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Release and Evaporation of Volatiles during Boiling of Unhopped Wort

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The release and evaporation of volatile compounds was studied during boiling of wort. The observed parameters were boiling time, boiling intensity, wort pH, and wort density. The effect of every parameter was discussed and approached chemically, with an eye on beer-aging processes. The results indicated that pH highly influenced the release of flavor compounds and that the formation of Strecker aldehydes was linear with boiling time. However, because of evaporation of volatiles, information about the applied thermal load on wort is lost when using a volatile heat load indicator. The thiobarbituric acid (TBA) method, which includes the nonvolatile precursors of volatile aging compounds, proved to be a more reliable method to determine all kinds of heat load on wort. Finally, it was discussed how the obtained insights could help to understand the mechanism of beer aging.

KEYWORDS: Wort boiling; volatiles; Maillard reaction; thermal load; TBA

INTRODUCTION

The wort boiling process is generally recognized as a key role player in the flavor stability of beer (*1–4*). Nevertheless, the boiling process can not be omitted, because it serves many objectives, as isomerization of hop α-acids into iso-α-acids, protein coagulation, evaporation of unwanted flavor compounds, sterilization, etc. The extreme conditions during wort boiling (100 °C), together with the complex mixture of reactants, provide an excellent environment for a cascade of reactions, such as Maillard reactions. These reactions could affect the flavor stability of beer by, for example, the formation of furfural and furfuryl alcohol, which lead to furfuryl ethyl ether, an important aging flavor in specialty beers (*4, 5*), or 5-hydroxymethylfurfural (HMF) (*6*). Precursors formed during the Maillard reactions are also found to affect flavor stability because of their reactive nature, such as α-dicarbonyl compounds (*7*). Reducing the boiling time is known to lead to a decreased formation of Strecker aldehydes during beer aging (*8*). Suppliers of brewing equipment responded to the need for reduced thermal load by developing innovative boiling systems (*9–11*). The evaporation efficiency of those new systems was recently discussed by Hertel and Sommer (*12*). On the other hand, it is known that the potent

aging flavor (*E*)-2-nonenal can react with amino compounds during boiling, resulting in imine linkages (*13*). This imine adduct is further protected against reduction by yeast during the fermentation process and allows (*E*)-2-nonenal to be released again during beer aging (*1*). Therefore, Yano et al. (*2*) investigated which boiling parameters affected the formation of (*E*)-2-nonenal and how the formation could be controlled.

However, no fundamental approach regarding the influence of boiling parameter variation on the formation and evaporation of flavor compounds can be found. This provokes the need for a profound chemical investigation of the wort boiling process under variable conditions. The obtained insights can then help to improve beer flavor stability. In previous work, the identification of wort volatiles has already been undertaken to gain insight in the chemical background of wort (*14*). In the present work, the formation and evaporation of a selection of volatiles were monitored during boiling under variable conditions. The selected volatiles represented major flavor-generating reactions occurring during wort boiling (*8*). The Strecker degradation leads to the formation of 2-methylpropanal, 3-methylbutanal, 2-methylbutanal, methional, phenylacetaldehyde, and benzaldehyde. The latter is indirectly produced from phenylalanine, as proposed by Granvogl et al. (*15*). The oxidation of lipids leads to the formation of aldehydes, such as hexanal (*16*) and pentanal, while typical Maillard reaction products are furfural, furfuryl alcohol (*4, 17*), and acetylfuran (*18*). Finally, β-damascenone is formed by the hydrolysis of glycosides (*19*) or the direct oxidation of carotenoids (*20*).

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The aim of this study was to evaluate the influence of different wort boiling parameters on the behavior of aging-relevant volatiles in wort. The obtained results can provide deeper insight in the chemical implications of thermal load on wort. This information might help to understand the influence of the applied thermal load of wort on the flavor stability of beer.

MATERIALS AND METHODS

Chemicals. The compound 2-pentylfuran (98%) was obtained from Alfa Aesar GmbH (Karlsruhe, Germany). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) with the highest purity available: isobutyraldehyde, (E)-2-nonenal, 3-methylthiopropionaldehyde, 3-methylbutanal, 2-acetylfuran, 2-ethyl-3,5(or 6)-dimethylpyrazine, phenylacetaldehyde, β-damascenone, hexanal, 2-heptanol, 2-furfural, furfuryl alcohol, pentanal, benzaldehyde, 2-methylfuran, 2-ethylfuran, 2-methylbutanal, 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 up 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 collected in 1.5 L containers and immediately cooled down 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 (originally 17.2 °P) was adjusted to 12 °P, and the pH was adjusted to the appropriate pH with sulfuric acid (20%). Where needed, sodium hydroxide (20%) was used to increase the pH. When the effect of wort density was studied, the wort was adjusted to the appropriate density with deionized water.

Boiling of Wort in Sealed Pyrex Tubes. Pyrex tubes were filled with 25 mL of wort and incubated in a boiling water bath. Each experiment was conducted in duplicate. After holding the samples in the water bath for the predetermined time, they were immediately cooled down on ice and subsequently stored at −25 °C, prior to analysis.

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 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 feedback 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) and Analysis by GC/MS. The SPME extraction was executed according to the optimized procedure of De Schutter et al. (*21*). 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 pre-incubated at 45 °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 45 °C.

