

Evolution of Chemical and Sensory Properties during Aging of Top-Fermented Beer

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The aging and consequent changes in flavor molecules of a top-fermented beer were studied. Different aging conditions were imposed on freshly bottled beer. After 6 months of aging, the concentration changes were recorded for acetate esters, ethyl esters, carbonyls, Maillard compounds, dioxolanes, and furanic ethers. For some flavor compounds, the changes with time of storage were monitored at different temperatures, either with CO₂ or with air in the headspace of the bottles. For some molecules a relationship was determined between concentration changes and sensory evaluation results. A decrease in volatile esters was responsible for a reduced fruity flavor during aging. On the contrary, various carbonyl compounds, some ethyl esters, Maillard compounds, dioxolanes, and furanic ethers showed a marked increase, due to oxidative and nonoxidative reactions. A very high increase was found for furfural, 2-furanmethanol, and especially the furanic ether, 2-furfuryl ethyl ether (FEE). For FEE a flavor threshold in beer of 6 μg/L was determined. In the aged top-fermented beer, FEE concentrations multiple times the flavor threshold were observed. This was associated with the appearance of a typical solvent-like flavor. As the FEE concentration increased with time at an almost constant rate, with or without air in the headspace, FEE (and probably other furanic ethers) is proposed as a good candidate to evaluate the thermal stress imposed on beer.

KEYWORDS: Top-fermented beer; beer aging; 2-furfuryl ethyl ether; furanic ethers; flavor stability

INTRODUCTION

The limited flavor stability of beer is still one of the major and less understood problems in the brewery industry. For beers, there is a need to optimize production processes toward shelf life. This is certainly necessary in a continuously competitive market. Most research on beer aging has been done on lager beer, which represents the largest part of the beer market. Especially, lipid oxidation during brewing and the appearance of *trans*-2-nonenal in beer have received much attention (1–3). The latter reaction is considered to be responsible for the development of papery and cardboard-like flavors during beer aging (4). However, most top-fermented beers do not show the same type of aging characteristics as found for lager beer (5, 6). Top-fermented beers, with their typically higher fermentation temperatures (20–24 °C) and different yeast strains, usually contain more esters and fusel alcohols, and they often have a higher alcohol content (6–10% v/v) (7). Although very little research has been done on the aging of such beers, it is often mentioned that the development of Madeira- or port wine-like flavors is typical. The objective of this study was an in-depth investigation on the chemical mechanisms involved in the aging

of top-fermented beer. Using advanced purge and trap extraction techniques combined with gas chromatography and mass spectrometry, the changes in the volatile fraction of beer were examined, as were the effects of temperature and oxygen. In addition, a relationship between the sensory evaluation of beer aging and the appearance of specific compounds was sought and discussed.

MATERIALS AND METHODS

Chemicals. The following substances with corresponding purity were supplied by Sigma Aldrich Chemie GmbH (Munich, Germany): ethyl acetate (99.9%), propyl acetate (99.9%), butyl acetate (99.7%), pentyl acetate (99.7%), hexyl acetate (99.7%), heptyl acetate (98%), octyl acetate (99+%), isobutyl acetate (99.8%), isoamyl acetate (99.7%), ethyl propionate (99.7%), ethyl butyrate (99.7%), ethyl pentanoate (99.7%), ethyl hexanoate (99+%), ethyl heptanoate (99%), ethyl octanoate (99+%), ethyl nonanoate (98+%), ethyl decanoate (99%), diethyl succinate (99.5%), ethyl phenylacetate (99+%), ethyl pyruvate (98%), ethyl lactate (98%), ethyl 3-methylbutyrate (99.7%), ethyl 2-methylbutyrate (99%), ethyl 2-methylpropionate (99%), acetaldehyde (99.5%), hexanal (98%), octanal (99%), nonanal (95%), *trans*-2-nonenal (97%), benzaldehyde (99.5%), phenylacetaldehyde (98+%), 3-methylbutanal (98%), 2-methylbutanal (95%), diacetyl (99.5%), 2,3-pentanedione (97%), 4-methylpentan-2-one (99%), 2-furfural (99%), 5-methyl-2-furfural (99%), 2-acetylfuran (99+%), 2-furanmethanol (99%), thiazole (99+%), 2-heptanol (99%), and 2,2-diphenyl-1-picrylhydrazyl (95%).

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The furanic ether 2-furfuryl ethyl ether with a purity of 95% was purchased from Narchem Corp. (Chicago, IL).

Beer Aging Conditions. A fresh pale top-fermented beer (7.5% v/v alcohol) was obtained from a Belgian brewery and subjected to five different aging conditions. Initially, the dissolved oxygen concentration was 0.5 mg/L, and the headspace contained <0.1 mg of oxygen. The beer was aged in bottles containing 250 ± 0.2 mL of beer and 10 ± 0.5 mL of headspace volume, filled with either CO₂ or air. Air was brought into the headspace by opening the bottles, flushing the headspace shortly with air, and capping it again. Beer samples with a CO₂ headspace were stored at 0 ± 0.2 , 20 ± 0.2 , and 40 ± 0.2 °C, whereas beer samples with air in the headspace were stored at 20 ± 0.2 and 40 ± 0.2 °C. After 0, 12, 25, 51, 84, 119, and 187 days of aging, two samples for each storage condition were analyzed in duplicate.

Beer Analysis. Total polyphenols were quantified according to the method of De Clerck and Jerumanis (8).

Flavonoids were quantified according to the method of Delcour and Janssens de Varebeke (9).

The reducing power of beer was measured using the method described by Kaneda et al. (10). Degassed beer (0.2 mL) was added to 2.8 mL of 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical solution (1.86×10^{-4} mol/L). The reducing power was defined as the discoloration of the DPPH solution (relative decrease in absorbance) after 5 min at 525 nm.

Beer color was measured at 430 nm according to the method of Seaton et al. (11).

Analysis of Volatile Compounds. Prior to analysis, beer was degassed by kieselguhr filtration. Then, 200 μ L of internal standard (250 mg/L 2-heptanol) and 200 μ L of a 10% antifoam solution Sigma Aldrich Chemie GmbH (Munich, Germany) were added to 50 mL of degassed beer. Five milliliters was then transferred into the Tekmar Dohrman 3000 (Emerson, St. Louis, MO) 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 steps 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, i.d. = 0.32 mm, film thickness = 1.2 μ m; Varian, Palo Alto, CA). The temperature program was as follows: 1 min at 50 °C/4 °C min⁻¹ to 120 °C/2.5 °C min⁻¹ to 165 °C/15 °C min⁻¹ to 240 °C and 5 min at 240 °C. Total ion mass chromatograms were obtained in the Fisons MD 800 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 of Volatile Compounds. Quantification was performed for compounds for which a standard reference compound was commercially available. 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 each component. For each compound a coefficient of variance (CV) was calculated from the areas obtained from eight consecutive injections of the same beer sample.

