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Furfuryl Ethyl Ether: Important Aging Flavor and a New Marker for the Storage Conditions of Beer

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Recently, it was reported that furfuryl ethyl ether is an important flavor compound indicative of beer storage and aging conditions. A study of the reaction mechanism indicates that furfuryl ethyl ether is most likely formed by protonation of furfuryl alcohol or furfuryl acetate followed by S_N2-substitution of the leaving group by the nucleophilic ethanol. For the reaction in beer, a pseudo-first-order reaction kinetics was derived. A close correlation was found between the values predicted by the kinetic model and the actual furfuryl ethyl ether concentration evolution during storage of beer. Furthermore, 10 commercial beers of different types, aged during 4 years in natural conditions, were analyzed, and it was found that the furfuryl ethyl ether flavor threshold was largely exceeded in each type of beer. In these natural aging conditions, lower pH, darker color, and higher alcohol content were factors that enhanced furfuryl ethyl ether formation. On the other hand, sulfite clearly reduced furfuryl ethyl ether formation. All results show that the furfuryl ethyl ether concentration is an excellent time−temperature integrator for beer storage.

KEYWORDS: Furfuryl ethyl ether; flavor; beer; aging; staling; furanic ethers; kinetics

INTRODUCTION

Consumers consider aroma and taste to be the most important quality-determining characteristics of a beer. Consequently, brewers must continuously direct efforts toward the production of a beer with a characteristic, yet balanced, flavor. Although brewers often succeed in producing a beer with the desired flavor profile, the flavor of the finished beer may change during storage. Moreover, the flavors that develop during aging are mostly experienced as unpleasant. Consequently, this flavor instability makes it difficult to ensure a constant product quality.

Much research has therefore focused on the reactions that occur in beer during its storage. Many studies are mainly related to the formation of (*E*)-2-nonenal and other alkenals, which are considered to be responsible for the development of the papery and cardboard-like flavors of aged beer (*1*). It has been shown that nonenal originates from lipid oxidation during brewing followed by its release by nonoxidative mechanisms in bottled beer (*2*, *3*); the last step is accelerated at lower pH (*4*).

Although alkenals may contribute to the staling flavor of some beers, recent studies (*5*−*7*) have demonstrated that their formation does not always occur and that the process of beer aging is much more complex than just nonenal and cardboard flavor appearance. Indeed, other molecules may be equally important for the aging process. In this respect, (*E*)-β-damascenone (*7*, *8*), dimethyl trisulfide (*9*), ethyl 2-methylbutyrate, and ethyl 3-methylbutyrate (*10*) have been mentioned. For a top-fermented beer, we recently reported the detection of furfuryl ethyl ether (FEE) and its marked increase in aged beer (*11*). A flavor threshold in beer of 6 μg/L was found, and in the aged top-fermented beer concentrations multiple times the threshold value were measured. FEE contributes to a sharp solvent-like flavor in beer. Moreover, other furanic ethers such as 5-(ethoxymethyl)-2-furfural were also formed. The latter molecule is found in sweet fortified wines and is part of the overall aging flavor of these wines (*12*, *13*). This sustains the hypothesis that furanic ethers can also be regarded as a class of aging-reflecting compounds, with a significant effect on stored beer flavor. Therefore, the pathway of furfuryl ethyl ether synthesis and the reaction kinetics were now studied more in depth. The effects of several parameters on its formation are described, and its concentration change during the aging of several commercial beers is investigated.

MATERIALS AND METHODS

Chemicals. Furfuryl alcohol (FALC) (99%), furfuryl acetate (FAC) (99%), and sodium sulfite were supplied by Sigma Aldrich Chemie GmbH (Munich, Germany). FEE with a purity of 95% was purchased from Narchem Corp. (Chicago, IL). Citric acid (100%), sodium hydroxide (50%), and ethanol (100%) were supplied by Merck (Darmstadt, Germany).

Beers. To study the behavior of FEE in beer, a fresh pale top-fermented beer (alcohol = 7.5% v/v; pH 4.2; color, 6.6 EBC) obtained from a Belgian brewery was used in the experiments. Furthermore, 10 commercial beers obtained from different Belgian breweries were used to examine the effect of prolonged natural aging.

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Analysis of Volatile Compounds. Prior to analysis, beer was degassed by kieselguhr filtration. Then, 200 μL of internal standard (250 mg/L 2-heptanol) and 200 μL of a 10% antifoam solution (Sigma Aldrich Chemie GmbH) were added to 50 mL of degassed beer. Only 200 μL of internal standard solution was added to the synthetic model media (see further). Five milliliters was then transferred into the Tekmar Dohrman 3000 (Emerson, Mason) purge and trap concentrator unit with a Vocarb 3000 trap (Supelco, Bellefonte, PA) in the following conditions; helium was the carrier gas, 10 min purge 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. The relatively high trap temperature of 140 °C during the purge and dry purge step avoided saturation of the trap with ethanol. Before entering the GC, volatiles were focused using a cold trap with an MFA 815 control unit (ThermoFinnigan, San Jose, CA) 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). The temperature program was 3 min at 50 °C/6 °C min^{-1} and 3 min at 240 °C. Total ion mass chromatograms were obtained in the Fisons MD 800 quadrupole mass spectrometer (ionization energy = 70 eV; source temperature = 250 °C) and analyzed using the Masslab software program for identification and quantification of volatiles. Quantification was performed using standard reference compounds. Peak areas were normalized using 2-heptanol as an internal standard. Calibration factors were determined using the standard addition method and creating linear regression models. Target ions were used in the identification and quantification of the component.

Addition of Substances to Beer. Additions to beer were made by opening the bottle, adding the substance to the beer, and evacuating headspace air by foaming and capping the bottle again. To study the formation of FEE from its precursors, furfuryl alcohol and furfuryl acetate were added from a stock solution (1 g/L) in amounts of 0, 250, 500, 750, and 1000 μL. To study the effect of sulfite on FEE formation, sodium sulfite was added in concentrations of 0, 5, 15, 45, 140, and 400 mg/L to beer. After the substances had been added, beers were submitted to forced aging.

To study the effect on FEE formation of the beer ethanol content, the beer was diluted twice and ethanol was added to obtain concentrations of 4, 8, 12, and 16% (v/v). Beer was transferred to sealed 50 mL Pyrex tubes with a nitrogen atmosphere. These tubes were stored for 2 days at 60 °C to accelerate aging.