The analyses were performed using a Trace GC Ultra gas chromatograph, equipped with a dual stage quadrupole (DSQ) mass spectrometer (both Thermo, Waltham, MA). The GC system was equipped with a split−splitless injection port with an operation temperature of 250 °C. Helium was used as carrier gas with a constant flow rate of 1.5 mL/min. Desorption of the fiber occurred in split mode (split ratio of 7), with a desorption time of 2 min. The volatile compounds were separated on a capillary RTX-5SilMS column (60 m × 0.25 mm ID × 1 μm df) purchased from Restek (Bellefonte, PA). The column temperature was held at 30 °C for 2 min and increased to 70 °C at 10 °C/min, and then the temperature was increased to 200 °C at 4 °C/min, followed by an increase to 270 °C at 20 °C/min, with a final holding time of 2 min. The temperature of the transfer line to the mass spectrometer was invariably set at 270 °C. The mass spectrometer was operated in the electron impact ionization mode (70 eV) at 250 °C. Detection was performed in scan mode with *m/z* between 33 and 280 at 5.6 scans per second. The analyses were performed in triplicate, unless stated otherwise.

Quantification was performed by the standard addition method as described by De Schutter et al. (*21*).

Thiobarbituric Acid (TBA) Procedure. The procedure was adapted from Coghe et al. (*22*). A total of 5 mL of a TBA solution [288 mg of thiobarbituric acid in 100 mL of acetic acid (90%)] was added to 10 mL of wort in sealed pyrex tubes (in duplicate). The mixtures were kept in a water bath at 70 °C for 70 min. Hereafter, the samples were cooled on ice. Absorptions were measured spectrophotometrically at 448 nm, the maximum absorption of the HMF−TBA complex. If necessary, the samples were diluted to obtain a value in the linear range of the spectrophotometer. As a blank measurement, untreated wort with a TBA solution was measured at the same wavelength. Subsequently, the TBA value was calculated as follows (*D* = dilution factor): TBA = $10(DA_{448} - A_{blank})$.

RESULTS AND DISCUSSION

Although wort is a very complex mixture, it is essentially a mixture of reducing sugars with amino compounds. The most abundant sugars in wort are glucose, maltose, and maltotriose. The reaction of these sugars with amino compounds leads to the formation of Maillard reaction products. The initiation of the Maillard reaction has a great influence on the overall reaction rate (*23, 24*). The first step is a nucleophilic addition of an amino compound to the carbonyl group of the reducing sugar. If the amino group is protonated, the nucleophilic nature of the amine will be lost however. Because the pK_a values of most amino groups of amino acids are situated around 9, the amount of unprotonated amino groups will be about 0.01% of the protonated ones at wort pH (5−5.5). In addition, the open-chain form of the sugar is considered to be the reactive species, and the ratio of open chain versus ring structure increases with pH (*25*).

After the initial nucleophilic addition, a Schiff base is formed, which can convert to the corresponding 1,2-enaminol. The Amadori rearrangement product (ARP) is formed after Amadori rearrangement of the enaminol. The ARP is believed to be the central intermediate of the early Maillard reaction pathway and is in equilibrium with the Schiff base, the cation form of the Schiff base, or the 1,2-enaminol (*24*). The ARP leads to the formation of 3-deoxyglucosone (3-DG) via 1,2-enolization and to 1-deoxyglucosone (1-DG) via 2,3-enolization. At higher pH, the formation of 1-deoxyglucosone and retro-aldol reactions are favored, while at lower pH, the formation of 3-deoxyglucosone becomes more important (*26*).

Flavor Generation during Boiling of Unhopped Wort in a Closed System. *Effect of Boiling Time on the Formation of Volatiles.* In this experiment, unhopped wort was adjusted to pH 5.2. Sulfuric acid was used instead of phosphoric acid to avoid the potential catalytic effect of the latter on Maillard reactions (*27*). The formation of volatiles during 8 h of boiling was monitored as shown in **Table 1**.

All observed Strecker aldehydes, 2-methylpropanal (2-MP), 2-methylbutanal (2-MB), 3-methylbutanal (3-MB), phenylacetaldehyde, methional, and benzaldehyde, evolved linearly with

Table 1. Evolution of Volatile Compounds during 8 h of Wort Boiling (*y*, concentration; *x*, time)

	time (min)														
	CV[a] (%)	0	30	60	90	120	150	180	240	300	360	420	480	increase (min)	R
		concentration (μg/L)													
2-methylpropanal	1.1	51.0	66.1	63.7	67.9	77.7	82.5	85.1	96.5	103.1	125.3	120.4	132.6	$y = 0.167x$	0.9797
2-methylbutanal	1.7	44.0	55.4	54.1	57.4	67.8	75.9	75.7	90.7	92.1	107.8	119.8	126.2	$y = 0.169x$	0.9857
3-methylbutanal	0.7	98.0	127.8	144.9	170.9	208.7	228.8	245.2	309.7	349.1	410.0	438.8	502.2	$y = 0.834x$	0.9817
phenylacetaldehyde	0.5	42.0	49.8	57.0	63.2	76.3	84.9	91.9	115.1	122.6	134.4	141.8	173.7	$y = 0.261x$	0.9927
methional	4.8	16.0	18.8	21.8	22.5	30.3	32.9	34.8	49.7	50.4	56.2	58.1	75.5	$y = 0.117x$	0.9797
benzaldehyde	3.9	1.8	1.8	1.9	1.9	2.6	2.8	2.8	4.1	4.2	5.2	5.0	6.6	$y = 0.010x$	0.9853
β-damascenone	2.8	0.3	4.4	9.6	15.7	22.7	30.2	36.0	49.4	58.1	72.3	74.6	83.9	$y = 0.184x$	0.9940
pentanal	1.7	6.0	6.2	5.9	6.2	6.5	6.7	6.5	7.1	5.7	6.4	5.7	7.0		
hexanal	0.4	33.1	34.6	33.2	33.8	38.1	38.1	37.4	44.1	36.9	42.9	37.3	40.0		
furfuryl alcohol	5.7	33.5	40.4	63.7	89.6	138.7	220.7	319.3	588.6	770.5	1282.1	1420.9	1857.4	$y = 0.0067x^2 + ...$	0.9956
furfural	4.2	1.0	16.4	35.6	60.2	87.5	126.6	152.6	228.9	270.2	388.1	430.8	530.6	$y = 0.0008x^2 + ...$	0.9979
acetylfuran	4.9	1.0	1.6	1.9	2.6	3.0	3.7	4.4	5.8	6.3	8.0	9.3	12.2	$y = 2 \times 10^{-5}x^2 + ...$	0.9946