Sensory Analysis of Beer. Sensory tests on aged beers were carried out using a trained panel of 11 members. Five beers were randomly presented in one session to the panelists. Aroma and taste were each evaluated for six aspects by giving a score from 0 to 8. The selected aroma aspects were fruity, solvent, papery/cardboard, port/Madeira, red fruit, and caramel, whereas the selected taste aspects were sweet, bitter, after-bitter, pungent, astringent, and warming. A score of 0 meant the particular flavor aspect was not present, whereas a score of 8 meant the particular flavor aspect was extremely strong.

Table 1. Aging Conditions Imposed on a Top-Fermented Beer

aging condition	storage temp (°C)	headspace contents
I	0	CO ₂
II	20	CO ₂
III	20	air
IV	40	CO ₂
V	40	air

Flavor Threshold of 2-Furfuryl Ethyl Ether. The flavor threshold of 2-furfuryl ethyl ether added to a neutral lager beer was determined according to EBC Analytica (12) method 13-9, using a trained panel of 15 members. Triangle tests were performed with concentrations of 1.2, 2.3, 4.7, 9.4, 18.7, 37.2, 75.6, and 149.9 μ g/L of 2-furfuryl ethyl ether.

RESULTS AND DISCUSSION

Sensory Analysis of Aged Beer. Table 1 summarizes the five different conditions used to study beer aging.

For sensory analysis, in a first session, the panel was asked to describe the characteristics of aroma and taste of each aged beer. On the basis of the results of the first degustation, six major aroma and taste descriptors were selected and used for further sensory evaluation. In a second session the panelists were asked to quantify their intensities. The mean of the scores for each flavor aspect is given in Figure 1. It appeared that after 6 months, aroma and taste were strongly affected by the type of storage condition. Samples stored at 40 °C lost the initial fruity estery flavor, and strong Madeira- or port-like flavors developed (Figure 1B). In these conditions, a significant solvent and a minor papery flavor appeared. Samples stored at this temperature showed an increased pungency (Figure 1D).

For samples with air in the headspace, the aroma was described as more pronounced caramel-like and red fruit-like (Figure 1A). Oxidation caused the beer's bitter taste to decrease and evolve toward sweet and warming flavors (Figure 1C).

Changes in Beer Color, Polyphenol Concentration, and Reducing Power. Figure 2A shows the increase of beer color during aging. An almost linear increase was observed for samples stored at 40 °C in the absence of oxygen. The formation of colored Maillard products in these storage conditions is the predominant cause. In the presence of oxygen the color increase was rapid during the first days. This must mainly be due to oxidation and consequent degradation of polyphenols (13). Such degradation is confirmed by the decrease in flavanoids (Figure 2B) and the constant total polyphenol concentration (Figure 2C). The assay for the total polyphenol concentration is based on the measurement of the total amount of phenol cores, and consequently polymerization does not affect the determination. In contrast, the assay for flavanoids measures the amount of flavanoid structures regardless of their polymerization degree and expresses the concentration in terms of catechin equivalents. During aging, a decrease in the number of flavanoid structures was observed. According to the most accepted theory (14), oxidative mechanisms cause flavanoids to polymerize during beer storage, which explains the concentration decrease in Figure 2B. Furthermore, the increased astringency of some aged beer samples (Figure 1) is related to polyphenol degradation (15). Due to the oxidation of antioxidative molecules such as polyphenols, sulfite, and reductones, the reducing power of beer decreased, especially during the first days, at 40 °C in the presence of headspace air (Figure 2D).

Evolution of Volatile Compounds. Table 2 shows the changes in volatiles after 6 months of storage under five conditions. The molecules belong to six chemical classes:

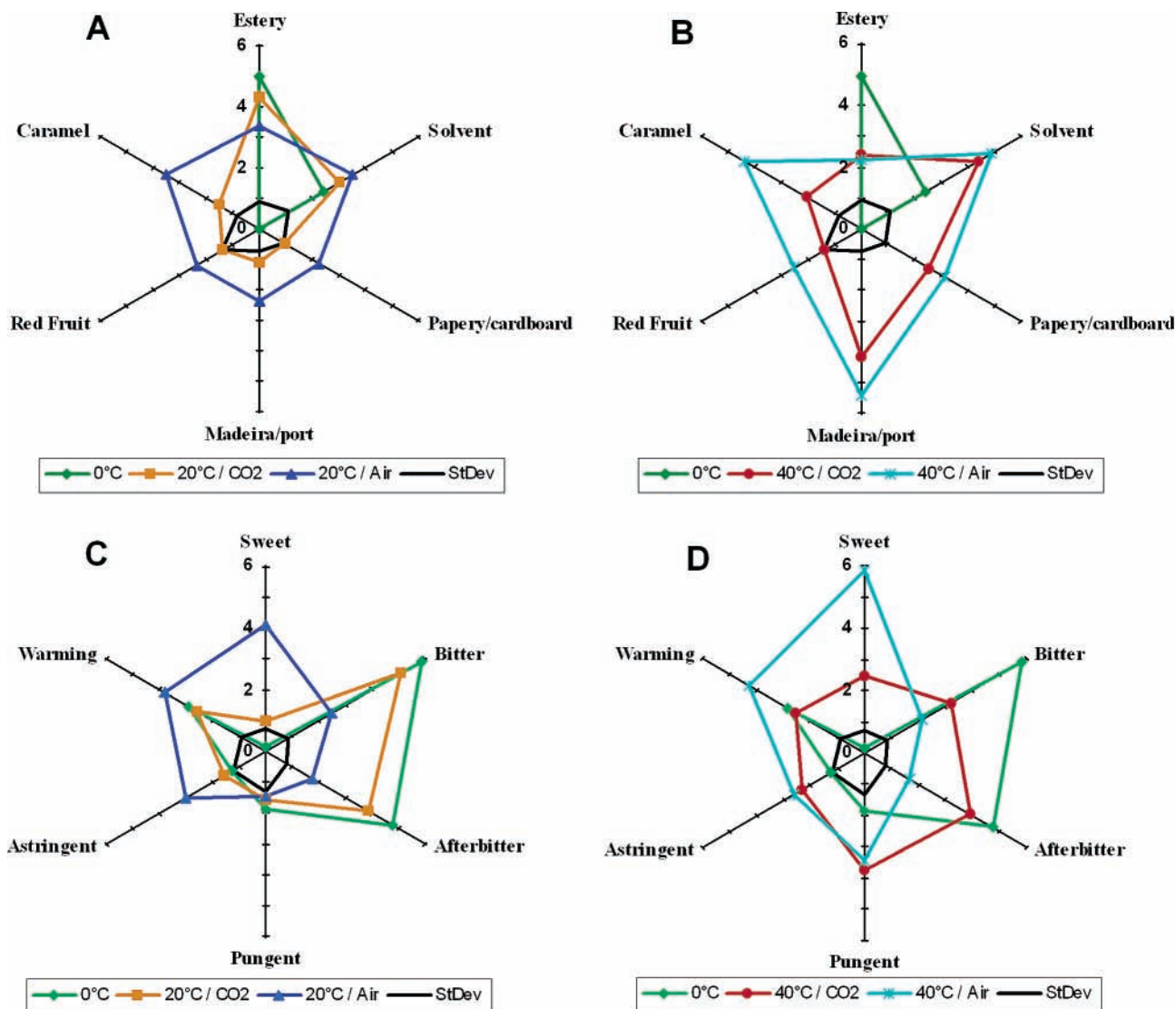


Figure 1. Aroma profiles of beer samples stored during 6 months at 20 °C (A) and 40 °C (B) and taste profiles of beer samples stored during 6 months at 20 °C (C) and 40 °C (D). Each diagram gives also the aroma or taste profile of beer stored at 0 °C and the mean standard deviation for each aroma and taste descriptor.

acetate esters, ethyl esters, carbonyl compounds, Maillard compounds, dioxolanes, and furanic ethers. Their behavior during aging and their impact on beer flavor are discussed below.

Acetate Esters. During aging, the concentration of all acetate esters decreased (**Table 2**). As an example, the hydrolysis of isoamyl acetate during beer aging is shown in **Figure 3A**. The hydrolysis appears to be independent of the presence of air in the headspace, at either 20 or 40 °C. Acetate esters in beer are secondary yeast metabolites formed in excess of their equilibrium concentration during the main fermentation. During aging, besides chemical hydrolysis, extracellular esterases released from the yeast may catalyze the breakdown of esters, as shown by Neven et al. (16). Two of the most important esters for beer flavor are isoamyl acetate, with a fruity banana flavor, and ethyl acetate, with a fruity solvent flavor (17). Only these esters appear in beer above their flavor threshold, but synergism between other esters present below their individual threshold is possible (18). Consequently, ester hydrolysis during storage may be the main cause of the loss of the fruity estery flavor note of beer. In top-fermented beers particularly, such loss of an initial strong estery flavor may also cause other components to dominate the flavor profile. **Figure 3B** shows the rate of hydrolysis of esters to be

dependent on their molecular weight: larger molecular weight esters are hydrolyzed to a higher degree. This behavior may be explained by the inverse variation of the reaction activation energy with the molecular weight of the ester (19), although reaction kinetics also depend on the initial ester, alcohol, and acid concentrations.

Ethyl Esters. The results shown in **Table 2** indicate that both the formation and hydrolysis of ethyl esters occurred during aging. Hydrolysis was generally found with linear ethyl esters synthesized by yeast during the main fermentation. Some of these, for example, ethyl hexanoate, were present in concentrations above or near their flavor threshold in fresh beer but below the threshold values after aging. The effects of ethyl ester hydrolysis on estery flavor may be similar to those described for acetate esters. The effect of the molecular weight is also comparable (**Figure 3D**).

In contrast to hydrolysis, the esterification of ethanol with organic acids also occurred during beer aging (**Table 2**). In fresh beer, the concentration of these ethyl esters is relatively low and well under the equilibrium concentration. It is therefore likely that they were little or not formed by yeast during fermentation. The precursor acids mainly originate from hop or yeast. Acids extracted from hops are 3-methylbutyric acid,

