

Release of Deuterated Nonenal during Beer Aging from Labeled Precursors Synthesized in the Boiling Kettle

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The use of labeled nonenal enabled the demonstration that the appearance of the cardboard flavor in finished beer comes from lipid auto-oxidation during wort boiling and not from lipoxygenase activity during mashing. Free *trans*-2-nonenal produced by linoleic acid auto-oxidation in the kettle disappears, owing to retention by wort amino acids and proteins. This binding linkage protects *trans*-2-nonenal from yeast reduction but is reversible, allowing release of the compound at lower pH during aging. Labeled *trans*-2-nonenal is detected after aging when deuterated precursors form in the boiling kettle. The amount of alkenal released correlates with the concentration of reversible associations in the pitching wort. This work brings new illumination to the formation of *trans*-2-nonenal and overturns many previous hypotheses. It also explains why a reduction in the beer pH intensifies the cardboard flavor.

Keywords: Flavor; beer; aging; oxidation; wort proteins

INTRODUCTION

Improving flavor stability of packaged beer remains a goal of many brewers. *trans*-2-Nonenal is considered the major aldehyde responsible for a cardboard flavor (Jamieson and Van Gheluwe, 1970; Collin and Noël, 1994). Bottled oxygen has long been considered the main source of staling (Narziss, 1987), but no significant difference in *trans*-2-nonenal concentration has been observed after aging between oxygen-receiving and oxygen-free beers (Collin et al., 1997; Lermusieau et al., 1999). Grigsby et al. (1974) have shown that samples stored with higher levels of oxygen do develop a more pronounced oxidized character, but the chief flavor change is the appearance of a sweet, caramelized note, quite different from the cardboard character usually associated with beer staling. Moreover, $^{18}\text{O}_2$ appears not to incorporate into the carbonyl fraction, indicating that the cardboard flavor in beer is not due to lipid oxidation in the bottle (Collin et al., 1997; Lermusieau et al., 1999). Nor can nonenol oxidation and sulfite adduct degradation account for the appearance of *trans*-2-nonenal, as shown by adding deuterated *trans*-2-nonenal to the pitching wort (Lermusieau et al., 1999). Recently, a nonoxidative mechanism has been proposed for the appearance of *trans*-2-nonenal in aged beer. Wort amino acids and proteins can bind *trans*-2-nonenal (Noël and Collin, 1995) and protect it against yeast reduction and then release it at the beer pH (Collin et al., 1997; Lermusieau et al., 1999).

Linkages between alkenals and proteins (Michael adducts, imines...) have been characterized for many aqueous model systems containing bovine serum albumin (Amarnath et al., 1998), cytochrome C (Houston et al., 1997; Zidek et al., 1997) or human hemoglobin (Kautiainen, 1992). Such association can be further

stabilized by specific interactions (Garcia del Vado et al., 1991). The nonenal potential measurement proposed by Drost et al. (1990) appears as a quick means of assessing the amount of reversible nonenal/nitrogen compound linkage (Lermusieau et al., 1999).

In the present work, we have used labeled *trans*-2-nonenal to clarify at which step in the brewhouse the precursors of the cardboard aroma are mainly synthesized.

EXPERIMENTAL PROCEDURE

Chemicals. Dichloromethane was from Prosan (Belgium). Deuterated butan-1-ol, dibromoethane, oxetane, chloroacetaldehyde (50%), triphenylphosphine, triethylamine, benzene, and hexane were from Aldrich Chemicals (Belgium). Phosphoric acid (85%), hydrobromic acid (48%) p.a., sulfuric acid (98%) p.a., anhydrous calcium chloride, magnesium turnings, diethyl ether p.a., ammonium chloride p.a., anhydrous sodium sulfate, G60 silica, chromium trioxide p.a., chloroform, ethyl acetate, and absolute ethanol p.a. were purchased from Merck (Belgium). Dichloromethane HPLC grade came from Prosan (Belgium). Argon was from Air Liquide (Belgium).

