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Characterization of Volatiles in Unhopped Wort

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The volatile fraction of wort components was studied during boiling. Not less than 118 volatile compounds were identified when unhopped pilsner wort was boiled and samples of wort and condensed vapors were analyzed with headspace SPME-GC/MS, of which 54 were confirmed with reference compounds. The wort samples contained 61 identifiable compounds, while the vapor condensate yielded 108 different compounds. Almost 30 known compounds were found that have not been described before in unhopped pilsner wort. One previously unknown aldol reaction product was tentatively identified as 2-phenyl-2-octenal. The detection of branched 2-alkenals underlines the importance of the aldol condensation in Maillard-type reactions, while the tentative identification of alkyloxazoles and alkylthiazoles could once more accentuate the central role of α-dicarbonyl compounds, aldehydes, and amino acids in flavor generation. The condensation of wort vapors joined with the SPME-GC/MS technique has proven to be a useful tool in volatile analysis.

KEYWORDS: Wort boiling; volatiles; evaporation; condensate; aldehydes; ketones; 2-alkenals; 2-phenyl-2-octenal; oxazoles; thiazoles; furans; Maillard reaction; flavor compounds

INTRODUCTION

In the world of brewing technology, no topic is more controversial than wort boiling. The reason for this is simple: wort is a complex mixture of sugars, amino acids, lipids, proteins, and polyphenols. Hundreds of reactions occur simultaneously, such as Maillard reactions, lipid oxidation reactions, the thermal decomposition of *S*-methylmethionine to dimethyl sulfide, and the isomerization of hop α-acids into iso-α-acids. Unfortunately, it is not yet known to which extent every single reaction accounts for the quality of the final product and the flavor stability of the beer during storage.

Brewing researchers agree that the reduction of thermal load during wort boiling improves beer flavor stability (*1, 2*). Apart from that, the evaporation of dimethyl sulfide is often the sole quality criterion used by suppliers to prove the value of their wort boiling system. Progress in this field can only be made through a more profound knowledge of the presence of the volatiles in wort and the chemical background of these compounds. In the past, analysis of volatiles has already been carried out on malt extract (*3*), ale malt (*4*), caramel malts (*5*),

roasted malts (*6*), ale wort (*7*), and pilsner-type beer (*8, 9*). Other papers focused primarily on odor-active compounds of hops in beer (*10*) or described new techniques to identify more than 120 different compounds in hop oil, while probably over 1000 compounds are still waiting to be discovered in the hop oil fraction (*11*).

The presence of these abundant hop volatiles in wort could impede a proper detection and identification of the malt-derived and boiling process related volatiles. Therefore, it was decided to investigate the flavor profile of unhopped pilsner wort together with the condensate of the vapors generated while boiling. The advantage of the analysis of vapor condensates is obvious: volatiles are generally enriched in the vapor phase, depending on the volatility of the particular compound. If this flavor-rich mixture is combined with a very sensitive analysis technique, such as solid-phase microextraction (SPME) in combination with GC/MS (*12, 13*), a powerful identification tool is created. The increased sensitivity offers opportunities for the detection of new compounds in wort and, consequently, for unraveling new reaction pathways that may occur during boiling. As the volatility of every volatile compound is determined and expressed by its vapor–liquid equilibrium (*14*), there must be a strict correlation between the presence of a compound in the condensate and in the wort. Variations in wort will therefore be reflected in the vapor condensate.

The purpose of the present study is the identification of a broad range of volatiles in order to obtain a better understanding

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of the chemical background of unhopped wort. A crucial factor in this identification is obtaining an increased sensitivity for the detection of novel compounds. Therefore, the SPME GC/MS technique is combined with the analysis of wort vapor condensate, a combination that should greatly enhance the analysis sensitivity.

MATERIALS AND METHODS

Chemicals. Methyl isobutyl ketone RECTAPUR was bought from VWR International (West Chester, PA). The compounds 2-pentylfuran (98%) and (*E*,*E*)-2,4-decadienal (90%) were obtained from Alfa Aesar GmbH (Karlsruhe, Germany). All other chemicals were acquired from Sigma-Aldrich (St. Louis, MO): isobutyraldehyde, 2-phenyl-2-butenal, (*E*)-2-nonenal, 5-hydroxymethyl-2-furfural (HMF), decanal, 5-methyl-2-furfural, 3-methylthiopropionaldehyde, nonanal, (*E*)-2-hexenal, 3-methylbutanal, dimethyl trisulfide (DMTS), 2-ethyl-3,5- (or 3,6-) dimethylpyrazine, 5-methyl-2-phenyl-2-hexenal, 4-methyl-2-phenyl-2-pentenal, 2-acetylfuran, 2-isopropyl-5-methyl-2-hexenal, 2-methylpyrazine, 2,3-diethyl-5-methylpyrazine, phenylacetaldehyde, α-linalool, diacetyl, 2,3-pentanedione, β-myrcene, dimethyl sulfide, β-damascenone, 4-vinylguaiacol, hexanal, 2-heptanol, phenylethyl alcohol, 3-methylbutanol, 1-hexanol, isovaleric acid, 2-furfural, heptanal, propanal, furfuryl alcohol, pentanal, benzaldehyde, 2-methylbutanal, octanal, butanal, isoamyl acetate, and γ-nonalactone.