Model Beer Systems. In some experiments a model system was used to mimic the reaction conditions used with top-fermented beer during aging. The model medium was a buffered water/ethanol (7.5% v/v) solution. The pH was adjusted to 4.2, or other value when stated, using a citrate buffer (0.05 M). For experiments in which the formation of FEE was studied, furfuryl alcohol was added in a concentration of 5 mg/L. On the other hand, for experiments in which the degradation of FEE was studied, FEE was added in a concentration of 1 mg/L. In some experiments additional substances were added to the medium. To study the effect of ionic strength on FEE formation, NaCl was added in concentrations of 0, 0.125, 0.375, 1.13, 3.4, and 10.1 g/L. The effect of sulfite was investigated by adding sodium sulfite in concentrations of 0, 5, 15, 45, 140, and 400 mg/L. In refermented beers yeast autolysis products may affect the aging reactions. The effect of yeast autolysis products was studied by adding yeast lysate (see further) to the model medium. Following the additions, the media were stored for a certain period of time and at specific temperatures.

Beer Refermentation Conditions. A *Saccharomyces cerevisiae cerevisiae* strain (CMBS 212) was added to the top-fermented beer together with glucose (10 g/L) at an initial concentration of 10^5 cells/mL. Bottles were incubated for 2 weeks at 24 °C to complete the refermentation. To promote cell autolysis, following the refermentation, some bottles (series B) were shaken at 200 rpm at 30 °C for 3 weeks. Afterward, shaken and nonshaken bottles (series A) were further stored at 20 or 40 °C for 4 weeks. The degree of cell autolysis in both series was assessed by determination of the yeast cell concentration. Furthermore, a control series of non-refermented beer samples was submitted to the same temperature regimen as the refermented samples.

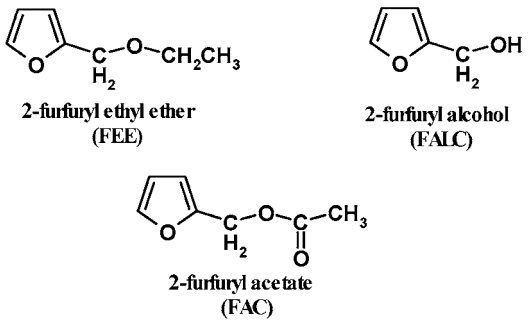

Figure 1. Chemical structures of furfuryl ethyl ether and its precursors.

Preparation of Yeast Lysate. Cultures of a *S. cerevisiae cerevisiae* strain (CMBS 212) were centrifuged at 4000*g* for 10 min, and cells were washed with model beer medium. Then 1 g of yeast was resuspended in 3 mL of the same medium, mixed with 10 g of glass beads, and mechanically disrupted by vortexing (five periods of 1 min with intervals of 1 min on ice). Repeating this procedure, 30 mL of yeast lysate was collected and then diluted to 500 mL with model beer medium. Furfuryl alcohol was then added to a concentration of 5 mg/L, and 200 mL of this solution was incubated at 20 or 40 °C for 4 weeks.

Beer Aging Conditions. Fresh top-fermented beer was subjected to three different aging conditions: storage at 0, 20, and 40 °C. After 0, 12, 25, 51, 84, 119, and 187 days of aging, two samples for each storage condition were analyzed in duplicate.

Ten fresh commercial beers were stored in an air-conditioned room at between 22 and 24 °C. After 4 years of aging, two samples of each beer were analyzed in duplicate.

RESULTS AND DISCUSSION

Pathway of Synthesis of Furfuryl Ethyl Ether in Beer. In alcoholic beverages, such as beer and wine, the most likely precursors of FEE are FALC, FAC, and ethanol (**Figure 1**). To study FEE formation from these precursors, FALC or FAC was added to fresh beer. The formation of FEE was measured after 1 day of storage at 60 °C (**Figure 2A**). The initial FEE concentration in fresh beer was 4 μg/L. Without addition of FAC or FALC, its concentration increased with 10.5 μg/L FEE during the forced aging. When FALC or FAC was supplemented, a linear relationship was found between FEE increase during aging and increased levels of added FALC or FAC. However, a higher synthesis rate of FEE was observed with FAC than with FALC. The original beer contained 24 μg/L FAC and 2493 μg/L FALC, indicating that almost all FEE in beer is probably formed from FALC. If this assumption is correct, and the FEE formation rate is directly proportional with the FALC concentration, the intercept of the FALC linear regression model (**Figure 2A**) with the horizontal axis should equal the measured FALC concentration in unsupplemented beer. With 25.9 μM (2539 μg/L), the intercept is close to the FALC concentration (2493 μg/L), which suggests that the assumptions made are indeed correct.

To examine the effect of ethanol on FEE formation, the beer was diluted and its ethanol content was then adjusted to increasing values and aged for 2 days at 60 °C. As shown in **Figure 2B**, the FEE increases were directly proportional to the ethanol concentration. In addition, the relationship between FEE formation and FALC and ethanol concentration was confirmed in experiments using the model system (results not shown).

These results suggest the following reaction mechanisms for FEE synthesis (**Figure 3**). FEE can be formed from FALC and FAC by a nucleophilic substitution reaction, replacing a leaving

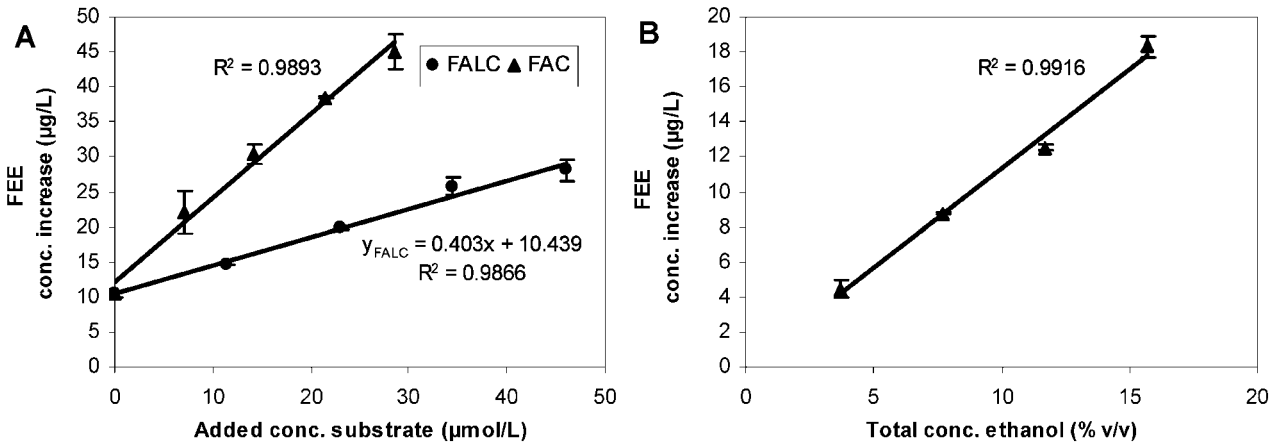

Figure 2. FEE increase in beer after 1 day of storage at 60 °C as a function of increased added amounts of FALC and FAC (**A**); FEE increase in beer after 2 days of storage at 60 °C as a function of the ethanol concentration (**B**). For each data point the standard deviation is given ($n = 3$).