[a] Coefficient of variance.

time in a pseudo-zeroth-order relation. This observation is in agreement with the results of Cremer and Eichner (*28*), who observed a zeroth-order relation for acetaldehyde, 2-MP, 3-MB, and 2-MB in low-moisture model systems. Hofmann et al. (*29*) also found that 3-DG was particularly efficient in Strecker aldehyde formation in comparison to other vicinal diketones (VDK). The levels of amino acids are known to remain approximately constant during wort boiling, except for glutamine (*30*). Therefore, the formation rate of 3-DG from ARPs (*31*) will be a determining step in the overall reaction rate.

β-Damascenone, a potential beer-aging flavor (*19*), also increased linearly with time, suggesting a pseudo-zeroth-order relation. Previously, experiments have been carried out to question its suitability as heat load indicator (*32*).

In contrast to the formation of Strecker aldehydes, hexanal derived from lipid oxidation was hardly formed with time. This could indicate that lipid autoxidation during wort boiling occurred rather slowly in comparison to the fast enzymatic oxidation during the mashing process (*33*). This could be due to the very low oxygen content of boiling wort. However, other processes, such as imine formation of the already present (*E*)-2-nonenal with amino acids, can also occur (*1, 13*). Presumably, other aldehydes could undergo imine formation as well. Furthermore, lipid oxidation products could be involved in a Strecker-like degradation of amino acids, as proposed by Hidalgo and Zamora (*34*).

The rate of formation of Maillard reaction products furfural, furfuryl alcohol, and acetylfuran increased with time (**Table 1**). This suggests that the formation rate is determined by the buildup of their precursors, presumably ARPs or intermediates in equilibrium with the ARPs. In fact, at pH 4.8 and 5.5, a steady buildup of ARP and 3-DG was observed by Martins and Van Boekel (*25*) during boiling of a glucose–glycine mixture. After 3–4 h, no maximum accumulation was reached, as the rate of formation of the ARP still exceeded the rate of degradation. Likewise, in wort, a steady buildup of ARP could take place, resulting in an ever-increasing formation of volatiles, such as furfural, with time. This observation implicates that wort boiling time could have a major impact on the formation of volatile compounds. More importantly, after a standard wort boiling period (1–1.5 h), the equilibrium in ARP formation is probably not reached. This lack of equilibrium could be transferred to aging beer and force the reactions to proceed further on, probably even at very low rates when stored at low temperature.

Effect of pH Variation on the Formation and Release of Volatiles in a Closed System. To study the effect of pH variation on the formation of volatile compounds, two experi-

Table 2. Level of Volatile Compounds after 60 min of Boiling of Wort with Adjusted pH

	CV (%)[a]		pH 5	pH 4	pH 3	pH 2
2-methylpropanal	0.8	μg/L	24.20	32.32	31.11	25.09
3-methylbutanal	0.6	μg/L	111.14	142.85	126.12	81.68
2-methylbutanal	0.9	μg/L	23.94	30.86	25.36	18.64
methional	6.5	μg/L	33.92	53.92	50.50	44.92
benzaldehyde	1.1	μg/L	2.97	4.45	5.24	4.28
phenylacetaldehyde	1.8	μg/L	86.26	127.05	157.59	146.82
pentanal	1.2	μg/L	1.43	2.34	2.87	3.27
hexanal	1.0	μg/L	5.91	10.60	16.08	34.88
2-furfural	3.6	μg/L	97.78	231.89	265.32	234.57
acetylfuran	4.8	μg/L	0.53	0.98	1.05	1.09
β-damascenone	2.2	μg/L	13.02	41.16	80.77	123.56
2-methylfuran[b]	5.5	%	100%	201%	250%	127%
ethylfuran[b]	3.7	%	100%	137%	125%	57%
2-pentylfuran[b]	2.4	%	100%	206%	245%	123%
4-cyclopenten-1,3-dione[b,c]	4.9	%	100%	124%	163%	338%
2-ethyl-3,5-dimethylpyrazine[b]	2.1	%	100%	99%	30%	15%
(*E*)-2-nonenal[b]	1.6	%	100%	300%	843%	2329%
γ-nonalactone[b]	1.3	%	100%	132%	226%	538%

[a] Coefficient of variance. [b] Values expressed relative to the values of pH 5. [c] Compound tentatively identified.

ments were set up. In a first experiment, the pH of wort was adjusted to different pH values, ranging from a normal wort pH to very acidic, and subsequently boiled for 1 h. In **Table 2**, the levels of volatiles are listed after 1 h of boiling at pH 5, 4, 3, and 2. In a second experiment, more attention was paid to the formation of volatiles when the wort pH was slightly varied in the interval of 5–5.5 and two extra outer limits. In **Figure 1**, the evolution of volatiles representing the most important reaction families is shown during boiling at different pH.