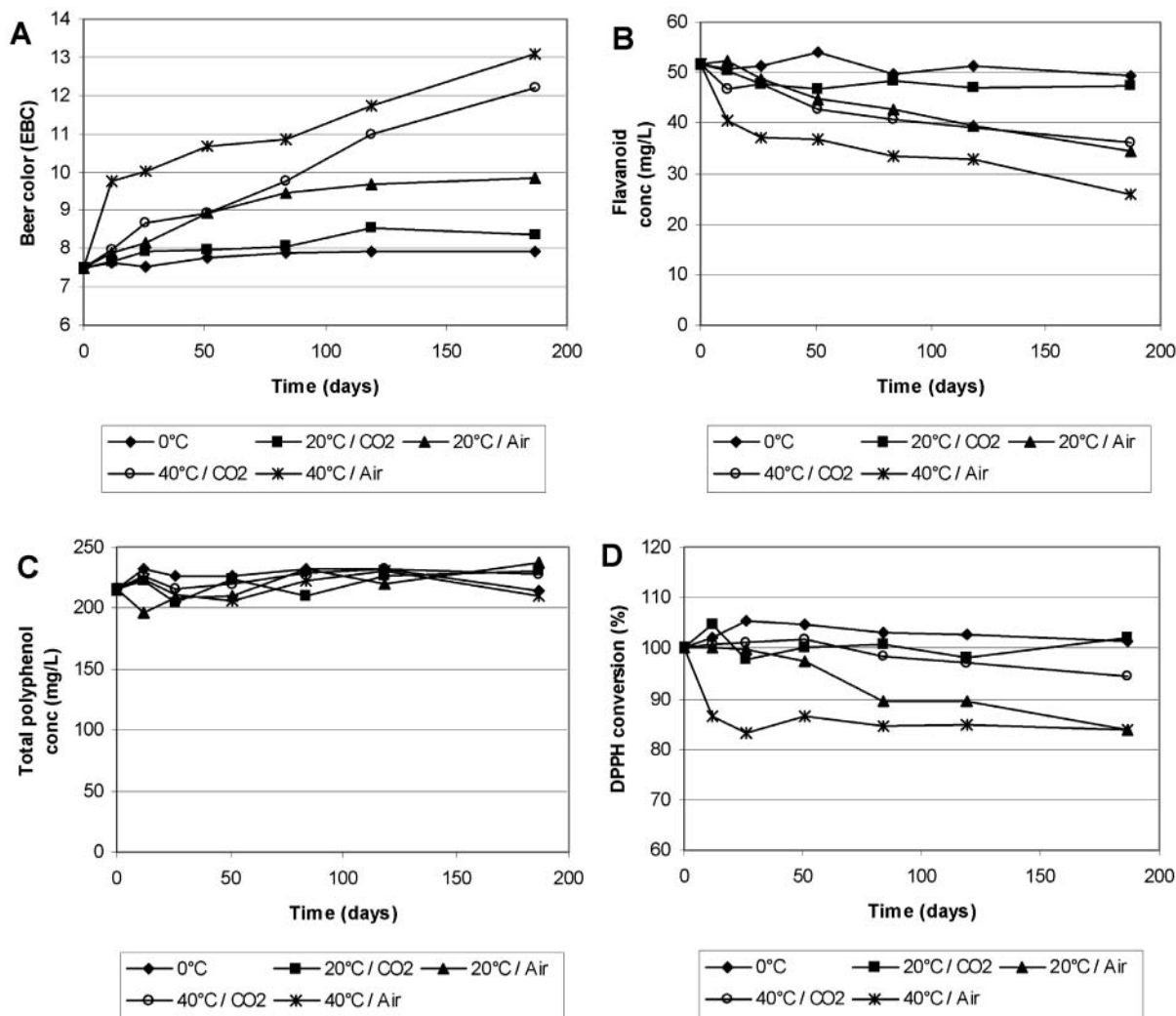


Figure 2. Evolution of beer color (A), flavanoid concentration (B), total polyphenol concentration (C), and reducing power (D) during aging of beer in various conditions.

2-methylbutyric acid, and 2-methylpropionic acid. Oxidative degradation of hop α -acids and β -acids in beer may also increase the concentration of precursor acids (20, 21). This may explain the increased levels of the corresponding ethyl esters during aging in the presence of air in the headspace, as shown for ethyl 3-methylbutyrate in Figure 3C. Furthermore, recent studies (22) indicate that certain precursor acids could also be formed due to a Strecker-type reaction.

In addition to hop acids, yeast acids are present in fresh beer. This may explain the increased concentration of diethyl succinate, ethyl pyruvate, ethyl lactate, ethyl nicotinate, and ethyl phenylacetate during aging (Table 2). Although these ethyl esters remain under their individual flavor thresholds, the effect on the flavor of their combined increase requires further research.

Carbonyl Compounds. Carbonyl compounds form an important and heterogeneous group of molecules that increased during beer aging. Some of them (Table 2) specifically increase in the presence of air in the headspace and can be used as markers for oxidation processes in bottled beer. The synthesis of acetaldehyde, mainly formed from ethyl alcohol, is very rapid, again especially at 40 °C in the presence of air (Figure 4A). At 20 °C the oxidation continues during 120 days. This persistence of oxidative reactions at 20 °C was already mentioned with respect to flavanoids, beer color, and reducing power.

Benzaldehyde may originate from the oxidative degradation of phenylpyruvic acid (23) released in beer during the phenylalanine metabolism of yeast.

Other carbonyl compounds increased through oxidative and nonoxidative reactions. Strecker degradation of the amino acids leucine, isoleucine, and phenylalanine may partly be responsible for the formation of 3-methylbutanal, 2-methylbutanal, and phenylacetaldehyde.

The vicinal diketones diacetyl and 2,3-pentanedione clearly increased during beer aging. The formation of diacetyl by a Maillard reaction (24) can explain its strong increase at 40 °C (Figure 4B). The oxidation of acetoin or 2,3-butanediol is probably responsible for increases in more oxidative conditions. The presence of air and a high storage temperature may cause diacetyl to finally increase above its flavor threshold and affect the beer flavor.

Other ketones strongly increasing during beer aging were 4-methylpentan-2-one (Figure 4C) and 3-methyl-3-buten-2-one. These molecules may originate from the degradation of hop compounds, because they are more abundant during aging when more hops are used in beer production (unpublished results). Also, the furanone 5,5-dimethyl-2(5H)-furanone has been associated with degradation of hop compounds (25).

The ketone (*E*)- β -damascenone, with a fruity-flowery flavor, increased at a constant rate at 20 and 40 °C, with or without air in the headspace (Figure 4D). The hydrolysis of glycosides and