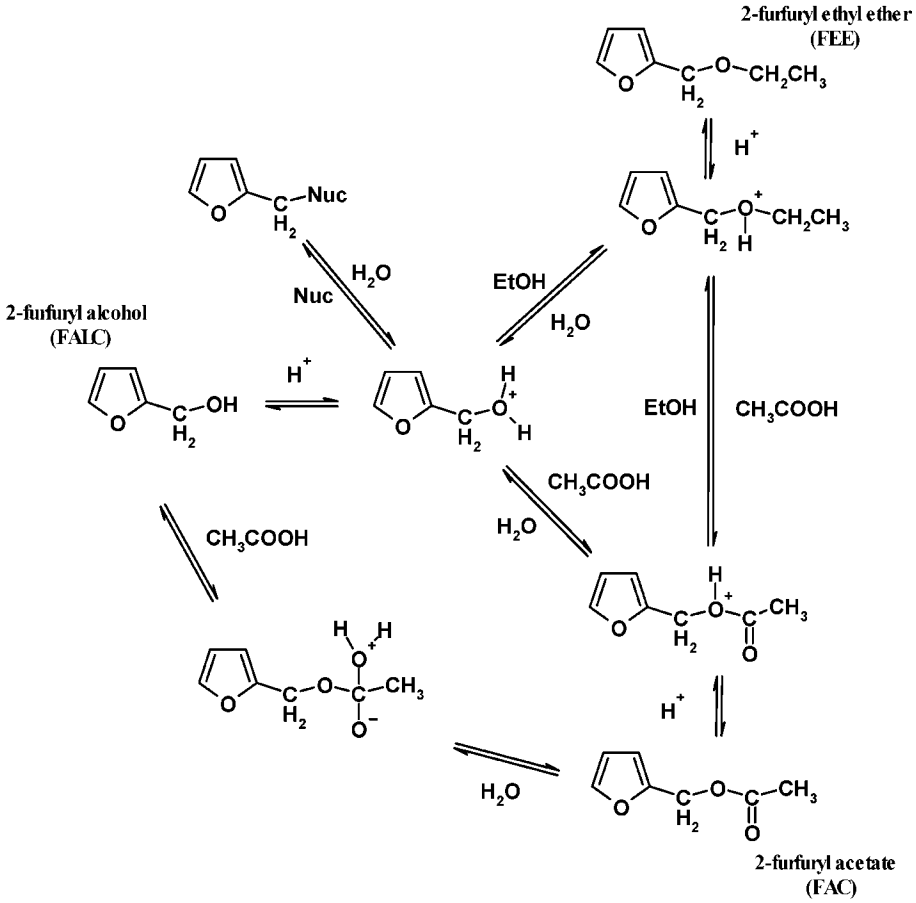

Figure 3. Proposed formation mechanism of FEE in beer during aging.

group by ethanol. FEE is, however, not directly formed from FAC and FALC, because OH^- and CH_3COO^- are not good leaving groups. Good leaving groups are created by protonation of an oxygen atom of FALC and FAC. The higher reactivity of FAC in FEE formation (**Figure 2A**) may be due to delocalization of the positive charge over the carboxyl group. This increases the stability of $FACH^+$, compared to the stability of $FALCH^+$. Furthermore, the higher reactivity of FAC compared to FALC and the linear relationship between FAC and FEE formation (**Figure 2A**) make a direct solvolysis of FAC much more plausible than hydrolysis of FAC and subsequent formation of FALC, a mechanism suggested by Harayama (*14*).

Because FEE formation is proportional to the ethanol (nucleophile) concentration, the substitution reaction in beer is most likely of the S_N2 type. However, reaction conditions in beer may also allow an S_N1 reaction, because a furfuryl carbocation is resonance stabilized and ethanol is a weak nucleophile. On the other hand, a high concentration of ethanol in beer (>5%) and the primary carbon atom on which substitution takes place favor an S_N2 reaction type. Furthermore, the aromatic pi cloud of the furan ring reduces the energy of the S_N2 transition state. This explains the sensitivity of furanic alcohols [furfuryl alcohol and 5-(hydroxymethyl)-2-furfural] toward etherification in beer.

In complex media such as beer, the presence of other nucleophiles may lead to several side reactions. Their effect on the formation rate of FEE is likely to be limited because, in beer, ethanol is much more abundant than any other nucleophile.

