	+	+	+	diacetyl, butterscotch	I
15	1011	3-methyl-3-buten-2-one	+	-	-	+		
16	1025	2-ethoxy-2,5-dihydro-furan	-	-	-	+		
17	1026	4-methyl-pentan-2-one	+	+	+	+		I
18	1031	isobutyl acetate	+	+	+	-	banana, sweet, fruity	I
19	1045	thiophene	+	-	-	+		
20	1054	propanol	+	+	+	0	alcoholic	I
21	1057	ethyl butanoate	+	+	+	-	papaya, butter, apple, perfumed	I
22	1063	2-isopropyl-4,5 dimethyl-1,3-dioxolane	-	-	-	+		
23	1071	ethyl 2-methyl-butyrate	+	+	+	+	sweet, fruity, grape-like, candy	I
24	1073	S-methyl-thioacetate	+	-	-	-		
25	1091	butyl acetate	+	+	+	-	solvent-like, banana, acetone, sweet	I
26	1092	ethyl 3-methyl-butyrate	+	+	+	+	fruity, grape-like, sweet, isovaleric	I
27	1106	hexanal	+	+	+	0	bitter, vinous, aldehyde	I
28	1109	isobutanol	+	+	+	0	alcoholic	I
29	1125	2-methyl-2-butenal	+	+	+	0	green fruit	V
30	1148	isoamyl acetate	+	+	+	-	banana, estery, solvent-like, apple	I
31	1156	ethyl pentanoate	+	+	+	-	papaya, fruity, apple, sweet	I
32	1158	1-butanol	+	+	+	0	alcoholic	I
33	1171	2-isobutyl-4,5-dimethyl-1,3-dioxolane	-	-	-	+		
34	1175	2-sec butyl-4,5-dimethyl-1,3-dioxolane	-	-	-	+		
35	1182	myrcene	+	+	+	0	sweet, balsamic, plastic	I
36	1191	pentyl acetate	+	+	+	-		
37	1218	isoamyl + act. amyl alcohol	+	+	+	0	alcoholic, vinous, banana, sweet	I
38	1252	ethyl hexanoate	+	+	+	-	apple, fruity, aniseed, sweet	I
39	1259	ethyl tiglate	+	-	-	-	fruity, caramel	V
40	1260	1-pentanol	+	+	+	0	alcoholic, iodoform	I
41	1263	3-methyl-3-buten-1-ol	+	-	-	0		
42	1272	thiazole	+	+	+	+	sulfury, grainy, carbon sulfide	II
43	1276	methyl 2-ethyl-hexenoate	+	-	-	0		
44	1282	styrene	+	+	-	-	plastic	V
45	1285	ethyl pyruvate	+	+	+	+	vegetable, caramel	V
46	1288	hexyl acetate	+	+	+	-	sweet, aromatic, perfumed	
47	1300	ethyl ester?	-	-	-	-		
48	1302	furfuryl ethyl ether	-	-	+	+	solvent, alcoholic, stale	
49	1308	octanal	+	+	+	0	orange, bitter, aldehyde	I
50	1316	ethyl (*Z*)-3-hexenoate	+	-	-	+		
51	1325	2-heptanol	+	+	+	0		
52	1330	ethyl (*E*)-3-hexenoate	+	-	-	+		
53	1348	ethyl heptanoate	+	+	+	-	fruity, perfumed, caprylic	I
54	1356	ethyl lactate	+	+	+	+	artificial raspberry	I
55	1360	1-hexanol	+	+	+	0	coconut, green leaves, unpleasant	I
56	1387	heptyl acetate	+	+	+	-	pear, fruity, aromatic, sweet	I
57	1398	ethyl ester?	-	-	-	-		