Wort Production. Wort production was performed in the pilot scale (5 hl) brewery of the Centre for Malting and Brewing Science (Heverlee, Belgium). An amount of 80 kg of milled pilsner malt (Dingemans, Stabroek, Belgium) was added to 220 L of water at 55 °C. The pH was adjusted to 5.5 with lactic acid, and the mash was subjected to a rest of 10 min. Next, the temperature was raised to 63 °C (35 min of rest) and the saccharification temperature of 72 °C (20 min). After the saccharification rest, the mash was heated to 78 °C and subsequently transferred to the lauter tun, in which the separation of spent grains and sweet wort takes place. The filtered sweet wort was collected in the boiling kettle, where it was heated up to 90 °C. At 90 °C, the wort was taken in 1.5 L containers and immediately cooled to room temperature. Hereafter, the containers with sweet wort were frozen at −25 °C. Prior to the boiling experiments, the wort was defrosted; the density was adjusted to 12 °P, and the pH was adjusted to 5.2 with sulfuric acid.

Laboratory-Scale Boiling. The boiling device consisted of a 6 L stainless steel vessel, equipped with a wort sample valve, a paddle stirrer, and a temperature sensor, which was immersed in the wort. A glass cover was used with inlets for the paddle stirrer, the temperature sensor, and an outlet chimney for wort vapors. A Liebig cooler (cooling water at 2 °C) was placed on the outlet in order to condense the produced vapors. This condensate was collected in 50 mL tubes on ice. The heating device consisted of a stainless steel plate with an integrated temperature sensor, and feed-back temperature regulation was performed with the immersed temperature sensor. While boiling, the heating plate temperature was kept constant to guarantee uniform heating during the boiling experiment, while continuous stirring ensured a homogeneous heat distribution.

Sample Preparation and Solid-Phase Microextraction (SPME). A 20 mL headspace vial was filled with a 10 mL aliquot of wort or vapor condensate, together with 3.5 g of NaCl (analytical grade) and 50 μL of a 200 ppm 2-heptanol solution, used as an internal standard. The vial was placed in the tray of the CombiPAL (CTC Analytics, Zwingen, Switzerland), where it was preincubated at 40 °C for 10 min. Hereafter, a carboxen−polydimethylsiloxane−divinylbenzene fiber (CAR-PDMS-DVB; Supelco, Bellefonte, PA) was used for extraction of volatiles in the headspace of the vial during 20 min at 40 °C.

GC/MS Analysis. The injection of the CAR-PDMS-DVB fiber was performed in a split/splitless injector of a Trace GC Ultra (Thermo, Waltham, MA), and desorption was executed for 2 min at 250 °C in split mode (with a split ratio of 8). The used columns were of the type RTX 200MS (30 m × 0.25 mm i.d., 0.5 μm df) and of the type RTX 5SilMS (60 m × 0.25 mm i.d., 1.0 μm df), both from Restek (Bellefonte, PA). A constant flow of 1.5 mL of He/min was applied in both cases. The following temperature program was used with the RTX

200MS column: 30 °C for 2 min, followed by a 3 °C/min increase to 120 °C, then a 10 °C/min rise to 200 °C, and finally with a 30 °C/min rise to 260 °C with a 1 min hold. The following temperature program was used with the RTX 5SilMS column: 30 °C for 2 min, then a rise to 70 °C at a rate of 10 °C/min with 1 min hold, followed by a rise to 200 °C at a rate of 4 °C/min, and finally a rise to 270 °C at a rate of 20 °C/min with a 2 min hold. After separation, the volatiles were analyzed with a dual stage quadrupole (DSQ) MS (Thermo, Waltham, MA), which was set to detect positive ions with a mass to charge ratio (*m*/*z*) of 33–260 in the electron impact mode. The data were analyzed using Xcalibur software (Thermo, Waltham, MA).

Identification of Volatiles. The identification of wort volatiles was performed by GC-MS spectral analysis, and their Kovat's retention indices were calculated for a RTX 200MS column and a RTX 5MS column. Where possible, chemical standards were injected and compared with identified compounds.

Aldol Condensation Reactions. Deionized water was supplemented with 500 ppb of each reagent. The pH was adjusted to pH 5.2 with lactic acid, and 20 mL of the solution was incubated in sealed Pyrex tubes for 1 h in a water bath at 100 °C (in duplicate). After incubation, the samples were cooled on ice and prepared for SPME-GC/MS analysis as described above.

RESULTS AND DISCUSSION

Headspace SPME-GC/MS is a very sensitive technique for trace compound analysis in the headspace of beverages, without the need for preliminary extraction and, consequently, without the risk of losing information with time-consuming extraction procedures (*13*). The only consideration that has to be made is the choice of the appropriate fiber. In this case, a three-phase CAR-PDMS-DVB fiber was chosen in order to obtain the broadest extraction spectrum of volatiles.

When wort is boiled, an important concern of the brewer is the quality of the wort after boiling. The evaporated fraction of the wort is of minor importance, except for energy recuperation by condensation. However, wort vapors contain high levels of volatiles due to the higher volatility of those compounds in comparison with the volatility of water. Therefore, the condensed vapors contain volatiles in levels that are related of the levels of those volatiles in the remaining wort. Hertel and co-workers have determined the vapor–liquid equilibrium of dimethyl sulfide as 75.6, whereas the vapor–liquid equilibrium of phenylacetaldehyde is no more than 5.6 (*14*). Hence, analyzing the condensed vapor fraction of the wort reveals more information than analysis of the same wort. While only 4 peaks have an intensity of more than 10^7 in wort (chromatogram A), there are more than 20 peaks with the same intensity in the condensate (chromatogram B). Therefore, it was chosen to take samples from pilsner wort during boiling, as well as from the vapors produced during the same wort boiling. The vapors were collected in subsequent fractions, the first condensate fraction containing the highest total concentration of volatiles as a mirror of the high content of volatiles in the unboiled wort.