In **Table 2**, it can be seen that levels of most volatiles (except for 2-ethyl-3,5-dimethylpyrazine) increased when wort pH was lowered from 5 to 4. The level of certain compounds increased when the pH was further lowered to pH 3 but decreased again at pH 2. Other compounds, such as hexanal, pentanal, (*E*)-2-nonenal, β-damascenone, 4-cyclopenten-1,3-dione, and γ-nona-lactone, reached the highest level at the lowest pH. In boiling wort, β-damascenone is formed predominantly by acid hydrolysis of glycoside precursors (*35*). This process is clearly favored at lower pH, leading to a 9-fold increase at pH 2. Remarkably, because hexanal did hardly increase after 8 h of boiling at pH 5, a strong pH drop liberated large amounts after 1 h. This effect was even stronger for (*E*)-2-nonenal. These observations are well in agreement with the findings of Lermusieau et al. (*13*) that (*E*)-2-nonenal can be trapped in an imine linkage with amino acids or proteins and subsequently released at lower pH during beer aging (*1*). Analogously, hexanal could form adducts

Release and Evaporation of Volatiles in Unhopped Wort

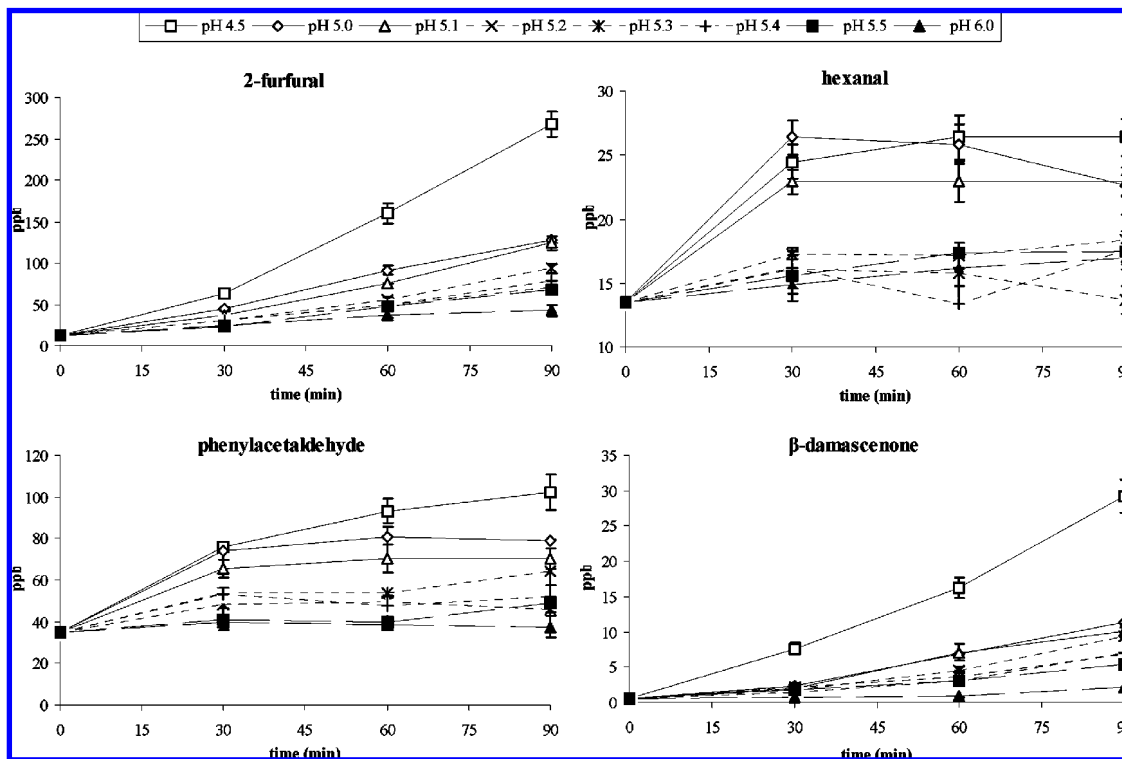*J. Agric. Food Chem.,* Vol. 56, No. 13, 2008 **5175**

Figure 1. Evolution of volatile compounds during 90 min of boiling with different initial wort pH. For each data point, the standard deviation (SD) is given ($n = 4$).

with amino compounds and survive in the beer, where a lower pH could facilitate its release. On the other hand, according to Zameo et al. (*36*), the release of imines is again inhibited at very low pH because the driving force for imine hydrolysis comes from the zwitterion conformation. At pH 2, this zwitterion is not favored and the rate of aldehyde release from the imine linkage will decrease. Therefore, it is likely that at pH 2 the high concentrations of hexanal and (*E*)-2-nonenal were also due to an increased autoxidation of lipids or an enhanced breakdown of already present hydroperoxides (*16*).