58	1406	2-nonanone	+	+	-	0	ketone, varnish	I
59	1413	nonanal	+	+	+	0	astringent, bitter, aldehyde	I
60	1453	(*E*)-2-octenal	+	+	-	0	bitter, aldehyde, stale	II
61	1460	ethyl octanoate	+	+	+	-	apple, sweet, fruity	I
62	1462	1-heptanol	+	+	-	0	coconut, unpleasant, ketonic solvent	I
63	1470	acetic acid	+	+	+	0	acetic, acidic, vinegar	I
64	1485	furfural	+	+	+	+	papery, husk-like	I
65	1487	octyl acetate	+	+	+	-	coconut, vegetable oil, aromatic	I
66	1495	2-ethyl-1-hexanol	+	-	-	0	mild, oily, sweet, slight rose	V
67	1510	2-decanone	+	+	-	0	ketone, floral, sweet	I
68	1513	decanal	+	+	+	0	bitter, aldehyde, orange peel	I
69	1528	2-acetyl furan	+	+	+	+	papery, nutty, almonds	I
70	1537	S-methyl-thiooctanoate	+	-	-	0		
71	1549	ethyl nonanoate	+	+	+	-	slightly fatty, oily, nutty, fruity	V
72	1553	linalool	+	+	+	0	citrus, floral, aniseed, terpenoid	I
73	1554	benzaldehyde	+	+	+	+	bitter almonds	I
74	1558	(*E*)-2-nonenal	+	+	+	0	papery, cardboard	I
75	1559	α-ionone	+	+	-	+	artificial raspberry, violets, woody	I
76	1561	β-ionone	+	+	-	+	artificial raspberry, cedarwood, violets	I
77	1564	1-octanol	+	+	-	0	coconut, walnut, oily	I
78	1597	5-methyl-2-fufural	+	+	+	+	spicy (vanilla, almond)	I
79	1618	isobutyric acid	+	+	-	+	sweaty, butyric, bitter	I
80	1618	4-cyclopentene-1,3-dione	+	-	-	+		
81	1639	butanoic acid	+	+	-	0	butyric, butter, cheesy	I
82	1641	5,5-dimethyl-2(5H)-furanone	+	-	-	+		
83	1641	ethyl 2-furoate	+	+	-	+	fruity, floral	V
84	1650	ethyl decanoate	+	+	+	-	caprylic, fruity, apple, solvent	I
85	1655	L-(-)-menthol	+	+	-	0	minty, woody	V
86	1665	1-nonanol	+	+	-	0	coconut, walnut, oily	I
87	1672	phenylacetaldehyde	+	+	+	+	hyacinth, lilac, aldehyde	I
88	1675	furfuryl alcohol	+	+	+	+	sugarcane, woody	I
89	1685	isovaleric acid	+	+	-	+	cheese, old hops, sweaty	I
90	1687	diethyl succinate	+	+	+	+		
91	1692	ethyl benzoate	+	+	-	0	heavy, floral, fruity	V
92	1704	ethyl 9-decenoate	+	+	-	-		
93	1721	linalyl acetate	+	+	-	0	sweet, floral, fruity, bergamot	V
94	1753	3-methylthio-1-propanol	+	+	-	0		
95	1797	1-decanol	+	+	-	0	coconut, walnut, oily	I
96	1836	ethyl phenylacetate	+	+	+	+	sweet, honey	V
97	1861	phenylethyl acetate	+	+	+	0	roses, honey, apple sweet	I
98	1864	ethyl nicotinate	+	+	+	+	grainy, slightly papery, perfumed	I
99	1868	damascenone	+	+	-	+	blackcurrant, raspberry, menthol	I
100	1870	hexanoic acid	+	+	-	0	caprylic, vegetable oil, sweaty	I
101	1897	benzyl alcohol	+	+	-	0	almond, bitter	I
102	1925	phenylethyl alcohol	+	+	+	0	roses, bitter, perfumed	I
103	1932	2-ethoxymethyl-5-furfural	-	+	-	+	nutty, cinnamon	IV
104	2018	octanoic acid	+	+	-	0	caprylic	I

a : RI, Kovats' Retention Index on CP-WAX column. b : Identification was possible (+) or not (-) with mass spectral library (LIB), Kovats' retention index (RI) or reference compound (RC). c : Appearance in aged beer compared to fresh beer: increased (+), decreased (-) or unchanged (0). d : Flavour description of component according to I Moll (17), II Meilgaard (18), III Peppard and Halsey (15), IV Cutzach et al. (19), V the supplier of the reference compound.