Table 2. Concentration of Flavor Compounds Important in Beer Aging^a

volatile compound	CV (%)	FT ($\mu\text{g/L}$)	fresh beer ($\mu\text{g/L}$)	6-month-stored beer ($\mu\text{g/L}$)					
				0 °C		20 °C		40 °C	
				CO ₂		CO ₂	air	CO ₂	air
acetate esters									
ethyl acetate	2.2	21000 ^b	28099	27578	27747	26711	22340	22650	
propyl acetate	10.7	30000 ^b	12.2	12.7	11.2	10.9	9.3	9.7	
butyl acetate	4.8	7500 ^b	17.4	17.8	14.9	14.3	9.0	9.5	
pentyl acetate	3.1		14.6	15.0	13.0	11.5	8.5	6.3	
hexyl acetate	4.9	3500 ^b	7.5	6.8	4.9	4.7	2.3	1.5	
heptyl acetate	7.1	1400 ^b	15.7	10.4	6.7	7.0	1.3	0.8	
octyl acetate	9.6	500 ^b	23.0	15.8	9.3	8.9	0.7	0.7	
isobutyl acetate	7.5	1600 ^b	41.2	42.5	39.0	38.6	34.2	30.5	
isoamyl acetate	3.6	1200 ^b	1967	1972	1712	1659	760.2	788.6	
ethyl esters									
ethyl propionate	2.5	1000 ^b	58.7	60.4	66.4	68.4	99.5	86.7	
ethyl 2-methylpropionate	3.9	5000 ^b	5.5	5.6	13.8	14.8	24.5	29.1	
ethyl 3-methylbutyrate	2.1	18–20 ^c	2.8	2.9	5.0	6.1	12.3	14.3	
ethyl 2-methylbutyrate	4.5	7–20 ^c	2.7	2.7	6.8	7.1	10.7	12.1	
ethyl phenylacetate	7.5		2.7	2.9	12.3	12.7	19.8	18.5	
diethyl succinate	7.5	1200 ^b	9.8	10.2	36.8	38.9	65.5	60.8	
ethyl lactate	21.9	25000 ^b	10.7	12.2	84.0	83.5	157.5	126.7	
ethyl pyruvate	17.2		7.2	8.3	27.6	31.0	60.3	51.4	
ethyl nicotinate	12.3	2000 ^b	6.5	6.5	40.3	42.3	72.0	68.6	
ethyl butanoate	7.5	400 ^b	199.4	180.4	165.9	173.0	149.1	127.3	
ethyl pentanoate	3.7	900 ^b	10.1	9.9	9.0	8.8	7.8	7.4	
ethyl hexanoate	4.6	210 ^b	264.7	266.2	239.3	214.5	125.9	100.0	
ethyl heptanoate	7.1	400 ^b	29.3	21.3	14.5	16.1	5.7	4.3	
ethyl octanoate	11.5	900 ^b	498.7	443.9	333.7	378.4	84.4	55.4	
ethyl nonanoate	14.2	1200 ^b	10.5	8.3	3.3	2.2	1.0	0.6	
ethyl decanoate	12.9	570 ^b	118.8	91.6	51.5	57.9	9.1	9.6	
carbonyl compounds									
acetaldehyde	3.5	25000 ^b	1052	1258	1377	3211	1943	3961	
benzaldehyde	6.6	2000 ^b	5.7	5.7	5.1	11.2	6.7	12.4	
3-methylbutanal	7.9	600 ^b	7.2	7.3	7.9	37.8	14.1	43.2	
2-methylbutanal	6.3	1250 ^b	3.4	3.2	4.6	10.4	6.5	14.2	
phenylacetaldehyde	13.2	1600 ^b	19.2	20.0	29.5	49.9	53.5	67.0	
diacetyl	4.0	150 ^b	2.0	6.4	24.8	58.6	72.0	109.7	
2,3-pentanedione	6.3	900 ^b	2.0	2.6	4.9	11.3	10.6	15.5	
4-methylpentan-2-one	4.1	60000 ^b	7.3	7.5	8.5	10.5	22.3	25.0	
3-methyl-3-buten-2-one ^e	3.4		1.0	1.0	1.1	1.4	1.9	2.4	
5,5-dimethyl-2(5H)-furanone ^e	7.1		1.0	1.0	1.1	1.2	1.9	2.2	
(E)- β -damascenone ^e	13.6	150 ^b	1.0	1.0	2.0	2.6	6.8	6.4	
hexanal	4.4	350 ^b	22.4	18.8	24.0	20.1	23.9	21.7	
octanal	8.5	40 ^b	1.1	1.3	1.1	1.2	1.0	1.2	
nonanal	6.9	18 ^b	4.7	4.9	5.1	4.2	4.4	4.7	
trans-2-nonenal	18.7	0.11 ^b	0.08	0.07	0.10	0.10	0.10	0.10	
Maillard compounds									
2-furfural	5.1	15000 ^b	48.1	65.0	258.5	361.0	2329	2535	
5-methyl-2-furfural	11.4	17000 ^b	6.8	7.6	7.3	6.1	34.7	27.3	
2-acetylfuran	5.6	80000 ^b	8.4	9.1	14.0	16.1	69.5	59.0	
2-furanmethanol	13.6	3000000 ^b	2342	2353	2816	2837	4321	4254	
thiazole	4.7	23000 ^b	3.5	3.4	4.5	4.9	6.2	6.6	
dioxolanes									
2,4,5-trimethyl-1,3-dioxolane ^e	7.8	900 ^d	1.0	1.1	1.0	5.0	1.6	4.8	
2-isopropyl-4,5-dimethyl-1,3-dioxolane ^e	7.3		1.0	1.0	1.1	6.3	2.5	6.0	
furanic ethers									
2-furfuryl ethyl ether	7.4	6	2.7	3.3	16.1	18.0	187.2	178.7	
5-(ethoxymethyl)-2-furfural ^e	20.5		1.0	1.2	5.4	7.1	61.7	82.7	
2-ethoxy-2,5-dihydrofuran ^e	15.6		1.0	1.5	12.0	12.5	138.0	130.8	

^a For each component the coefficient of variance (CV), the flavor threshold (FT) ($\mu\text{g/L}$), and the concentration ($\mu\text{g/L}$) in fresh beer and in beer stored for 6 months in five different conditions are given. ^{b-d} Flavor threshold in beer according to Moll et al. (17) (b), Williams et al. (21) (c), or Peppard et al. (38) (d). ^e Product commercially unavailable as pure substance; instead of concentrations, ratios of a particular peak area to the peak area in fresh beer are given.

conversion from allene triols and dihydroxyacetylene have been suggested to explain the (E)- β -damascenone increase (26).

Although, in the past 30 years, lipid oxidation and the formation of linear aldehydes have received much attention (27–29), no significant increase of such compounds could be detected. Particularly, trans-2-nonenal, which is suggested (4) to be responsible for the cardboard flavor of aged beer, did not significantly increase. This sustains the opinion of several authors (30–34) that the historical focus on trans-2-nonenal as

the key indicator for beer aging may have to change, from both analytical and sensory standpoints. This could partly be related to the technological advances in brewing equipment, which reduce lipid oxidation in the production of beers used for these studies.

Maillard Compounds. During beer aging, various heterocyclic compounds were detected, which are intermediates of the Maillard reaction between sugars and amino acids. Although 2-furfural is often cited as an indicator for thermal damage