The volatiles that were identified in the wort and vapor condensates are shown in **Table 1**. In the unhopped wort, it was possible to identify 61 volatile compounds: 6 sulfur compounds, 10 furans, 11 linear aldehydes, 2 esters, 14 ketones, 8 alcohols, 4 branched 2-alkenals, 2 pyrazines, a terpenoid compound, an acid, a lactone, and a phenolic compound. On the other hand, analysis of the vapor condensate revealed 114 identifiable compounds: 6 sulfur compounds, 14 furans, 13 linear aldehydes, 5 esters, 18 ketones, 14 alcohols, 19 2-alkenals, 13 pyrazines, 5 terpenoids, 2 oxazoles, 3 thiazoles, 1 lactone, and a phenolic compound. Some of these compounds have never been reported before as part of the volatile fraction of unhopped

Table 1. Volatile Compounds Identified in Wort and Vapor Condensate

| no. | compound | Kovat's RI | | detection[a] | | identification[b] |
		RTX 200MS	RTX 5SilMS	wort	cond	
	sulfur compounds					
1	methanethiol	<500	463	*	*	MS, RI
2	dimethyl sulfide	550	519	*	*	MS, RI, RC
3	thiophene	795	639	*		MS, RI
4	dimethyl disulfide	949	768	*	*	MS, RI
5	dimethyl trisulfide	1207	974	*	*	MS, RI, RC
6	3-(methylthio)propionaldehyde (methional)	1303	913	*	*	MS, RI, RC
7	dimethyl tetrasulfide	1465	1236		*	MS, RI
	furans					
8	furan	504	522	*	*	MS, RI
9	2-methylfuran	714	608	*	*	MS, RI, RC
10	2-ethylfuran	832	705	*	*	MS, RI, RC
11	2-vinylfuran	921	765	*	*	MS, RI
12	2-butylfuran	1111	896		*	MS, RI
13	furfuryl alcohol	1154	859	*	*	MS, RI, RC
14	2-pentylfuran	1219	996	*	*	MS, RI, RC
15	2-furfural	1226	841	*	*	MS, RI, RC
16	2-(2-pentenyl)furan[d]	1233	1003		*	MS
17	acetylfuran	1317	924	*	*	MS, RI, RC
18	5-methyl-2-furfural	1392	978	*	*	MS, RI, RC
19	3-phenylfuran	1523	1224		*	MS, RI
20	2-hexanoylfuran[d]	1650	1283		*	MS
21	5-hydroxymethyl-2-furfural	1679	1295	*	*	MS, RI, RC
	alkanals and Strecker aldehydes					
22	propanal	701	512	*	*	MS, RI, RC
23	2-methylpropanal	767	557	*	*	MS, RI, RC
24	butanal	823	604	*	*	MS, RI, RC
25	3-methylbutanal	947	664	*	*	MS, RI, RC
26	2-methylbutanal	952	668	*	*	MS, RI, RC
27	pentanal	1009	695	*	*	MS, RI, RC
28	hexanal	1133	806	*	*	MS, RI, RC
29	heptanal	1249	902	*	*	MS, RI, RC
30	benzaldehyde	1335	972	*	*	MS, RI, RC
31	octanal	1358	1006		*	MS, RI, RC
32	phenylacetaldehyde	1429	1049	*	*	MS, RI, RC
33	nonanal	1453	1108	*	*	MS, RI, RC
34	decanal	1546	1212		*	MS, RI, RC
	esters					
35	methyl acetate	721	528		*	MS, RI
36	vinyl acetate	763	574	*	*	MS, RI
37	isoamyl acetate	1202	879		*	MS, RI, RC
38	methyl geranate	1608	1329		*	MS, RI
39	isopropyl laurate[d]	1802	1603	*	*	MS
	ketones					
40	acetone	736	483	*	*	MS, RI, RC
41	2-butanone	886	612	*	*	MS, RI, RC
42	diacetyl	896	604	*	*	MS, RI, RC
43	2-pentanone	1024	698	*	*	MS, RI
44	2,3-pentanedione	1038	701	*	*	MS, RI, RC
45	methyl isobutyl ketone	1091	732	*	*	MS, RI, RC
46	3-penten-2-one	1122	738		*	MS, RI
47	5-methyl-3-hexen-2-one	1309	906		*	MS, RI
48	4-cyclopentene-1,3-dione[d]	1348	920	*	*	MS
49	6-methyl-5-hepten-2-one	1356	987	*	*	MS, RI
50	2-octanone	1369	998	*	*	MS, RI
51	3-octen-2-one	1446	1044	*	*	MS, RI
52	acetophenone	1459	1078	*	*	MS, RI
53	2-nonanone	1463	1096	*	*	MS, RI
54	3,5-octadien-2-one	1472	1092		*	MS, RI
56	(*E*,*E*)-3,5-octadien-2-one	1501	1088	*	*	MS, RI
57	β-damascenone	1722	1398	*	*	MS, RI, RC
58	3-hydroxy-β-damascone[d]	1727	1630		*	MS
	alcohols					
59	1-penten-3-ol	843	689	*	*	MS, RI
60	3-methylbutanol	958	744	*	*	MS, RI, RC
61	2-methyl-2-buten-1-ol	982	749		*	MS, RI
62	1-pentanol	1006	769	*	*	MS, RI, RC
63	*cis*-2-pentenol	1016	766		*	MS, RI
64	3-hexen-1-ol	1117	852		*	MS, RI
65	1-hexanol	1128	868	*	*	DE, RI, RC
66	1-octen-3-ol	1240	979	*	*	MS, RI
67	2-ethylhexanol	1300	1036	*	*	MS, RI
68	1-octanol	1348	1083		*	MS, RI