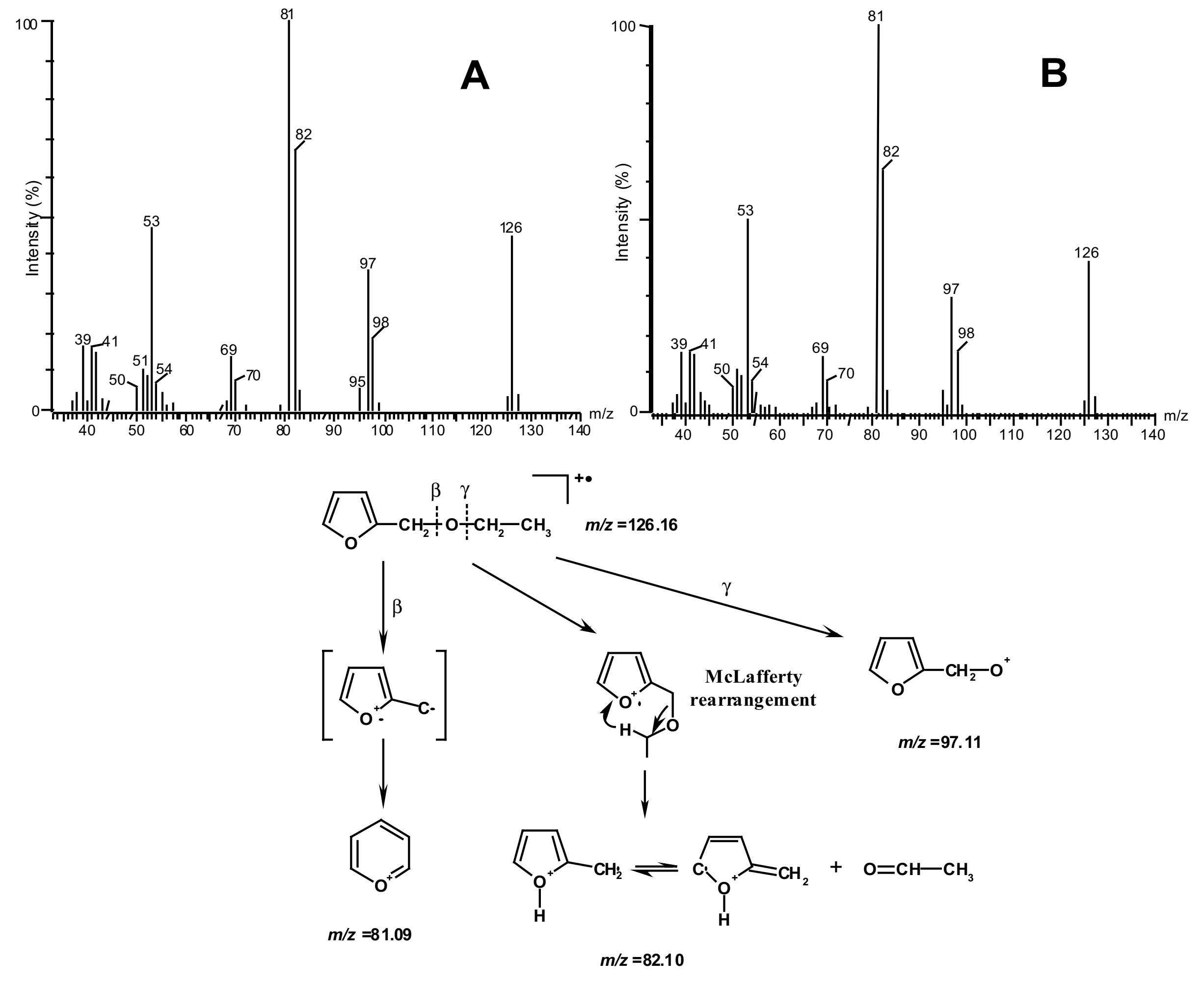

Fig. 1 Mass spectra of chromatogram peak 48 (A) and of a reference solution of furfuryl ethyl ether (B) together with the proposed fragmentation pattern of furfuryl ethyl ether.