Between pH 5 and 4, furfural increased with 137%, while the level of Strecker aldehydes was raised with 30−50%. As mentioned before, the initiation of the Maillard reaction is enhanced at higher pH. However, the subsequent reactions are acid−base-catalyzed, leading the reactions toward directions that are enhanced at that particular pH, while others are inhibited. It has been documented that ARPs accumulate at pH 5.5 and 4.8, while they are quickly degraded at pH > 6 in a glucose/ glycine model system (*25*). Because studies at lower pH have not been made, one can only suspect that experiments at lower pH would also result in the accumulation of ARPs. The formation rate of 3-DG could then be comparably favored because of the accumulated ARP, while at the same time, the rates of melanoidin formation and retro-aldol reactions with 3-DG are significantly reduced at the same pH, as demonstrated by Martins and Van Boekel (*25*). Consequently, when 3-DG formation is enhanced, while the rate of melanoidin formation and retro-aldol reactions is decreased, the Maillard reaction pathway is directed in favor of furan formation. Therefore, a greater proportion of the accumulated ARPs lead to flavor formation, as can be seen in the case of furfural. In addition, it might be possible that already a significant amount of ARPs is produced during malting in circumstances different from the wort boiling process (higher pH). The accumulation of ARPs can further continue during boiling of wort, while more flavor compounds are released at low pH.

The enhanced effect of lower pH on Strecker degradation is more ambiguous. The increase in Strecker aldehyde formation (or release) with lower pH was already observed in the case of phenylacetaldehyde by Hofmann et al. (*29*). In **Figure 2**, a detailed Strecker reaction scheme is depicted, emphasizing the occurrence of different intermediates. An enhanced generation of 3-DG and other α-dicarbonyl compounds provides more available reactants for the Strecker reaction, while at the same time, a lower pH causes a decrease in the nucleophilic character of the amino compounds involved. There might also be some intermediates of the Strecker reaction already present in the wort (derived from the malting process), which could be more easily released at lower pH. These intermediates could either be the imine or hemiaminal form, resulting from decarboxylation of the amino acid, or the imine form of the direct pseudo-Strecker degradation product originating from ARPs (*31*). These assumptions are not unrealistic, because Suda et al. (*37*) found that only 15% of the formed Strecker aldehydes during beer aging are originating from newly initiated Strecker degradation reactions. The other 85% must therefore come from intermediates formed during the malting and brewing process, possibly in imine form or bound to ARPs. Protonation of the imine is enhanced at lower pH, which allows the nucleophilic attack of water, resulting in the hemiaminal. This hemiaminal is in equilibrium with two ionic structures. The zwitterionic form of the hemiaminal facilitates the release of the Strecker aldehyde (*36*). This could explain why the formation (or release) of Strecker aldehydes decreases again at pH 2. Therefore, in addition to the observation involving boiling time, the formation of Strecker degradation products seems to be highly dependent upon the pH. The observations for furfural and Strecker aldehyde formation in **Table 2** are well in correlation with the results given in **Figure 1**. Compounds of different reaction families are depicted: furfural as Maillard compound, phenylacetaldehyde as Strecker aldehyde, hexanal from lipid oxidation, and β-dama-scenone. Furfural, β-damascenone, and phenylacetaldehyde were
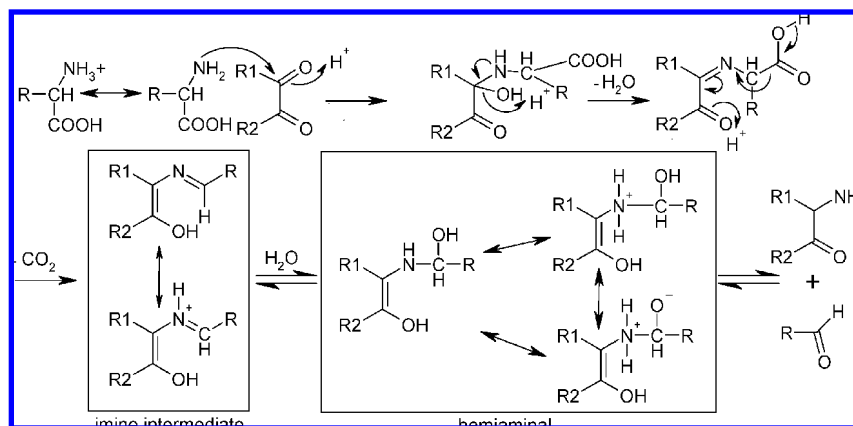
Figure 2. Strecker degradation including the different intermediates. The imine intermediate is in equilibrium with the cationic form, which facilitates transfer to the hemiaminal. Rearrangement of the hemiaminal into its zwitterionic form provides the driving force for aldehyde release [on the basis of the imine theory by Zameo et al. (*36*)].

increasingly formed at lower pH, and the formation or release of hexanal also improved.

Concerning Maillard reaction products and Strecker aldehydes, it could be interesting to boil wort at a rather low pH. This measure will reduce the initiation of the Maillard reaction, while in the same time, more aging-relevant volatiles are created. The volatiles can then be evacuated by evaporation, while the total amount of precursor compounds is kept to a minimum.

Interplay between Formation and Evaporation of Volatiles during Wort Boiling. All experiments that included evaporation were performed by monitoring the levels of volatiles in wort during boiling, while the produced wort vapors were condensed and collected. In this way, over 100 different wort volatiles were detected as described in previous work (*14*).

In addition to the formation or release rate, the evaporation rate of volatiles during wort boiling is a determining factor. If the rate of evaporation exceeds the formation of a compound, a net decrease is observed. Every compound is characterized by its own vapor−liquid equilibrium, which determines the volatility of the compound. When the equilibrium of a certain compound is known, one can predict how much a volatile is enriched in the vapor phase (*38*). The compounds considered in the present paper can be subdivided in highly volatile (2-methylpropanal, 3-methylbutanal, 2-methylbutanal, pentanal, hexanal, and DMS), medium volatile (methional, phenylacetaldehyde, benzaldehyde, acetylfuran, and β-damascenone), and components with low volatility (2-furfural and furfuryl alcohol).