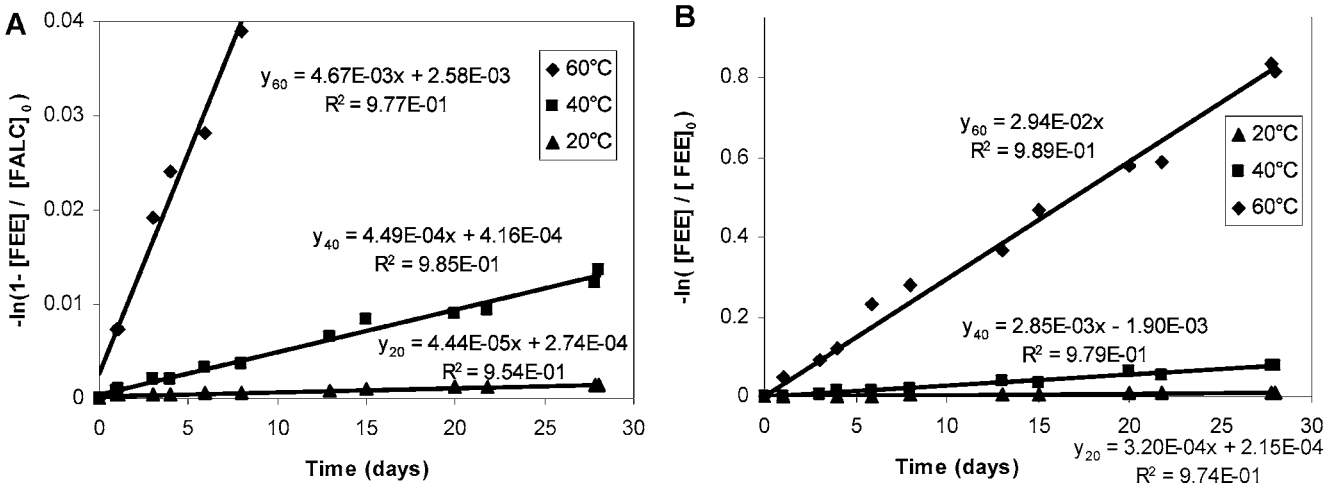

Figure 4. Formation of FEE from FALC in a model system as a function of time and at various storage temperatures (**A**); degradation of FEE in a model system as a function of time at various storage temperatures (**B**).

However, this does not exclude the possibility that such nucleophiles may lead to the formation of molecules with a very low threshold. Furfurylthiol, with a roasted, coffee-like aroma and with an extremely low flavor threshold (0.01 μg/L in water), was found to be formed by certain Maillard reactions and in furfural/H_2S systems (*15*), but a nucleophilic substitution with FALC and H_2S as nucleophile might also lead to its formation. Little is known of the flavor effects of this molecule in beer. It may affect the flavor of some aged wines (e.g., Champagne) (*16*). Further research is needed to elucidate the formation of this compound and its precise impact on flavor of beer.

Kinetics of Furfuryl Ethyl Ether Formation in Beer. To explain FEE synthesis in beer, the kinetics of its formation were studied. Because in beer FEE seems to be completely formed from FALC, the reaction can be reduced to

$$\mathrm{FALC} + \mathrm{EtOH} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \mathrm{FEE} + \mathrm{H_2O}$$

Ethanol and water are present in excess concentrations and can be considered constant in the course of the reaction. The rate law (eq 1) for this reaction in beer is therefore pseudo-first-order and depends on the concentrations of FALC ([FALC]) and FEE ([FEE]) with the respective rate constants k_1 and k_2.

$$\frac{\mathrm{d[FEE]}}{\mathrm{d}t} = k_1[\mathrm{FALC}] - k_2[\mathrm{FEE}] \qquad (1)$$

Integration of this rate equation results in eq 2, taking the initial ($t = 0$) FALC and FEE concentrations as $[\mathrm{FALC}]_0$ and $[\mathrm{FEE}]_0$, respectively.

$$[\mathrm{FEE}] = \left(1 - \frac{k_2 + k_1\,\mathrm{e}^{-(k_1+k_2)t}}{k_1 + k_2}\right)[\mathrm{FALC}]_0 + \left(\frac{k_1 + k_2\,\mathrm{e}^{-(k_1+k_2)t}}{k_1 + k_2}\right)[\mathrm{FEE}]_0 \qquad (2)$$

In reaction conditions not close to chemical equilibrium, and with no initial FEE, the approximate formation of FEE is given by eq 3.

$$[\mathrm{FEE}] = (1 - \mathrm{e}^{-k_1 t})[\mathrm{FALC}]_0 \qquad (3)$$

Table 1. Rate Constants k_1 and k_2 for the Formation or Degradation of FEE in a Model System at Different Storage Temperatures; Equilibrium Constant *K* Is Also Given

T (°C)	k_1 (1/days)	k_2 (1/days)	*K*
60	4.67E–03	2.84E–02	1.64E–01
40	4.49E–04	2.85E–03	1.58E–01
20	4.44E–05	3.20E–04	1.39E–01

Alternatively, for its degradation, eq 2 can be simplified to eq 4 with $[\mathrm{FALC}]_0$ considered as zero.

$$[\mathrm{FEE}] = \mathrm{e}^{-k_2 t}[\mathrm{FEE}]_0 \qquad (4)$$

To determine the rate constants, experiments were carried out with a model medium (same alcohol content and pH as the top-fermented beer). For the determination of k_1 an initial amount of FALC was added, and for k_2 FEE was added. Formation or degradation of FEE in time was monitored at three storage temperatures (20, 40, and 60 °C). Plots of a logarithmic relative FEE concentration (**Figure 4A**,**B**) versus storage time showed a linear relationship at all three temperatures. This observation is in accordance with eqs 3 and 4. Consequently, the slope of the fitted linear regression model gives the *k* value at a certain storage temperature.

Table 1 summarizes the values of the rate constants at different storage temperatures. The ratio of k_1 to k_2 gives the equilibrium constant *K*. At all three temperatures k_1 was smaller than k_2. A possible explanation can be deduced from the proposed reaction mechanism (**Figure 3**). In beer, the concentration of the protonated form ($FALCH^+$ and $FEEH^+$) depends on the basicity of FALC and FEE. Although the basicity constants for FEE and FALC are not known, the electron-donating effect of the ethyl group may shift the acid−base equilibrium more to the protonated form in the case of FEE. The overall reaction rate is proportional to the concentration of the protonated form because this is the actual substrate of the nucleophilic substitution. Furthermore, the rate constants are also influenced by the concentration and the strength of the nucleophiles. Ethanol is a stronger nucleophile than water, but water is more abundant in beer than ethanol.

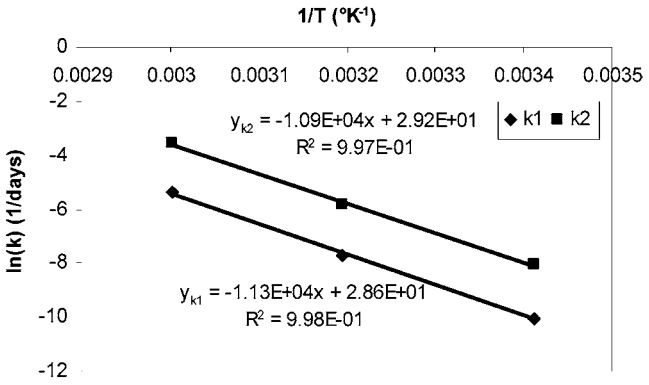

Figure 5. Arrhenius plot for rate constants k_1 and k_2.