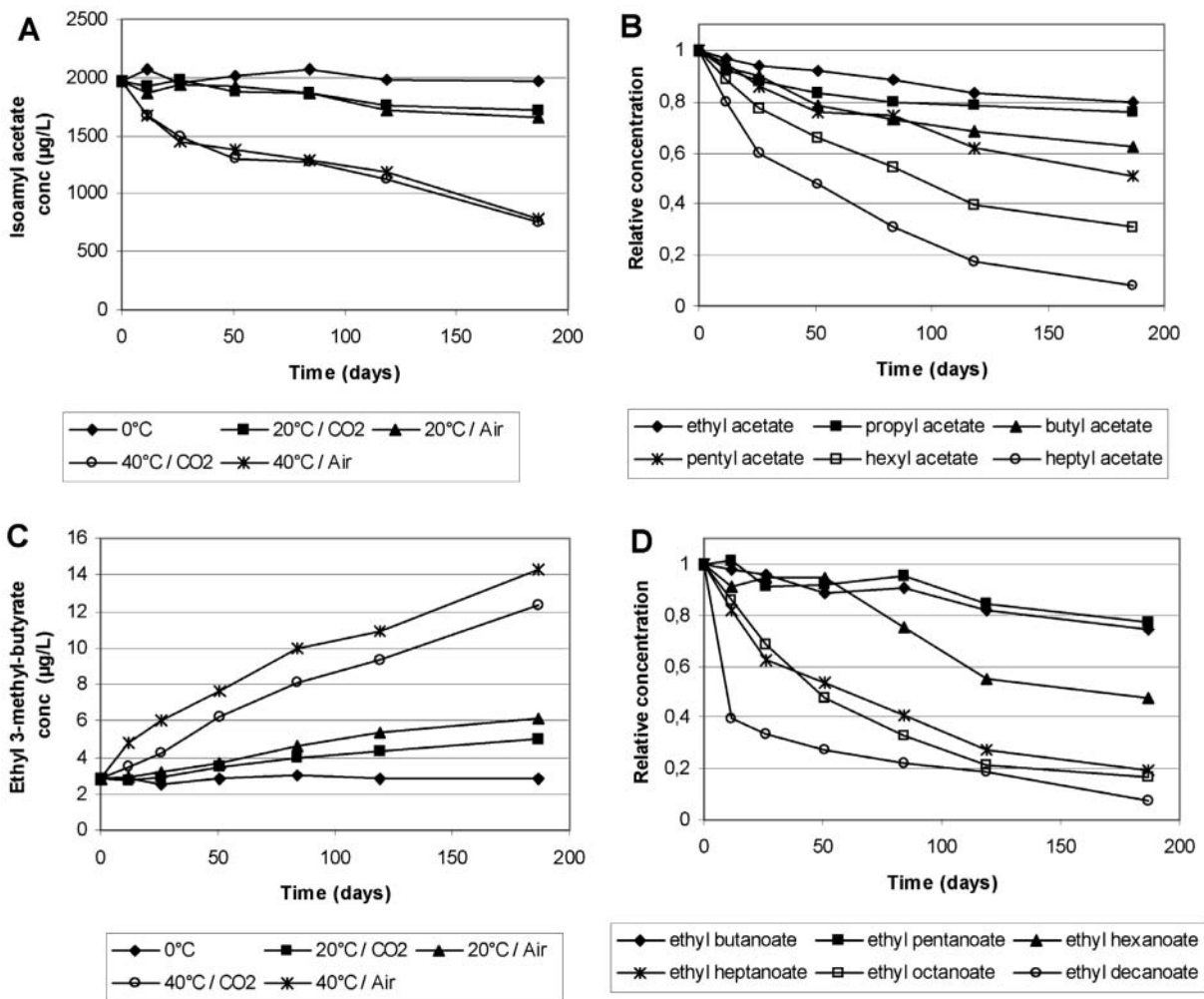


Figure 3. Concentration evolution of esters during beer aging: (A) hydrolysis of isoamyl acetate in different storage conditions (B) hydrolysis of linear acetate esters in beer stored at 40 °C (C) synthesis of ethyl 3-methylbutyrate in different storage conditions; (D) hydrolysis of linear ethyl esters in beer stored at 40 °C.

during certain stages of the production process (30, 35, 36), its increase during aging appeared also to be slightly oxygen dependent (**Figure 5A**). This was not found for the other Maillard products mentioned in **Table 2**. **Figure 5B** shows the changes for 2-furanmethanol. Formation through the Maillard reaction (37) and to a small extent through hydrolysis of 2-furfuryl acetate (maximum concentration in beer = 60 µg/L) is responsible for the increase of 2-furanmethanol. Reduction of 2-furfural by yeast during fermentation may influence the initial concentration in fresh beer. All concentrations reached after 6 months were well below the threshold values.

Dioxolanes. Two detected dioxolanes, 2,4,5-trimethyl-1,3-dioxolane and 2-isopropyl-4,5-dimethyl-1,3-dioxolane, increased significantly during aging. These molecules originate from a condensation reaction between 2,3-butanediol (up to 280 mg/L in beer) and an aldehyde (acetaldehyde or 3-methylbutanal). In beer an equilibrium between 2,4,5-trimethyl-1,3-dioxolane, acetaldehyde, and 2,3-butanediol may be reached quite rapidly but due to the increase in the acetaldehyde concentration (**Figure 4A**) the dioxolane concentration increased very similarly (**Figure 6A**). A flavor threshold of 900 µg/L and a maximum concentration in beer of ~100 µg/L were reported for 2,4,5-trimethyl-1,3-dioxolane (38). Consequently, this molecule may become a suitable marker for bottled beer oxidation during aging.

Furanic Ethers. The furanic ethers 2-furfuryl ethyl ether [FEE or 2-(ethoxymethyl)furan], 5-(ethoxymethyl)-2-furfural, and

2-ethoxy-2,5-dihydrofuran all markedly increased with aging. Although these molecules are not significantly formed during the production process, the precursor molecules ethanol and a furanic alcohol [2-furanmethanol, 5-(hydroxymethyl)-2-furfural or 2-hydroxy-2,5-dihydrofuran] are relatively abundant in the final beer. Etherification of two alcohols in an aqueous medium is an acid-catalyzed reaction involving the formation of a carbocation. The reactivity of furanic alcohols toward etherification can be explained by stabilization of the carbocation due to delocalization of the positive charge (**Figure 7**). Only 2-furfuryl ethyl ether has been detected in beer (39). In wine, 5-(ethoxymethyl)-2-furfural was found and linked with aging flavors in sweet fortified wines (40, 41).