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Table 1. (continued)

no.	compound	Kovat's RI		detection[a]		identification[b]
		RTX 200MS	RTX 5SilMS	wort	cond	
69	*cis*-2-octenol	1351	1064		*	MS, RI
70	phenylethyl alcohol	1433	1112	*	*	MS, RI, RC
71	1-nonanol	1442	1163	*	*	MS, RI
72	1-decanol	1533	1279		*	MS, RI
	2-alkenals					
73	2-butenal	1004	648		*	MS, RI
74	(*E*)-2-hexenal	1247	858		*	MS, RI, RC
75	2,4-heptadienal	1287	1029		*	MS, RI
76	(*E*)-2-octenal	1460	1068		*	MS, RI, RC
77	2-isopropyl-5-methyl-2-hexenal (iso1)	1467	1114		*	MS, RI, RC
78	2-isopropyl-5-methyl-2-hexenal (iso2)	1478	1117		*	MS, RI, RC
79	(*E*)-2-nonenal	1556	1172		*	MS, RI, RC
80	(*E,E*)-2,4-nonadienal	1624	1224		*	MS, RI
81	2-phenyl-2-butenal (iso1)[c]	1684	1277	*	*	MS, RI, RC
82	2,4-decadienal	1693	1322	*	*	MS, RI, RC
83	2-phenyl-2-butenal (iso2)[c]	1694	1281		*	MS, RI, RC
85	(*E,E*)-2,4-decadienal	1713	1328		*	MS, RI, RC
86	4-methyl-2-phenyl-2-pentenal (iso1)[c]	1752	1406	*	*	MS, RC
87	4-methyl-2-phenyl-2-hexenal (iso1)[d]	1795	1486		*	MS
88	5-methyl-2-phenyl-2-hexenal (iso1)[c]	1806	1496	*	*	MS, RC
89	5-methyl-2-phenyl-2-hexenal (iso2)[c]	1818	1502		*	MS, RC
90	4-methyl-2-phenyl-2-hexenal (iso2)[d]	1822	1510		*	MS
91	2-phenyl-2-octenal[d,e]	1858	1714		*	MS
	nitrogen compounds					
92	2-methylpyrazine	1126	822		*	MS, RI, RC
93	2,5-dimethylpyrazine	1208	925	*	*	MS, RI
94	2-ethyl-5-methylpyrazine	1292	1003		*	MS, RI
95	2-ethyl-6-methylpyrazine	1294	1008		*	MS, RI
96	2-ethyl-3-methylpyrazine	1295	1001		*	MS, RI
97	3-ethyl-2,5- (or 2,6-) dimethylpyrazine	1349	1083	*	*	MS, RI, RC
98	2-*sec*-butyl-3-methoxypyrazine	1391	1182		*	MS, RI
99	2,3-diethyl-5-methylpyrazine	1402	1155		*	MS, RI, RC
100	3,5-diethyl-2-methylpyrazine	1416	1157		*	MS, RI
101	2-isobutyl-3-methylpyrazine	1421	1122		*	MS, RI
102	3-isobutyl-2,5-dimethylpyrazine	1462	1192		*	MS, RI
103	2-isoamyl-6-methylpyrazine	1529	1266		*	MS, RI
104	3-isopentyl-2,5-dimethylpyrazine	1563	1304		*	MS, RI
	terpenoids					
105	β-myrcene[c]	1187	995		*	MS, RI, RC
106	linalool[c]	1365	1105	*	*	MS, RI, RC
107	α-terpineol	1465	1196		*	MS, RI
108	β-cyclocitral[d]	1582	1209		*	MS
109	α-cadinol[d]	1799	1645		*	MS
	oxazoles					
110	4,5-dimethyl-2-isopropyloxazole	1223	958		*	MS, RI
111	4,5-dimethyl-2-isobutyloxazole	1324	1091		*	MS, RI
	thiazoles					
112	2-isopropyl-4,5-dimethylthiazole	1387	1124		*	MS, RI
113	2-isopropyl-4-ethyl-5-methylthiazole	1424	1206		*	MS, RI
114	2-isobutyl-4,5-dimethylthiazole	1445	1219		*	MS, RI
	other					
115	isovaleric acid	1114	870	*		MS, RI, RC
116	γ-butyrolactone	1449	928	*		MS, RI
117	4-vinylguaiacol	1670	1323	*	*	MS, RI, RC
118	γ-nonalactone	1810	1382		*	MS, RI

[a] Compound detected in wort or vapor condensate (cond). [b] Identification by MS spectra (MS), Kovat's retention indices (RI), or comparison with a reference compound (RC). [c] Compound not described before in pilsner wort without hop addition. [d] Compound tentatively identified. [e] New compound, not previously identified.

wort or even in beer. One compound, 2-phenyl-2-octenal, that has not been identified previously, was identified by mass spectral analysis and confirmed by experiments with a model solution. This compound was never reported before in literature, to the best of our knowledge.

Sulfur Compounds. Methanethiol is generated from the degradation of methional or methionine by a retro-Michael reaction, and two molecules of methanethiol can further associate to yield dimethyl disulfide (*15*). The compound dimethyl sulfide is one of the most important quality parameters for brewers while boiling. It is formed by the thermal decom-

position of *S*-methylmethionine (*16*). The flavor threshold of this compound is around 50 ppb, and in high concentrations it has an unpleasant, cooked cabbage flavor. Thiophene is obtained as a result of the reaction of cysteine with Maillard reaction products (*17*). Dimethyl trisulfide was easily detected in both wort and condensate, despite the general belief that this flavor compound belongs to the typical constituents of the hop oil fraction (*18*). However, analogous to the production of dimethyl disulfide, dimethyl trisulfide can also be formed from methional, as this process was observed during beer aging as well (*19*). Dimethyl tetrasulfide, a known compound in hops (*20*), has