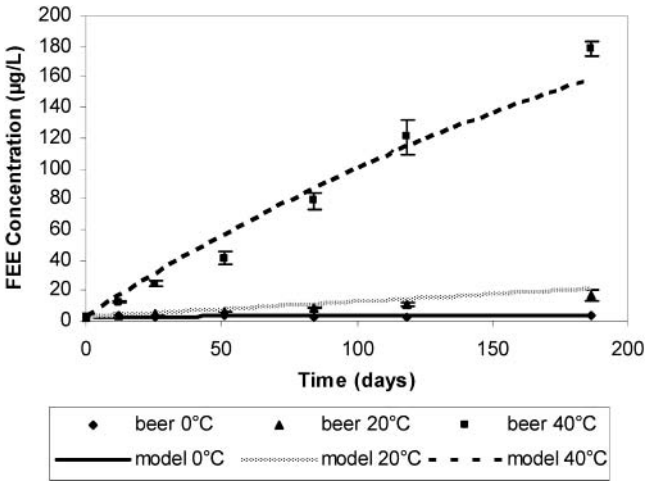

Figure 6. FEE concentration in beer during storage and values predicted by the model (eq 2) at various temperatures. For each data point from beer the standard deviation is given ($n = 4$).

The relationship between the rate constant and the storage temperature is given by the Arrhenius equation (*17*) as in eq 5.

$$\ln k = \ln A - \frac{E_A}{RT} \tag{5}$$

From the slope and intercept of the linear regression models in the Arrhenius plot (**Figure 5**), the reaction activation energy (E_A) and the pre-exponential factor (A) were calculated. For the formation of FEE, E_A was 94.3 kJ/mol and A was 2.72E+12, whereas for its degradation E_A was 90.8 kJ/mol and A was 4.61E+12. Knowledge of these kinetic parameters now allows the calculation of the concentration of FEE in beer at different storage temperatures and time intervals. In fresh beer, FEE is present in minor amounts because it is not extracted from raw materials or formed by yeast activity. Therefore, the FEE concentration found in a particular beer gives information on its storage time and temperature history. In other words, FEE can be used as a "time−temperature integrator" in beer. Similar time−temperature integrators have previously been described for milk, for which they are used to study the impact of thermal processes (*18*).

The data points in **Figure 6** represent the FEE concentration evolution in time of a top-fermented beer at various storage temperatures. To verify whether the FEE formation derived from the model system predicts well the FEE increase in beer during storage, the results calculated from the model system were compared with the results obtained by FEE determination. The initial FALC and FEE concentrations in beer were 2342 and 2.74 μg/L, respectively. From **Figure 6** it is clear that there is a close correlation between the model prediction using eq 2 and the FEE concentration measured in beer during storage. However, the model remains a simplification, as in beer FALC is formed during aging and FALC can react in side reactions with itself or with other nucleophilic molecules. Nevertheless, in our conditions of storage times and temperatures, the precision of the model prediction is apparently not affected by these possible reactions.

Inhibition and Stimulation of Furfuryl Ethyl Ether Formation in Beer. *pH Effect.* Like most other alcohols, FALC behaves as a weak base in an aquatic medium, and FALC can react with H_3O^+ to form a conjugate acid. For FEE formation, this conjugated acid is the actual substrate for nucleophilic substitution because, contrary to FALC, it has a good leaving group (i.e., H_2O). This acid−base reaction is the first step in FEE formation.

$$R{-}OH + H_3O^+ \rightleftharpoons R{-}OH_2^+ + H_2O$$

The acid−base reaction is very rapid compared to nucleophilic substitution, which is the rate-limiting step in FEE formation. An acid−base equilibrium exists in beer, and the relationship between the concentration of the conjugated acid ([FALCH$^+$]) and the FALC concentration ([FALC]) is determined by eq 6. The relationship depends on medium pH and the acidity constant of the conjugated acid (pK_{BH+}).

$$[FALCH^+] = [FALC]\frac{1}{10^{(pH-pK_{BH+})}} \tag{6}$$

A precise value of the pK_{BH+} for FALC has not yet been determined, but for conjugated acids of alcohols it is generally around −2 (*19*). The value of the pK_{BH+} is therefore much smaller than the pH range of alcoholic beverages (pH from 3 to 5), which means that [FALC] ≫ [FALCH$^+$]. As previously stated, the FEE formation rate (eq 7) is directly proportional with the FALC concentration and the rate constant (k_{pH}) is pH-dependent. The FEE formation rate is also proportional with the conjugated acid concentration, and this rate constant (k') is independent from the pH (eq 8).

$$\frac{d[FEE]}{dt} = k_{pH}\cdot[FALC] \tag{7}$$

$$\frac{d[FEE]}{dt} = k'[FALCH^+] \tag{8}$$

Using these rate laws, eq 6 can be transformed to eq 9, which shows the influence of pH on the rate constant of the FEE formation.

$$\frac{k_{pH}}{k_{4.2}} = \frac{k'}{k_{4.2}}\frac{1}{10^{(pH-pK_{BH+})}} \tag{9}$$

In eq 9, the rate constant is expressed relative to the rate constant at pH 4.2. Because k' is independent of the pH, $k'/k_{4.2}$ can be replaced by a constant, C, and rewritten.

$$\log\left(\frac{k_{pH}}{k_{4.2}}\right) = \log C + pK_{BH+} - pH \tag{10}$$

A similar equation can be derived for the rate constant (k_2) of FEE degradation. Expression 10 indicates an inversely proportional relationship between the logarithm of the rate

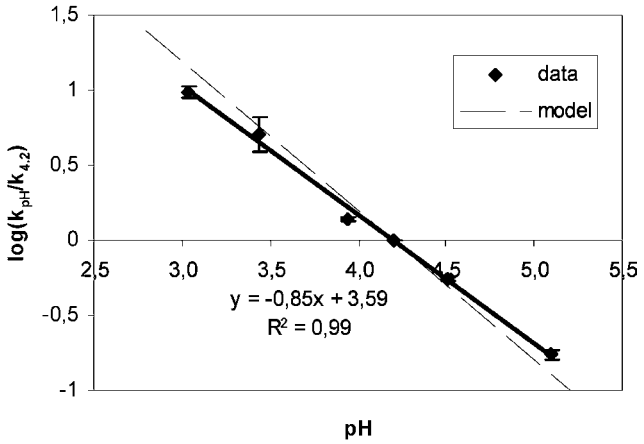

Figure 7. Effect of pH on the rate constant k_1 of FEE formation. The experimental data are compared to values calculated with eq 10. For each data point the standard deviation is given ($n = 3$).