FEE increased in time at almost constant synthesis rates of 0.9 µg/L/day at 40 °C and 0.08 µg/L/day at 20 °C. Air in the headspace did not affect the rate (**Figure 6B**). The linear behavior of its formation makes FEE an ideal marker for evaluating thermal stress imposed on beer. However, the reaction rate is also affected by the increase of the precursor 2-furanmethanol during aging (**Figure 5B**). The impact of FEE on beer flavor was assessed, and its flavor threshold was determined with a panel of 15 members. The distribution of the individual best estimate thresholds is shown in **Figure 8**. The group best-estimated threshold was 6 µg/L. A clearly distinguishable warming solvent-like flavor appeared at concentrations >20 µg/L. In conclusion, FEE affects the flavor of aged beer because

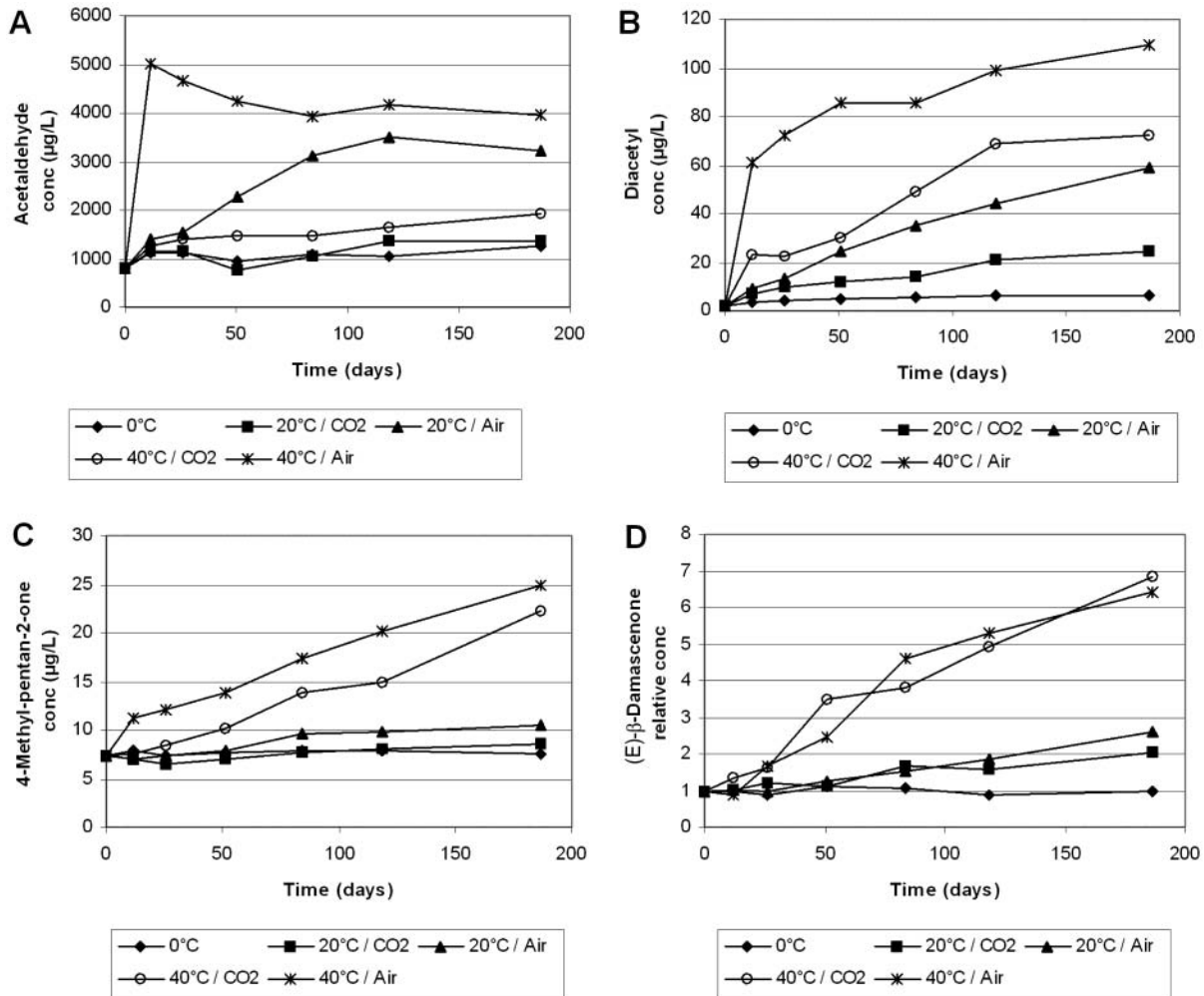


Figure 4. Concentration evolution of the carbonyl compounds acetaldehyde (A), diacetyl (B), 4-methylpentan-2-one (C), and (*E*)- β -damascenone (D) during aging of beer in various conditions.

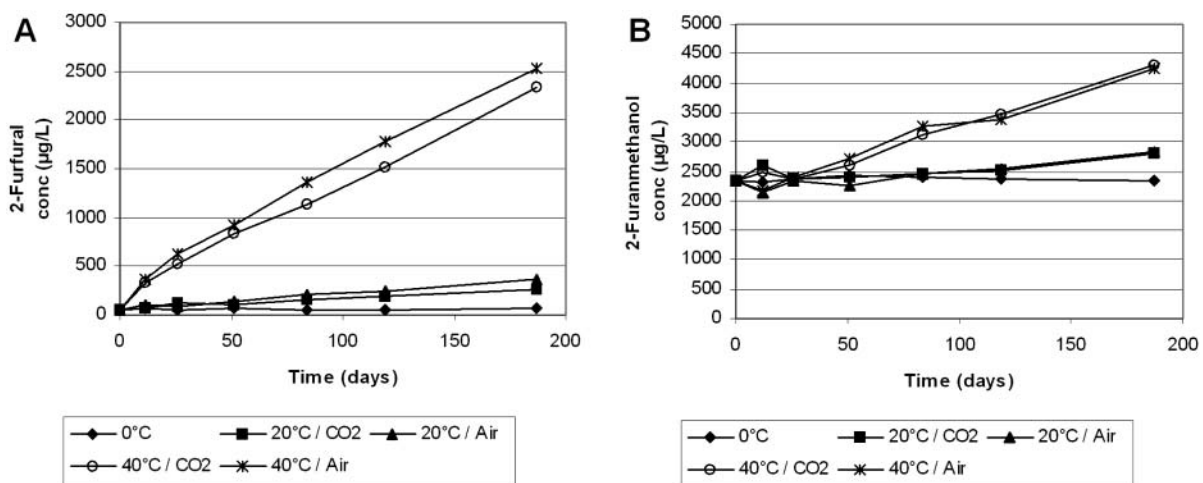


Figure 5. Concentration evolution of the Maillard products 2-furfural (A) and 2-furanmethanol (B) during aging of beer in various conditions.

in 6-month-old beer stored at 20 or 40 °C its concentration largely exceeds the threshold value. The concentration evolution of 2-ethoxy-2,5-dihydrofuran showed a behavior similar to that of FEE. In contrast, the formation of 5-(ethoxymethyl)-2-furfural was higher in the presence of air in the headspace. Evaluation of the flavor impact on beer of these newly identified aging compounds is currently in progress. Furthermore, as the etherification of ethanol with alcohols originating from the Maillard reaction is probably not limited to the compounds

above, the products formed can be regarded as a class of flavor active molecules especially important during beer aging.