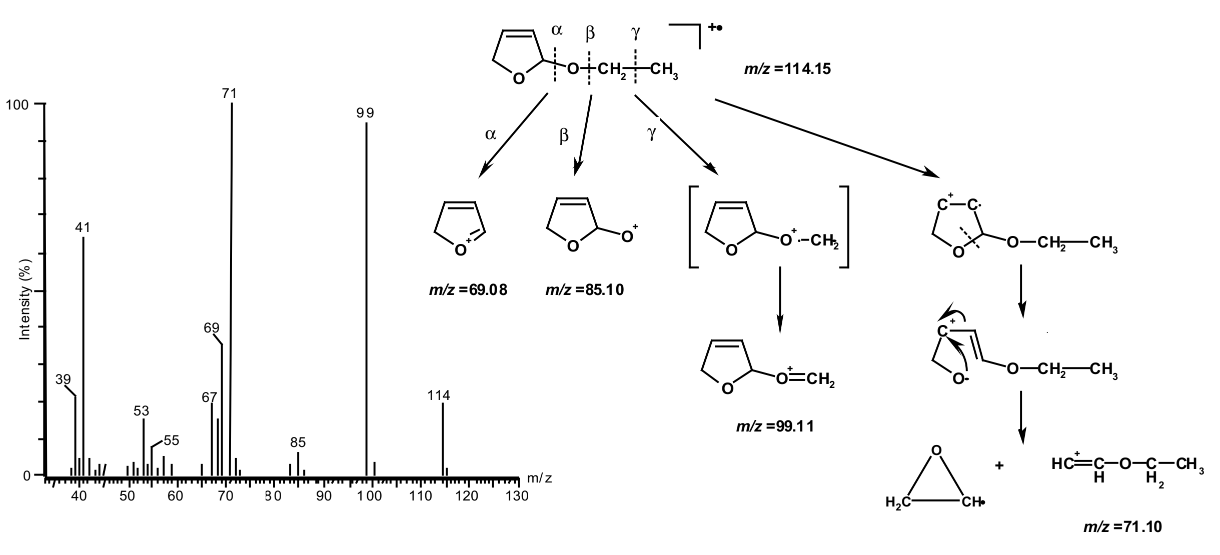

Fig. 2 Mass spectrum of chromatogram peak 16 and the proposed fragmentation pattern of 2-ethoxy-2,5-dihydro-furan.

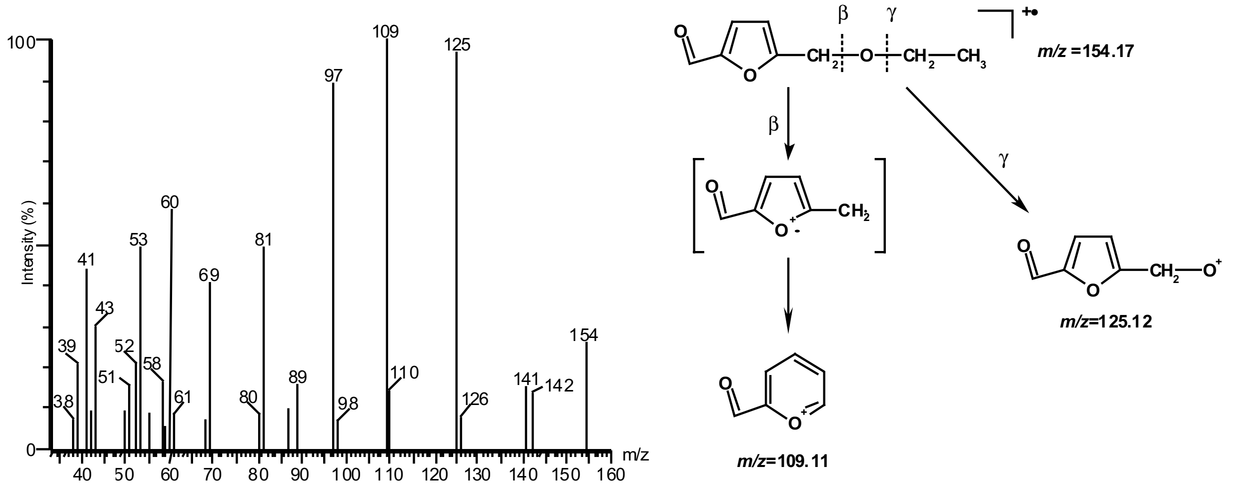

Fig. 3 Mass spectrum of chromatogram peak 103 and the proposed fragmentation pattern of 5-(ethoxymethyl)-2-furfural.