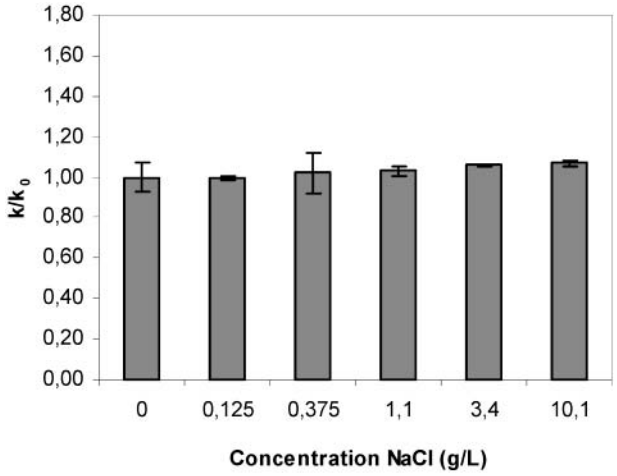

Figure 8. Effect of the ionic strength on the rate constant k_1 of FEE formation. For each data point the standard deviation is given ($n = 3$).

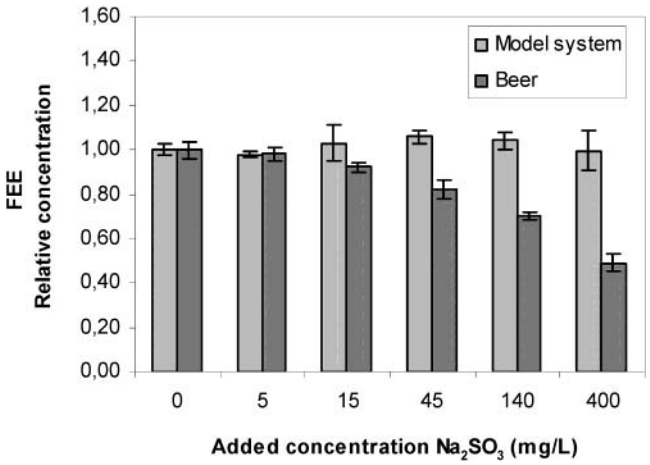

Figure 9. Effect of sodium sulfite addition on the formation of FEE in a model system and in beer after 7 days of storage at 60 °C. The ratio of a specific concentration to the concentration in beer or in the model system with no sulfite addition is given (relative concentration). For each data point the standard deviation is given ($n = 3$).

constant and the pH of the medium. A pH decrease of 1 unit causes the rate constant to increase 10 times. This was verified in experiments with the model system. The model medium with an ethanol content of 7.5% v/v was adjusted to pH values of between 3 and 5 using a citrate buffer. FEE formation was initiated by adding 5 mg/L FALC. After 1 day at 60 °C, the concentration of FEE was measured and the rate constant (k_1) at each pH was calculated using eq 3. The rate constant relative to the rate constant at pH 4.2 is given as function of the pH in **Figure 7**. For the pH range studied, a linear relationship ($r^2 = 0.99$) was observed between the medium pH and the logarithm of the rate constants. The slope of the linear regression model was −0.85, which is a little less than expected from eq 10. This could be related to increasing side reactions, mainly polymerization of FALC at lower pH values (*20*). Although the pH range in beer is mostly limited to between 4 and 4.5, the reaction rate can still vary by a factor 3, and a lower beer pH may significantly enhance the off-flavor formation due to FEE synthesis during storage. It is worth mentioning that other aging processes in beer, such as ester hydrolysis (*21*) and the release of (*E*)-β-damascenone, dimethyl trisulfide (*22*), or (*E*)-2-nonenal (*4*), are also enhanced by a lower pH, in accordance with the sensory observation that, during storage, a lower beer pH increases its flavor deterioration (*23*).

Ionic Strength. The effect of ionic strength was evaluated by adding different concentrations of NaCl to the model system. After 3 days of storage at 60 °C, the FEE concentration was measured and the rate constant was calculated using eq 3. The results given in **Figure 8** indicate that increased salt concentrations had only a very limited effect on the rate constant. As an increase in salt concentration assists S_N1 reactions in contrast to S_N2 reactions (*24*), this supports our conclusion that FEE formation in beer is an S_N2 type of reaction.

Sulfite. In contrast to wine, beer usually does not contain added sulfite, but various concentrations of sulfite can be produced by yeast. To study its effect on FEE formation, sodium sulfite was added to the model system and to the top-fermented beer. After 7 days of storage at 60 °C, the FEE concentrations were measured. The results are given in **Figure 9**. In the model system, addition of sodium sulfite did not influence the formation of FEE. In beer, on the other hand, FEE formation was lowered when more sodium sulfite was added to nonaged beer. The results of the model system indicate that sulfite does not interfere with the formation of FEE from FALC shown in **Figure 3**. FALC is formed in a Maillard reaction (*25*−*27*) and can increase during beer aging, especially at high temperatures or during long storage periods. It is known that sulfite inhibits the Maillard browning through reaction with certain intermediates such as 3,4-dideoxyhexosulos-3-ene (*28*). Hence, the reduced FEE levels in our conditions (60 °C) may result from inhibitory effects of sulfite on FALC formation rather than from a direct influence on the reaction kinetics of FEE formation.

Bottle Refermentation and Yeast Autolysis. A characteristic process applied to various top-fermented beers is a refermentation in the bottle, comparable to the bottle fermentation for the production of Champagne. Refermentation and the subsequent storage of beer in contact with the, mostly inactive, yeast sediment or its autolysis products can have profound effects on the flavor evolution of bottled beers (*29*). Therefore, the effects of a refermentation and yeast autolysis products on FEE formation were examined. In this experiment, a top-fermented beer and the model medium with added autolysis products were used. Beer samples were refermented and then first stored for 3 weeks at 30 °C followed by 4 weeks at 20 or 40 °C. One part of the samples was not shaken during storage (series A), whereas another part was shaken during the first 3 weeks (series B). The latter conditions increase yeast autolysis (*30*). The model medium with added yeast cell lysate was stored at 20 or 40 °C.