Effect of Boiling Intensity and pH on the Evolution of Volatile Compounds. Several factors define the thermal load on the final wort in the brewhouse. First of all, the initial composition of the wort is of utmost importance, because pH, amount of nitrogen compounds, and reducing sugars will highly influence the reaction rates. Apart from that, boiling time, boiling temperature (if top pressure is applied), and boiling intensity will influence the final wort composition, before it is subject to fermentation. Some compounds will be reduced by yeast (*39*), while others are protected against reducing activity, such as, for example, ARPs and (*E*)-2-nonenal as an imine adduct (*1*). Therefore, the thermal load of the final wort can be defined as the chemical memory of the entire brewing process.

It is difficult to visualize boiling intensity, because it is virtually impossible to determine the precise conditions at the interface of the heating surface and the wort. The interplay between convection and conduction results in a determined heat transfer and concurrently in increased chemical reactions.
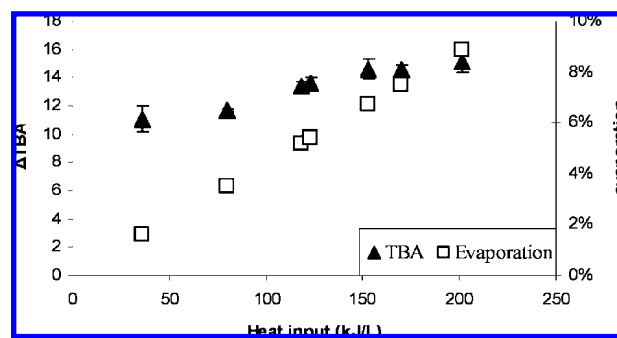

Figure 3. Evaporation rate as a function of the heat input. Accordingly, the thermal load is expressed as ΔTBA. For each data point of TBA, the SD is given (*n* = 3).

Therefore, boiling intensity has to be quantified using a bypass, the evaporation rate. In other words, at atmospheric conditions, the boiling temperature is constant, and if heat losses because of irradiation are neglected, boiling intensity is the energy input needed for the obtained degree of evaporation. In this experiment, the different degrees of boiling intensity led to an evaporation rate varying between 2 and 9%, thereby completely covering the range of modern boiling systems. The relation between boiling intensity and evaporation is depicted in **Figure 3**. Additionally, the increase in heat load because of a higher boiling intensity is expressed as ΔTBA. This value is based on the colorimetric reaction of HMF with TBA. During the procedure, ARPs and other intermediates leading to the formation of HMF are converted to HMF and subsequently bound to TBA. Because the reaction occurs under strong acidic conditions (30% acetic acid, pH ∼2), the initiation of the Maillard reaction will be inhibited, while acid-catalyzed reactions are favored. Although the yield of the reaction is not 100% (*40*), the procedure proves to be highly repeatable (CV = 1.47%). The ΔTBA increased with an increasing boiling intensity from 11 to 15.

In **Figure 4**, the evolution of some volatiles is presented as a function of the boiling time with varying boiling intensity and the initial pH was set at 5.2. Six flavor compounds representing the different chemical reaction families with different volatilities are depicted. Hexanal and 3-methylbutanal were easily evacuated after only 15 min of boiling. Only at the lowest boiling intensity, the evacuation of volatiles was delayed, which is in agreement with the findings of Hertel et al. (*38*) that a certain amount of evaporation is needed to efficiently remove volatiles. The levels at the end of boiling were very
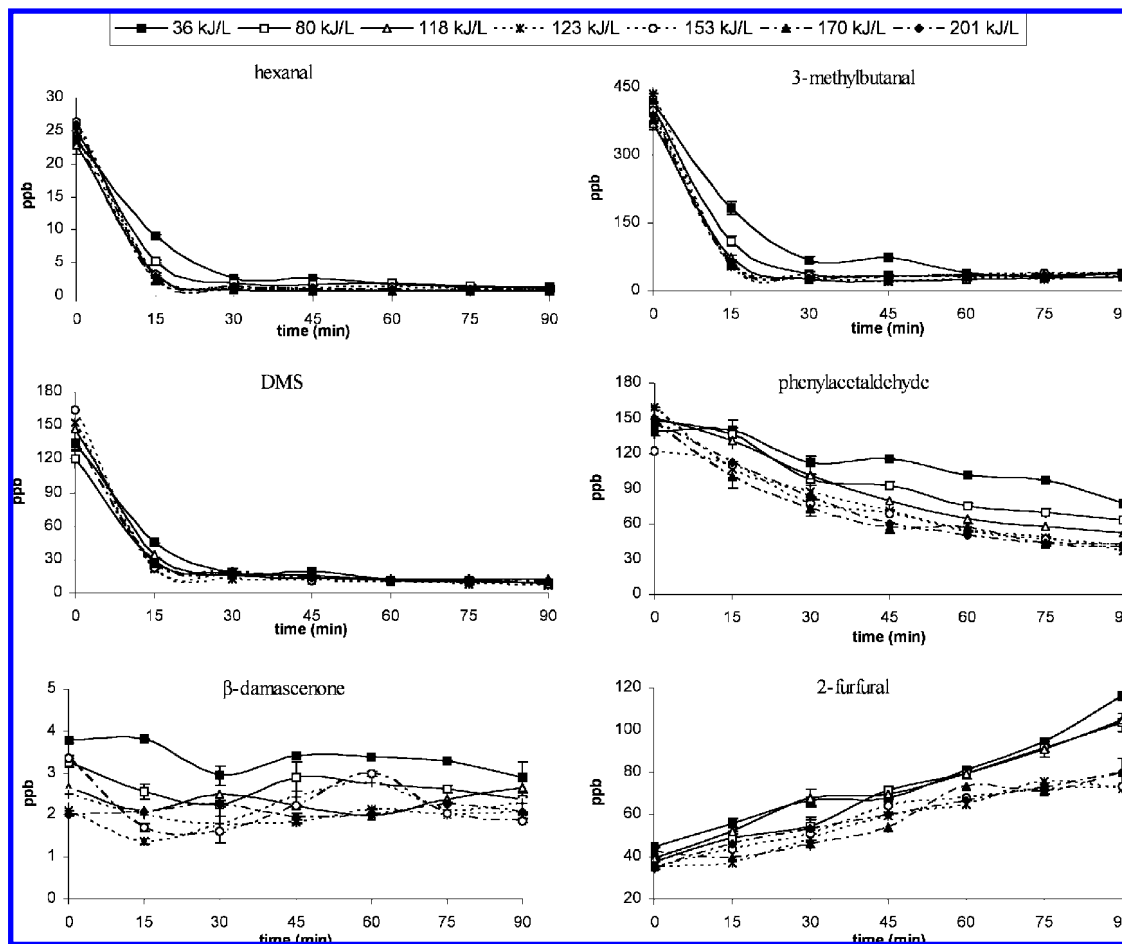
Figure 4. Evolution of flavor compounds in wort as a function of boiling intensity. Values are presented in ppb. For each data point, the SD is given (*n* = 3).