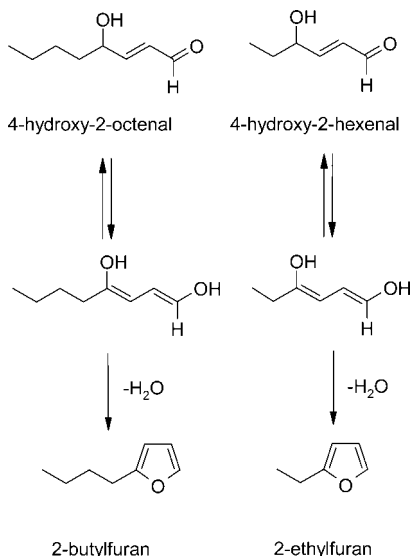**250** *J. Agric. Food Chem.,* Vol. 56, No. 1, 2008

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Figure 1. Formation of 2-butylfuran and 2-ethylfuran from their precursors (based on the formation pathway of 2-pentylfuran as proposed by Hidalgo et al. (*24*)).
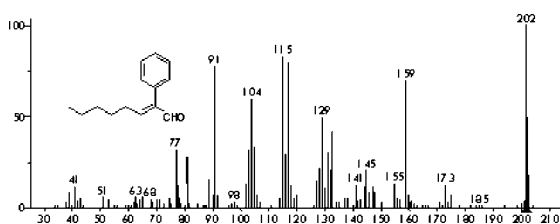

Figure 2. Mass spectrum of 2-phenyl-2-octenal.

never been detected in nonhopped beer constituents. The level of dimethyl tetrasulfide in wort was very low as this compound was only detectable in the vapor condensate.

Furans. Furans have always been associated with the Maillard reaction as this pathway is one of the main furan-generating pathways. Furfuryl alcohol (and to a lesser extent 2-furfural) is known as a precursor of furfuryl ethyl ether, an important beer-staling compound in beer (*2, 21*). Furfural and 5-hydroxymethylfurfural are generally considered as important beer aging markers (*22*). Acetylfuran and 5-methyl-2-furfural also tend to accumulate during beer aging (*23*), and their formation is due to the Maillard reaction as well. The alkylfurans 2-methylfuran, 2-ethylfuran, and 2-pentylfuran have already been observed in malt (*4*) and wort (*7*), while 2-butylfuran has only been reported in malt extract (*3*). Pentylfuran, with a "cough syrup"-like flavor characteristic, is recognized as a product of lipid oxidation and formed by a ring closure of 4-hydroxy-2-nonenal (*24*). Subsequently 2-butylfuran could be formed in an analogous manner starting from 4-hydroxy-2-octenal, and 2-ethylfuran could be formed starting from 4-hydroxy-2-hexenal as well (**Figure 1**). Both possible precursors of 2-butylfuran and 2-ethylfuran have previously been detected in vegetable oil (*25*). As these precursors have a polar nature, they will not easily be evaporated. Only after ring closure is a more volatile compound generated, which can be evacuated by evaporation.

The compounds (*E*)- or (*Z*)-2-(2-pentenyl)furan and 3-phenylfuran were tentatively identified. While 2-(2-pentenyl)furan has only been reported in the volatile fraction of cooked pork (*26*), the presence of 3-phenylfuran has already been observed in the volatile fraction of chocolate, and its flavor was described

as cocoa, green, or mint (*27*). The tentatively identified 2-hexanoylfuran has never been described in food-related literature so far.

Linear Alkanals and Strecker Aldehydes. The listed aldehydes were all identified and compared with their reference compounds. The well-known Strecker aldehydes (2-methylpropanal, 2-methylbutanal, 3-methylbutanal, methional, phenylacetaldehyde, and benzaldehyde) are considered as aging-relevant compounds and are frequently used as indicators for wort boiling performance (*1*). The parent amino acids of the Strecker aldehydes are easy to determine, except for benzaldehyde, as there is no direct correlation between benzaldehyde and any available amino acid. Granvogl et al. (*28*) suggested an imine−enamine tautomerization of the Schiff base of phenylethylamine and methylglyoxal, followed by the oxidation of the intermediate and a retro-aldol reaction, generating benzaldehyde and formaldehyde. Phenylethylamine can be formed out of phenylalanine. Consequently, direct formation of benzaldehyde from phenylalanine could occur analogously with an additional decarboxylation step.

The linear alkanals have already been described many times in wort (*29*) and beer (*30*). They originate from the enzymatic oxidation of fatty acids during the mashing process or from chemical autoxidation reactions during the wort boiling process. One of the most flavor active compounds is (*E,E*)-2,4-decadienal, which is formed by oxidation of linoleic acid and which is characterized by an unpleasant "fried oil" flavor (*31*). The oxidized form of (*E,E*)-2,4-decadienal, the 4,5-epoxy-2-decenal derivative, could also directly be involved in a Strecker-type reaction with amino acids (*32*).

Esters. The identified esters probably originate from the barley malt. The tentatively identified isopropyl laurate (characteristic ions 183, 200, 201) was never encountered before in beer or wort. Strikingly, this compound was detectable not only in the vapor condensate but in wort as well.

Ketones. Eighteen ketones were identified in the vapor fraction of the wort. Among them *β*-damascenone was found, which could be generated either by hydrolysis of glycosides (*33*) or by direct oxidation of carotenoids (*34*). Together with *β*-damascenone, its direct precursor 3-hydroxy-*β*-damascone was tentatively identified for the first time in the wort volatile fraction. Acetophenone was found in both the wort and vapor fraction. Its presence has already been observed in Maillard model systems (*35*). The presence of different isomers of 3,5-octadien-2-one was also encountered. Presumably this compound originates from lipid oxidation like most of the ketones listed.