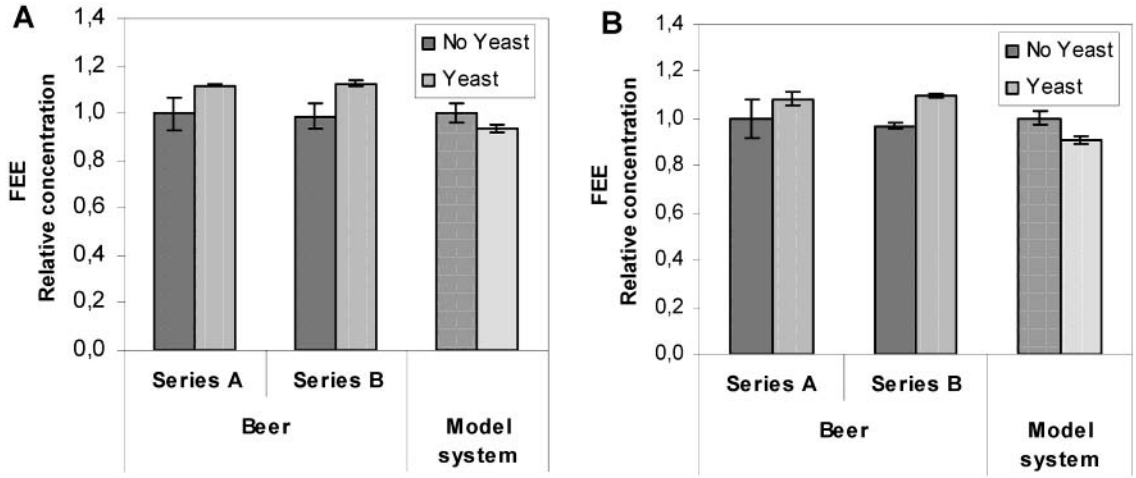

Figure 10. Effect of beer refermentation and yeast autolysis on FEE formation at 20 °C (**A**) and 40 °C (**B**). For beer, the ratio of FEE concentration in a sample to the concentration in not-refermented beer of series A is given (relative concentration). For the model system, the ratio of concentration with added lysate to the concentration with no yeast lysate is given (relative concentration). For each data point the standard deviation is given ($n = 3$).

Table 2. FEE Concentration in 10 Commercial Beers after 4 Years of Aging, Together with Some Parameters of the Fresh Beer

	beer type	FEE concn (μg/L)	SD FEE[a] (μg/L)	color (EBC)	eth %[b] (v/v)	pH	refermentation[c]
A	lager	76.5	2.1	5	5.1	4.17	–
B	blond ale	136.3	1.0	10	6.9	4.2	–
C	blond ale	148.7	3.1	12	6.9	4.3	–
D	blond ale	170.4	2.4	7	8.5	4.2	+
E	blond ale	179.7	0.6	12	7.8	4.17	+
F	wheat	216.7	8.7	11	8.6	4.28	+
G	dark ale	148.5	3.0	54	6.3	4.23	–
H	dark ale	180.7	3.9	62	6.4	4.21	–
I	dark ale	191.4	2.8	71	7.3	4.11	+
J	acidic ale	184.0	4.2	60	5.6	3.62	–

[a] SD FEE, standard deviation on FEE concentration ($n = 4$). [b] Eth % (v/v), ethanol percentage (v/v). [c] Refermentation: beer with (+) or without (–) bottle refermentation.

After 4 weeks of storage, with and without added lysate, FEE formation was measured. **Figure 10** summarizes the results. Although the differences were small at both 20 and 40 °C, the refermented beers showed an increase in FEE concentration compared to not-refermented beers. Refermentation causes the ethanol concentration of beer to increase by 0.5% v/v, which may explain the small differences. Shaking had only minor effects on FEE formation. In the model system, the presence of added yeast lysate caused a small decrease in FEE concentration. Considering the substantial amount of added lysate, this might be related to side reactions with nucleophilic compounds present in the lysate. In practice, it can be assumed that the effect of refermentation on FEE formation will largely be limited to small effects by an increased ethanol concentration.

Furfuryl Ethyl Ether in Naturally Aged Commercial Beers. Ten commercial Belgian beers, consisting of nine top-fermented beers and one lager beer, were aged for 4 years under normal storage conditions (22−24 °C). **Table 2** gives the FEE concentration, together with parameters of the respective fresh beer. Analysis of aged and fresh beer was always performed on samples from the same batch. It is clear that after 4 years of storage, the FEE concentration largely exceeded its flavor threshold of 6 μg/L. Although in beer the combined effect of various parameters determines the FEE concentration, it seems that FEE formation is positively correlated with a higher ethanol content, darker color, and lower pH. This confirms the effects found of a higher ethanol content and lower pH on the FEE formation rate from FALC. However, a lower pH generally inhibits Maillard reactions (*31*) and may thus produce less FALC during beer storage, thereby also reducing FEE formation. On the other hand, dark beers made from dark malts contain more Maillard products (*32*), and this may increase the FALC and furfural concentrations in the production wort. Furthermore, *S. cerevisiae* may reduce furfural to FALC during fermentation (*33*). Altogether, this can lead to higher initial FALC concentrations in dark beers. In lager beer, FEE formation was much less pronounced. This can partly explain the sensory observation that aging of specialty beers differs significantly from that of lager beers. Because FALC is the precursor of FEE, further research will be concentrated on the formation of this compound. The influence of brewing and fermentation parameters on the FALC concentration in beer will be investigated. This will lead to an overall insight into the parameters that determine FEE development during the storage of beer.

LITERATURE CITED

(1) Meilgaard, M. Stale flavor carbonyls in brewing. *Brew. Dig.* **1972**, *47*, 48−57.

(2) Noel, S.; Liegeois, C.; Lermusieau, G.; Bodart, E.; Badot, C.; Collin, S. Release of deuterated nonenal during beer aging from labeled precursors synthesized in the boiling kettle. *J. Agric. Food Chem.* **1999**, *47*, 4323−4326.

(3) Liegeois, C.; Meurens, N.; Badot, C.; Collin, S. Release of deuterated (*E*)-2-nonenal during beer aging from labeled precursors synthesized before boiling. *J. Agric. Food Chem.* **2002**, *50*, 7634−7638.

(4) Lermusieau, G.; Noel, S.; Liegeois, C.; Collin, S. Nonoxidative mechanism for development of *trans*-2-nonenal in beer. *J. Am. Soc. Brew. Chem.* **1999**, *57*, 29−33.

(5) Narziss, L.; Miedaner, H.; Lustig, S. The behaviour of volatile aromatic substances as beer ages. *Monatsschr. Brauwiss.* **1999**, *52*, 164−175.