close to each other, indicating that these compounds were evacuated regardless of the evaporation rate. The very volatile DMS was also easily evacuated. However, its concentration rose again during the whirlpool rest. The formation of DMS is highly determined by the breakdown of the DMS precursor in a first-order relationship (*41*). If the initial precursor content is present in excessive amounts, such as in highly modified malts, the evacuation efficiency is lower and the DMS level can substantially raise during the whirlpool rest. Less volatile compounds as phenylacetaldehyde decreased more slowly during wort boiling, and the highest levels at the end of boiling were obtained with the lowest boiling intensities. The level of β-damascenone fluctuated throughout the entire wort boiling process. Furfural increased steadily during the entire boiling process, regardless of the applied thermal load or evaporation rate. Therefore, the boiling time should be reduced at all times, because increased levels of furfural and the even less volatile furfuryl alcohol lead to an increased generation of furfuryl ethyl ether (*4*).

Strikingly, the lowest boiling intensities (lowest evaporation) led to the highest levels of the less volatile compounds at the end of boiling. These results indicate that there was no volatile compound suitable enough to bear the role of heat load indicator. Changes in boiling intensity leading to a variable heat load are always an interaction of formation rates and evaporation. Accelerated formation because of a higher boiling intensity is often compensated by the increased evaporation rate. The volatile content of wort can therefore not represent its chemical memory. Moreover, some volatiles are also reduced by yeast during fermentation, such as furfural (*4, 42*), HMF (*6*), and Strecker aldehydes (*39*). Because of the evaporation during

boiling and reduction by yeast, the volatile content of fresh beer will not reveal how wort was treated during the brewing process. Only heat load indicators that take into account the presence of nonvolatile intermediates, such as ARPs, can provide the necessary information to evaluate the effect of the applied heat load on wort.

In **Figure 5**, results are shown of the behavior of volatiles during boiling experiments at pH values ranging from 4.8 to 5.4, with an interval of 0.2. In each case, the same heating and evaporation rate was obtained. As shown in **Figure 1** and **Table 2**, the formation rate of most volatiles increased with decreasing pH. Despite this certain enhanced formation, the highly volatile compounds displayed no marked difference between the different pH values. Clearly, the evaporation compensated sufficiently for the increased formation rate. β-Damascenone levels were initially higher at the lowest pH values, but this difference decreased in the course of boiling. In contrast, furfural levels did increase with decreasing pH. Obviously, the least volatile compounds, such as furfural, were not so easily evacuated, resulting in a higher residual level in wort after boiling. Interestingly, when taking furfural as a heat load indicator, one might suspect that a higher heat load was applied to the wort at the lowest pH. However, exactly the same boiling intensity was used. Therefore, neither furfural nor any other volatile compound should be taken as a heat load marker.