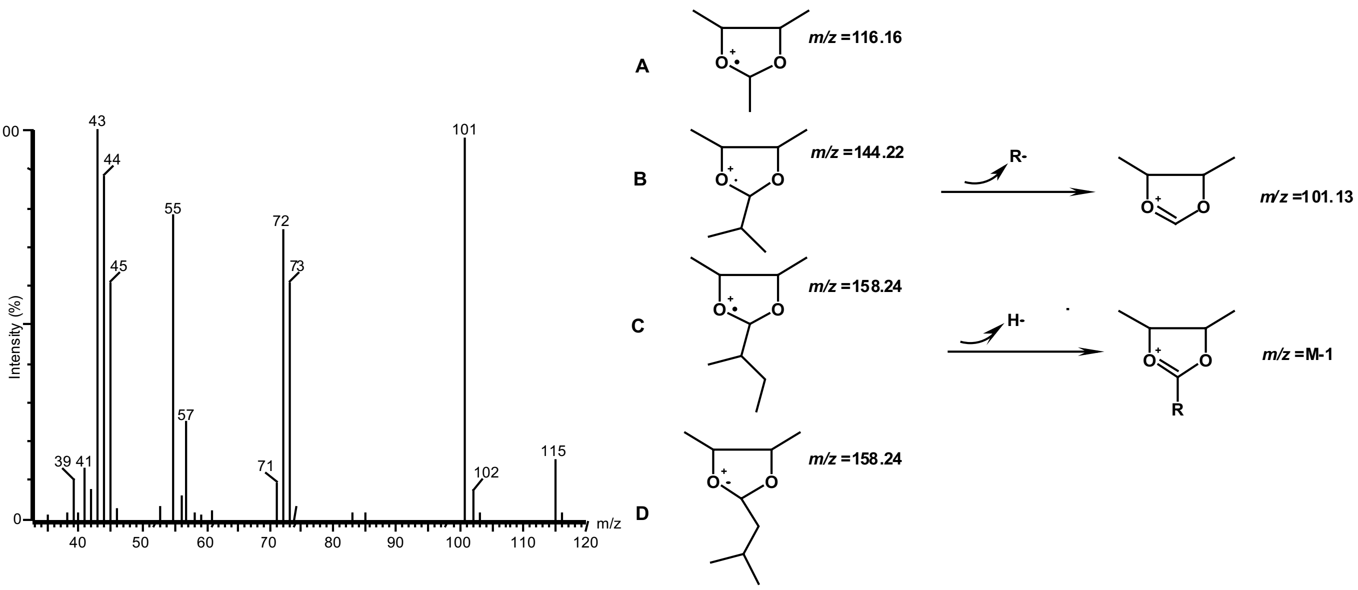

Fig. 4 Mass spectrum of chromatogram peak 8 and the proposed fragmentation pattern of 2,4,5-trimethyl-1,3-dioxolane (A) 2-isopropyl-4,5-dimethyl-1,3-dioxolane (B) 2-sec butyl-4,5-dimethyl-1,3-dioxolane (C) and 2-isobutyl-4,5-dimethyl-1,3-dioxolane (D).

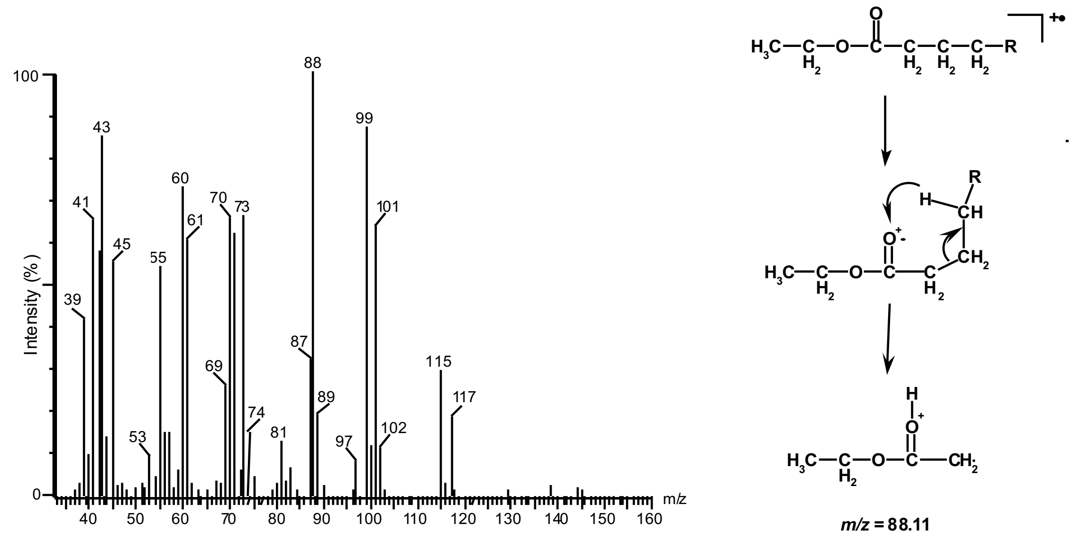

Fig. 5 Mass spectrum of ethyl hexanoate and McLafferty rearrangement of ethyl esters.