(6) Foster, R. T.; Samp, E. J.; Patino, H. Multivariate modeling of sensory and chemical data to understand staling in light beer. *J. Am. Soc. Brew. Chem.* **2001**, *59*, 201−210.

(7) Schieberle, P.; Komarek, D. Changes in key aroma compounds during natural beer aging. In *Freshness and Shelf Life of Foods*; Cadwallader, K. R., Weenen, H., Eds.; American Chemical Society: Washington, DC, 2002; pp 70−79.

(8) Chevance, F.; Guyot-Declerck, C.; Dupont, J.; Collin, S. Investigation of the β-damascenone level in fresh and aged commercial beers. *J. Agric. Food Chem.* **2002**, *50*, 3818−3821.

(9) Gijs, L.; Perpete, P.; Timmermans, A.; Collin, S. 3-Methylthiopropionaldehyde as precursor of dimethyl trisulfide in aged beers. *J. Agric. Food Chem.* **2000**, *48*, 6196−6199.
(10) Williams, R. S.; Wagner, H. P. The isolation and identification of new staling related compounds form beer. *J. Am. Soc. Brew. Chem.* **1978**, *36*, 27−31.
(11) Vanderhaegen, B.; Neven, H.; Coghe, S.; Verstrepen, K. J.; Verachtert, H.; Derdelinckx, G. Evolution of chemical and sensory properties during aging of top-fermented beer. *J. Agric. Food Chem.* **2003**, *51*, 6782−6790.
(12) Cutzach, I.; Chatonnet, P.; Dubourdieu, D. Study of the formation mechanisms of some volatile compounds during the aging of sweet fortified wines. *J. Agric. Food Chem.* **1999**, *47*, 2837−2846.
(13) Simpson, R. F. Volatile aroma components of australian port wines. *J. Sci. Food Agric.* **1980**, *31*, 214−222.
(14) Harayama, K.; Hayase, F.; Kato, H. Contribution to stale flavor of 2-furfuryl ethyl ether and its formation mechanism in beer. *Biosci., Biotechnol., Biochem.* **1995**, *59*, 1144−1146.
(15) Münch, P.; Hofmann, T.; Schieberle, P. Comparison of key odorants generated by thermal treatment of commercial and self-prepared yeast extracts: Influence of the amino acid composition on odorant formation. *J. Agric. Food Chem.* **1997**, *45*, 1338−1344.
(16) Tominaga, T.; Guimbertau, G.; Dubourdieu, D. Role of certain volatile thiols in the bouquet of aged Champagne wines. *J. Agric. Food Chem.* **2003**, *51*, 1016−1020.
(17) Arrhenius, S. About the reaction rate of the inversion of non-refined sugar at souring. *Z. Phys. Chem.* **1889**, *4*, 56−58.
(18) Claeys, W. L.; Van Loey, A. M.; Hendrickx, M. E. Intrinsic time temperature integrators for heat treatment of milk. *Trends Food Sci. Technol.* **2002**, *13*, 293−311.
(19) Levitt, L. S.; Levitt, B. W. Evaluation of the basic ionization constants of water and alcohols from their ionization potentials. *J. Phys. Chem.* **1970**, *74*, 1812−1814.
(20) Gandini, A.; Belgacem, M. N. Furans in polymer chemistry. *Prog. Polym. Sci.* **1997**, *22*, 1203−1379.
(21) Neven, H. Evolution of top fermented beer esters during bottle conditioning and storage: chemical versus enzymatic hydrolysis. Dissertationes de Agricultura 333, Katholieke Universiteit Leuven, Belgium, 1997.
(22) Gijs, L.; Chevance, F.; Jerkovic, V.; Collin, S. How low pH can intensify beta-damascenone and dimethyl trisulfide production through beer aging. *J. Agric. Food Chem.* **2002**, *50*, 5612−5616.
(23) Kaneda, H.; Takashio, M.; Tomaki, T.; Osawa, T. Influence of pH on flavour staling during beer storage. *J. Inst. Brew.* **1997**, *103*, 21−23.
(24) Bunton, C. A.; Del Pesco, T. W.; Dunlop, A. M.; Yang, K.-U. Specific salt effects upon the rates on SN1 solvolyses. *J. Org. Chem.* **1971**, *36*, 887−897.
(25) Chen, J. H.; Ho, C. T. Comparison of volatile generation in serine/threonine/glutamine-ribose/glucose/fructose model systems. *J. Agric. Food Chem.* **1999**, *47*, 643−647.
(26) Wnorowski, A.; Yaylayan, V. A. Influence of pyrolytic and aqueous-phase reactions on the mechanism of formation of Maillard products. *J. Agric. Food Chem.* **2000**, *48*, 3549−3554.
(27) Yaylayan, V. A.; Keyhani, A. Origin of carbohydrate degradation products in L-alanine/D- C-13 glucose model systems. *J. Agric. Food Chem.* **2000**, *48*, 2415−2419.
(28) Keller, C.; Wedzicha, B. L.; Leong, L. P.; Berger, J. Effect of glyceraldehyde on the kinetics of Maillard browning and inhibition by sulphite species. *Food Chem.* **1999**, *66*, 495−501.
(29) Vanderhaegen, B.; Neven, H.; Coghe, S.; Verstrepen, K. J.; Derdelinckx, G.; Verachtert, H. Bioflavoring and beer refermentation. *Appl. Microbiol. Biotechnol.* **2003**, *62*, 140−150.
(30) Fornairon-Bonnefond, C.; Camarasa, C.; Moutounet, M.; Salmon, J. M. New trends on yeast autolysis and wine aging on lees: A bibliographic review. *J. Int. Sci. Vigne Vin* **2002**, *36*, 49−69.
(31) Ajandouz, E. H.; Puigserver, A. Nonenzymatic browning reaction of essential amino acids: Effect of pH on caramelization and Maillard reaction kinetics. *J. Agric. Food Chem.* **1999**, *47*, 1786−1793.
(32) Coghe, S.; Vanderhaegen, B.; Pelgrims, B.; Basteyns, A.; Delvaux, F. R. Characterization of dark specialty malts: new insights in color evaluation and pro- and antioxidative activity. *J. Am. Soc. Brew. Chem.* **2003**, *61*, 125−132.
(33) Palmqvist, E.; Almeida, J. S.; Hahn-Hagerdal, B. Influence of furfural on anaerobic glycolytic kinetics of *Saccharomyces cerevisiae* in batch culture. *Biotechnol. Bioeng.* **1999**, *62*, 447−454.

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