The cyclic ketone 4-cyclopentene-1,3-dione was tentatively identified for the first time in wort and vapor condensate. However, since no reference compound is commercially available, its presence could not be confirmed. The compound has been described as quantitatively the most important carbonyl compound in melanoidin degradation model systems (*36*), and its formation pathway has been proposed by Yaylayan et al. (*37*). This pathway starts from a phosphorylated D-glucose, which is converted to 3-deoxyglucosone 6-phosphate. Via a phosphate-mediated retro-aldol reaction a 1,2,4-tricarbonyl compound is generated. This tricarbonyl compound undergoes cyclization via an intramolecular aldol condensation. However, it is not yet known whether this compound could contribute to the overall flavor of beer.

Alcohols. The listed alcohols are known to be present in wort or beer. Some of them, such as 3-methylbutanol and phenylethyl alcohol, are important flavor compounds of beer.
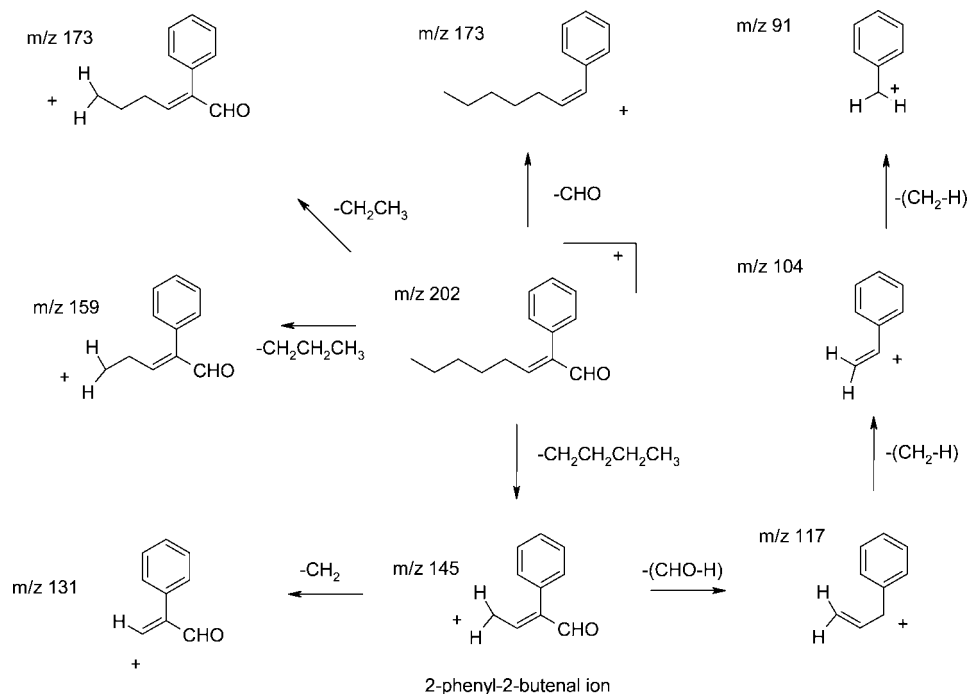
Volatiles in Unhopped Wort

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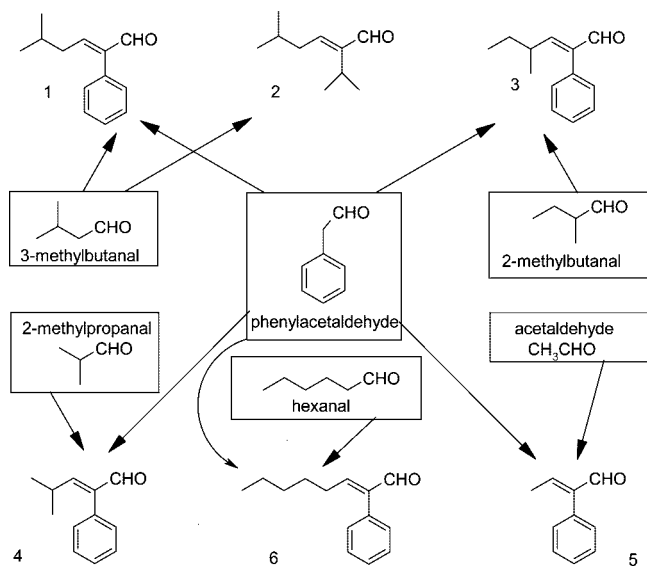**Figure 3.** Proposed spectral fragmentation pattern for 2-phenyl-2-octenal.

Figure 4. Formation of branched 2-alkenals by aldol condensation of Strecker aldehydes. The reaction products are 5-methyl-2-phenyl-2-hexenal (**1**), 2-isopropyl-5-methyl-2-hexenal (**2**), 4-methyl-2-phenyl-2-hexenal (**3**), 4-methyl-2-phenyl-2-pentenal (**4**), 2-phenyl-2-butenal (**5**), and 2-phenyl-2-octenal (**6**).

Alkenals. The linear 2-alkenals and 2,4-alkadienals have been described as important off-flavors in aged beers (*30*). The most intriguing of these linear 2-alkenals is (*E*)-2-nonenal, which is for decades held responsible for the aged flavor of beer, with a cardboard-flavor kind of characteristic. An important route to (*E*)-2-nonenal formation is the lipid oxidation pathway (*38*), but this 2-alkenal could also be generated by the aldol condensation of heptanal and acetaldehyde (*39*).