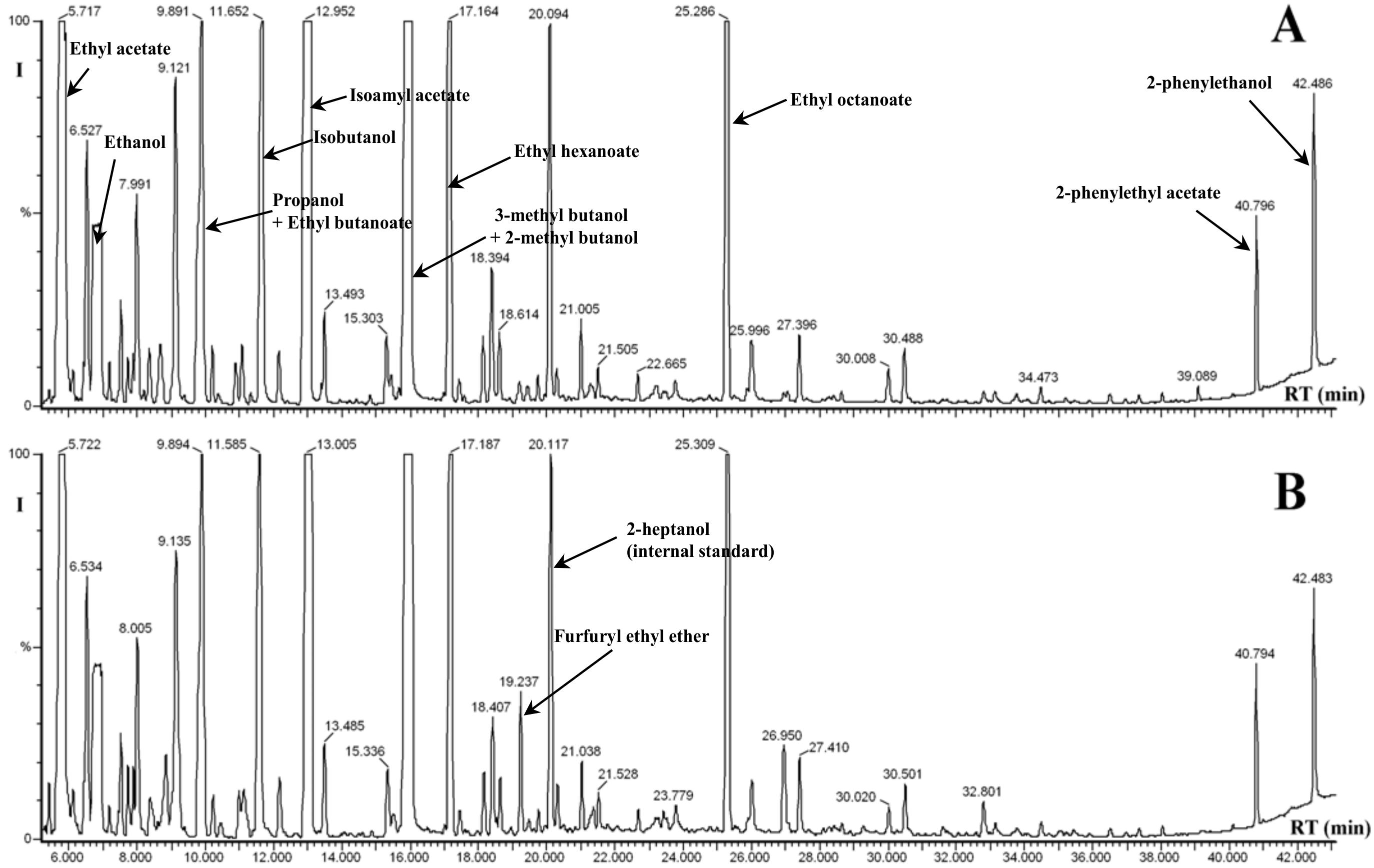

Fig. 6 Total ion chromatograms of fresh (A) and aged (B) beer.