Almost the same observations were made when the density of the wort was increased (results not shown). As expected, the highly volatile compounds did not increase with the density while boiling. The medium volatile compounds ended slightly
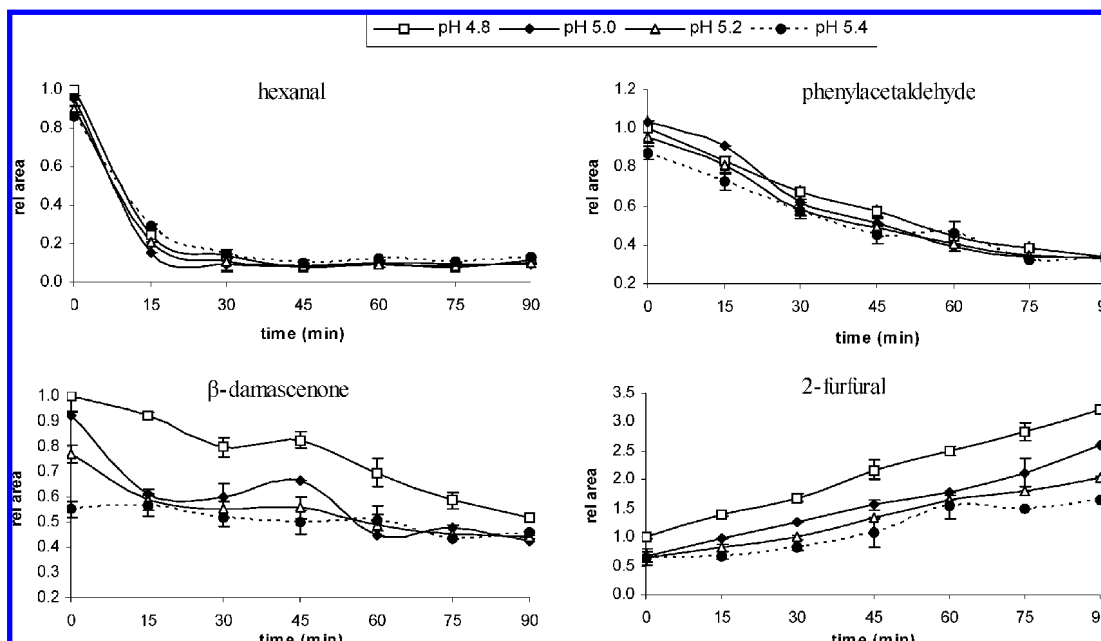
Figure 5. Evolution of volatiles during boiling of wort at different pH. Values are expressed relative to the starting value at pH 4.8. For each data point, the SD is given (*n* = 3).

Table 3. TBA Increase after 1.5 h of Wort Boiling with a Variation of Different Parameters (CV = 1.47%)

	begin boiling	end boiling	ΔTBA
pH Variation			
pH 4.8	22.8	40.1	17.3
pH 5.0	22.9	40.9	18.0
pH 5.2	23.8	40.2	16.4
pH 5.4	23.1	40.4	17.1
Density Variation			
12 °P	23.8	40.2	16.4
13 °P	22.7	41.6	18.9
14 °P	25.8	45.7	19.9
15 °P	27.4	48.7	21.3
16 °P	27.6	49.1	21.6
Variation in Boiling Intensity			
36 kJ/L	21.1	35.9	14.9
80 kJ/L	20.7	37.3	16.6
118 kJ/L	23.8	40.2	16.4
123 kJ/L	23.6	40.6	17.0
153 kJ/L	23.1	41.6	18.5
170 kJ/L	21.8	41.0	19.2
201 kJ/L	23.8	44.4	20.6

higher with higher density, and the least volatile compounds were found in increasing amounts after boiling at higher density.

In **Table 3**, the TBA value at the beginning and end of boiling (1.5 h) of the different boiling procedures is listed, together with the calculation of ΔTBA. Lowering the pH did not influence the ΔTBA markedly, suggesting that, in the observed pH range, no differences in precursor formation had taken place. Because no different boiling intensity was applied, the TBA value reflected the heat load very well in the observed pH range. When the density of the wort was increased from 12 °P (12 g of solids/100 g of wort) to 16 °P, the TBA value increased accordingly with 4 units. The higher concentration of reactants accelerated the reaction rates of the Maillard reaction in every way. Again, the TBA method was a reliable measure for the influence of thermal load on the wort, because the concentration of reactants determines the reaction rates. Finally, the TBA value increased also with the boiling intensity. The lowest applied energy resulted in an increase of 14.9 TBA units, while the highest

thermal load resulted in an increase of more than 20 TBA units. In every case, the TBA measurement was more consistent than any volatile available. Therefore, the best heat load indicator of wort is ΔTBA, which reflects the TBA difference between the beginning and end of boiling. The TBA value provides an indication of the total amount of Maillard intermediates formed and can therefore be regarded as an indicator of the chemical memory of the wort.

In conclusion, the results of this work provide insight in the great complexity of the formation of volatiles and their evaporation during wort boiling. The relationship of some important volatiles with boiling time was established. Strecker aldehydes developed linearly with time in a pseudo-zeroth-order relation, and furans increased in a first-order relation, while lipid oxidation products hardly developed. The essential and complex role of pH in the Maillard reaction also became apparent. While a lower pH inhibits the Maillard reaction at the very beginning of the reaction, the generation of most flavor compounds was clearly enhanced. This offers opportunities to brewers in such a way that, if more volatiles are generated, they can also be evacuated with wort vapors, while less precursor compounds are generated compared to wort at higher pH.

Furthermore, it was clear that an increased boiling intensity and thus heat load did not necessarily lead to an increased volatile content, while the TBA value did reflect the same changes in heat load. If a choice has to be made, clearly the boiling process with the lowest TBA added value is preferable. This can be obtained when excessive boiling (or hot holding) times are avoided and the boiling intensity is lowered. Because of the fact that evaporation highly influences the volatile content of wort, no volatile can be esteemed suitable as a heat load indicator. Instead, ΔTBA offers deeper insight in the chemical memory and thus the heat load of wort by converting intermediates that are involved in flavor generation. These intermediates, such as ARPs and imines, are protected against reduction by brewer's yeast, while flavor-active aldehydes and some α-dicarbonyl compounds are not. These compounds can then end up in the final beer, and a steady release of flavor compounds could occur from these intermediates during beer aging, facilitated by the pH decrease during fermentation. This could

Release and Evaporation of Volatiles in Unhopped Wort

J. Agric. Food Chem., Vol. 56, No. 13, 2008 **5179**

be the driving force of Maillard and Strecker volatile formation during beer aging. In future work, the effect of increased heat load on beer aging will be considered, to establish a more profound insight of the beer aging mechanism.

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5180 *J. Agric. Food Chem.,* Vol. 56, No. 13, 2008

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