Remarkable in the group of 2-alkenals was the detection of the branched 2-alkenals 2-isopropyl-5-methyl-2-hexenal, 2-phenyl-2-butenal, 4-methyl-2-phenyl-2-pentenal, 4-methyl-2-phenyl-2-hexenal, and 5-methyl-2-phenyl-2-hexenal. The phenyl-containing compounds are each characterized by a cocoa-like flavor and have been identified in malt, cocoa, and coffee according

to Tressl (*40*). The compound 5-methyl-2-phenyl-2-hexenal has also been found in ale wort (*7*) and malt extract (*3*) and is known as one of the most important flavor compounds in cocoa (*27, 41*). However, it has not been described before in pilsner wort, where pale malt is used. It was suspected that the branched 2-alkenals would hardly be detected due the apparent correlation between the concentration of these compounds and increasing malt color as stated by Coghe et al. (*6*). It was suggested that the formation rate is higher in dark malts that was attributed to the dehydrating conditions during malting and roasting. However, all known compounds were easily detected in the vapor condensate, while 2-phenyl-2-butenal, 4-methyl-2-phenyl-2-pentenal, and 5-methyl-2-phenyl-2-hexenal were also found in the wort fraction.

Logically, these compounds are formed by aldol condensation of two Strecker aldehydes. Coghe et al. (*6*) described the formation of 2-isopropyl-5-methyl-2-hexenal from two molecules of 3-methylbutanal. In the other cases phenylacetaldehyde seems to be the central compound, which can react with acetaldehyde, 2-methylpropanal, 2-methylbutanal, and 3-methylbutanal to give rise to 2-phenyl-2-butenal, 4-methyl-2-phenyl-2-pentenal, 4-methyl-2-phenyl-2-hexenal, and 5-methyl-2-phenyl-2-hexenal, respectively.

Another observation that has not been mentioned in the literature so far was the presence of two isomers of each branched 2-alkenal in the vapor fraction (except for 4-methyl-2-phenyl-2-pentenal, presumably because the isomer was under the detection level). Therefore, it is likely that the two isomers are obtained after reaction of the parent molecules. However, the ratio of isomers differs greatly. The isomers with the lowest RI (**Table 1**) displayed the highest peak intensity: about 98% of the sum of the two peak areas in the case of 5-methyl-2-phenyl-2-hexenal. Apparently, the formation of the same isomer every time is strongly favored during the reaction. This could be explained by steric hindrance of the bulky phenyl of isopropyl side chains, favoring either the cis or the trans configuration. However, it is unclear whether the cis or the trans configuration dominates.

In addition, a previously unknown compound in food systems was tentatively identified as 2-phenyl-2-octenal, which could
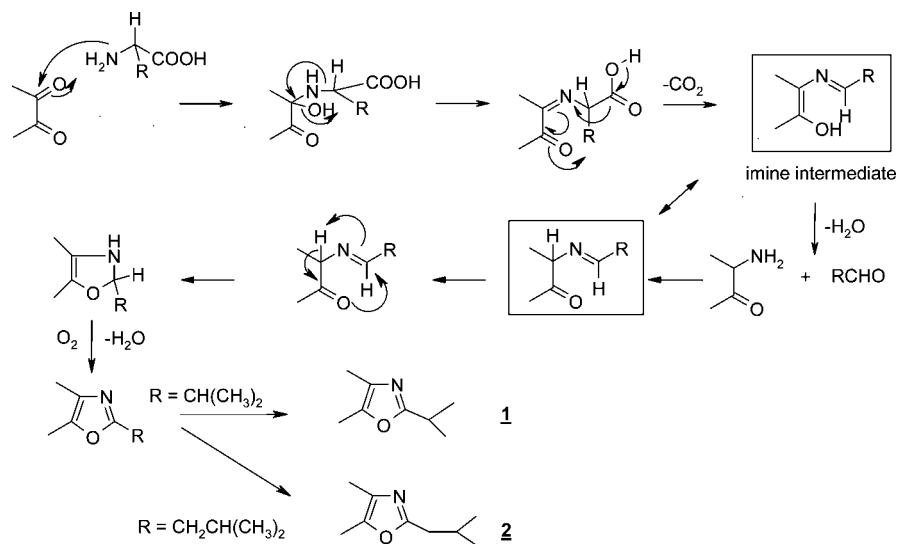
Figure 5. Possible reaction pathway for the formation of 4,5-dimethyl-2-isopropyloxazole (**1**) and 4,5-dimethyl-2-isobutyloxazole (**2**). The imine intermediate, straight from the Strecker degradation of valine or leucine with diacetyl or via the reaction of an α-amino ketone with 2-methylpropanal or 3-methylbutanal, undergoes cyclization and subsequent oxidation, yielding the respective oxazoles.

be the product of the aldol condensation reaction between hexanal and phenylacetaldehyde. When analyzing the mass spectrum (**Figure 2**), the base structure was comparable to those of all branched 2-alkenals disposing of a phenyl side group with characteristic peaks at *m/z* 115 and 117. A mass spectral fragmentation pattern of 2-phenyl-2-octenal is proposed in **Figure 3**. After breakdown of 2-phenyl-2-octenal to the 2-phenyl-2-butenal ion during mass spectral fragmentation, the smaller fragments are highly comparable to the fragments of 2-phenyl-2-butenal.

In order to investigate if the synthesis of branched 2-alkenals was possible under wort boiling conditions, model experiments were performed with the addition of equal amounts of Strecker aldehydes in a solution at pH 5.2. The following aldehydes were incubated together with phenylacetaldehyde at 100 °C: 3-methylbutanal, acetaldehyde, 2-methylbutanal, 2-methylpropanal, and hexanal. After 1 h of boiling, the reaction mixtures were prepared and analyzed with GC-MS. It was observed that the reaction occurred in each sample, nevertheless in considerably lower amounts compared to the concentration of the parent molecules (in the case of 5-methyl-2-phenyl-2-hexenal: peak area 8×10^6 vs 6×10^8 and 15×10^8 for 3-methylbutanal and phenylacetaldehyde, respectively). As the aforementioned reactions seem to occur in wort under boiling conditions, virtually every aldehyde present in wort must be able to undergo an aldol condensation with another aldehyde. These aldol condensations could give rise to a large group of merged aldehydes with different flavor characteristics (e.g., the cocoa flavor of 5-methyl-2-phenyl-2-hexenal). An overview of the detected branched 2-alkenals together with their parent compounds is depicted in **Figure 4**.