As a general conclusion it becomes clear that in a top-fermented beer, all examined components, present in fresh beer, are not in chemical equilibrium conditions. During aging, chemical and biochemical reactions initiate either a decrease (e.g., esters) or an increase (ethyl esters, carbonyl compounds, Maillard compounds, dioxolanes, and furanic ethers). Some reactions are intensified by the presence of air in the headspace

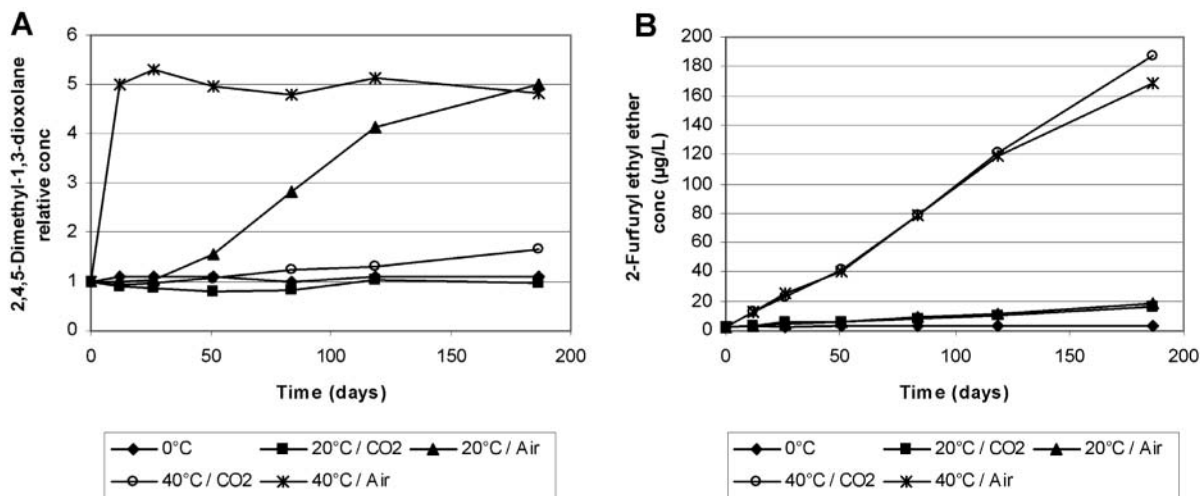


Figure 6. Concentration evolution of 2,4,5-dimethyl-1,3-dioxolane (A) and 2-furfuryl ethyl ether (B) during aging of beer in various conditions.

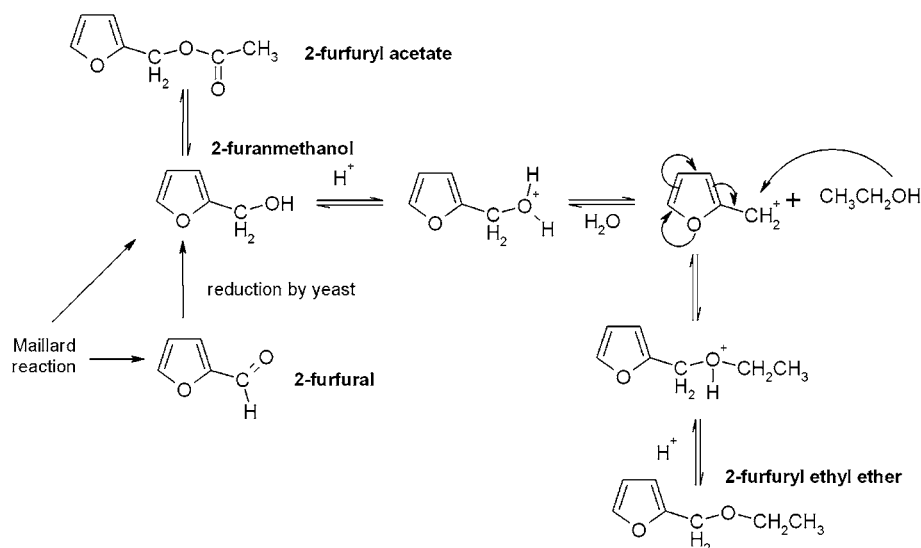


Figure 7. Proposed formation mechanism of 2-furfuryl ethyl ether in beer during storage.

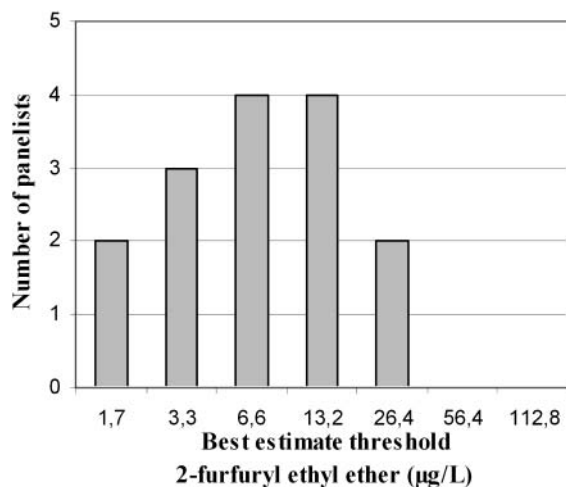


Figure 8. Distribution of the individual best estimate thresholds for 2-furfuryl ethyl ether in beer.

of bottled beer; others are not, for example, 2-furfuryl ethyl ether. Interestingly, contrary to what was found in lager (39), much larger amounts of FEE were formed during storage of the top-fermented beer. This can be due to the high amounts of 2-furanmethanol present in the fresh beer, its increase during

aging, and its continuous etherification with ethanol. As the rate of FEE formation is almost linear with time, this compound can be proposed as a marker for aging and thermal stress imposed on beer. The flavor threshold of FEE was estimated at 6 µg/L. The concentration detected during beer aging was much higher and could be related to the development of a solvent-like flavor. Harayama et al. (39) related FEE to the development of stale flavors, especially in the presence of *trans*-2-nonenal. Unexpectedly, *trans*-2-nonenal was not formed during our experiments. Further work has begun to study all parameters that may affect the formation of FEE in top-fermented beers. Special attention will be focused on the effects of yeast when top-fermented beers are subjected to bottle refermentation, a process very common in Belgium.

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