Pyrazines. The pyrazines were mostly found in the condensate fraction, although 2,5-dimethylpyrazine and 3-ethyl-2,5-(or 2,6-) dimethylpyrazine were detected in the pilsner wort as well. Hence, the concentration of the pyrazines appeared to be low in most cases, as the majority was not detectable in the pilsner wort. Pyrazines are characteristic compounds of roasted cocoa beans (*27, 40, 41*), coffee (*40, 42*), and roasted malt (*4, 40*), often characterized by nutty and roasted flavors. The presence

of these pyrazines in pilsner wort indicates that these compounds can be formed in more gentle conditions than a roasting process, although in very low quantities.

Terpenoids. Linalool was easily detected in both wort and condensate. β-Myrcene, α-terpineol, β-cyclocitral, and α-cadinol (tentatively identified) were only detected in the condensate. These compounds are typical constituents of hop essential oil (*11, 18*) and have never been associated with unhopped wort before. Hence it seems that malt also contains minimal amounts of terpenoids. The detection of these terpenoids demonstrates once more the high sensitivity of this technique, as their concentration must be extremely low.

Oxazoles. The oxazoles 4,5-dimethyl-2-isopropyloxazole and 4,5-dimethyl-2-isobutyloxazole were identified in the wort condensate. Oxazoles are formed when an α-amino ketone and an aldehyde react together (*15*) or when a Strecker degradation is not complete. They have also been described in model systems (*43*). The compound 4,5-dimethyl-2-isopropyloxazole probably originates from the reaction of the α-amino ketone of diacetyl with 2-methylpropanal, and consequently 4,5-dimethyl-2-isobutyloxazole probably originates from the α-amino ketone of diacetyl with 3-methylbutanal. Logically, the compounds could also be formed by the immediate reaction of diacetyl with valine and leucine, respectively. This oxazole formation could also be obtained immediately from the Strecker degradation pathway, starting from tautomerization of the imine intermediate. The hypothetical formation pathway of the detected oxazoles is depicted in **Figure 5**. After the reaction of the Strecker aldehyde with the α-amino ketone, a Schiff base is formed. This can undergo a cyclization, followed by an oxidation, and finally yielding the respective oxazoles.

Thiazoles. Alkylthiazoles were tentatively identified in the wort vapor fraction. As these compounds were only detected in the vapor condensate, the wort levels must have been considerably low. The identified compounds were 2-isopropyl-4,5-dimethylthiazole, 2-isopropyl-4-ethyl-5-methylthiazole, and 2-isobutyl-4,5-dimethylthiazole. Alkylthiazoles have already been found in the volatile fraction of cooked meat and are described as a product of the reaction of α-dicarbonyl compounds with H_2S, NH_3, and virtually every type of aldehyde (*44, 45*).

Possibly, the alkylthiazoles could also be formed, analogously to the oxazole formation, directly from α-amino ketones, aldehydes, and H$_2$S or from the reaction of an imine intermediate during Strecker degradation and H$_2$S.

Other. The lactones γ-butyrolactone and γ-nonalactone were found in wort and vapor condensate, respectively. The peach-like flavored γ-nonalactone is known as an important beer-aging compound (*33*). The phenolic compound 4-vinylguaiacol (smoked, dentist-like flavor) was detected in wort and vapor condensate with increasing intensity during the wort boiling process. It is formed by thermal decarboxylation of ferulic acid, which is released during the mashing process. However, the contribution of the thermal decarboxylation to the total formation of 4-vinylguaiacol in beer is low compared with the enzymatic production of 4-vinylguaiacol during fermentation (*46*).

Conclusion. The combination of headspace SPME-GC/MS combined with vapor condensate analysis was a successful tool for the detection of new volatile compounds. More than 110 different volatile compounds were identified with this technique in unhopped wort, from which 54 were confirmed with reference compounds. The study on wort vapors has revealed the importance of the aldol condensation mechanism between aldehydes for the generation of new compounds. Both isomers of the unsaturated condensation product can be formed with this reaction. However, the bulky side chains of the generated aldehyde seem to influence the balance between the two isomers.

It was demonstrated that Strecker aldehydes could combine together to generate branched 2-alkenals. Heating hexanal and phenylacetaldehyde together in a watery solution generated the previously unknown aldol condensation product 2-phenyl-2-octenal. Hence, virtually every aldehyde present in wort could undergo the same reaction, generating a large group of complex aldehydes with variable characteristics. Some of these generated aldehydes could cause off-flavors, like the cardboard flavor of (*E*)-2-nonenal, or they could be beneficial, like the cocoa flavor of 5-methyl-2-phenyl-2-hexenal. Further research must elucidate the relevance of these compounds in beer, as no information is present about actual concentrations and thresholds of these compounds.

The discovery of alkyloxazoles and alkylthiazoles in the vapor fraction could indicate once more the central role of dicarbonyl compounds, aldehydes, and amino acids. At present, the flavor characteristics of some oxazoles have been described as sweet, nutty, or cocoa (*47*), and they may have a low odor threshold.

This paper focused primarily on the complete identification of the wort volatile fraction. However, quantitative measures have not been taken, nor has there been a followup on volatiles during wort boiling. Further research will therefore pay attention to the evolution of the identified compounds in the course of wort boiling.

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