Chromatographic Analysis of Carbonyl Compounds. Carbonyl compounds were extracted by vacuum distillation, transferred to dichloromethane, and concentrated before analysis by gas chromatography and mass spectrometry as described by Lermusieau et al. (1999). For single ion monitoring analyses, $m/z = 46, 58, 71$ were selected for labeled *trans*-2-nonenal. All analyses were done in duplicate. All variation coefficients were under 10%.

Nonenal Potential Experiment (Based on the Method of Drost et al., 1990). Applied to Wort. The pH of 1.5 L of wort was adjusted to 4 with 85% phosphoric acid. After being purged for 15 min with argon to reduce the oxygen level, the wort was heated at 100 °C for 2 h in a 2 L closed vessel and then cooled to 4 °C and kept at that temperature overnight prior to *trans*-2-nonenal analysis.

Applied to Beer. The beer sample, already saturated with CO_2 , was purged with argon for only 5 min before the heat treatment (2 h at 100 °C).

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Deuterated *trans*-2-Nonenal Synthesis. Deuterated 1-Bromobutane Synthesis (Vogel, 1962). In a stirred 50 mL flask, 2.33 mL of concentrated sulfuric acid was added dropwise to 14.2 g of hydrobromic acid (48%). Deuterated 1-butanol (5 g) was further added to the mixture, followed by 3.41 g of concentrated sulfuric acid and a few pumice stones. A reflux cooler was fitted to the flask, and the mixture was slowly boiled for 3 h. After cooling, the organic phase was decanted and washed twice with 3 mL of concentrated sulfuric acid, once with 5 mL of distilled water, once with 5 mL of a 5% sodium bicarbonate solution, and twice again with 5 mL of distilled water. The perdeuterated bromobutane was stored on anhydrous calcium chloride.

Deuteriobutylmagnesium Bromide Synthesis (Vogel, 1962). To a well-dried 250 mL conical ground-glass flask containing a magnetic stirring bar was added 1.2 g of purified magnesium turnings and a very small iodine crystal. The flask was heated until violet iodine vapor was visible. Sodium-dried diethyl ether (10 mL) was added to the mixture, followed by 3 drops of 1,2-dibromoethane. When bubbles of ethylene began to appear, magnetic stirring was started and the deuterated bromobutane was slowly added, dissolved in 50 mL of dried ether. The flask was stoppered and stored overnight. Just before use, the solution was vacuum-filtered from the excess of magnesium on a fritted-glass funnel.

Deuterated Heptanol Synthesis (Huynh et al., 1979). In a well-dried 250 mL conical ground-glass flask was dissolved 10 g of oxetane in 50 mL of sodium-dried ether. Cuprous iodide (500 mg) was added and the suspension cooled to -30°C . The filtered Grignard solution, also cooled at -30°C , was added to the mixture with stirring. The flask was stoppered and stored for 24 h at room temperature. The resulting suspension was washed with a saturated ammonium chloride solution, provision being made for air access. The organic phase was then washed twice with 20 mL of water and dried with anhydrous sodium sulfate. The deuterated heptanol was obtained after ether evaporation.

Deuterated Heptanal Synthesis (Hudlicky, 1990). Chromatography-grade G60 silica (81 g) was added to 9.2 g of chromium trioxide dissolved in 170 mL of water. After water evaporation, the rotavapor water bath was brought to boiling for 1 h. The freshly prepared silica-supported chromium trioxide was then slowly added to 300 mL of dried ether in a flask placed in a 15°C water bath to moderate ether evaporation. The deuterated alcohol dissolved in 100 mL of dried ether was then poured at once into the mixture with stirring. After 1 h, the suspension was filtered on a fritted glass funnel. The funnel residue was washed twice with 20 mL of dried ether. After rotavapor evaporation, the deuterated heptanal was recovered.

Deuterated *trans*-2-Nonenal Synthesis (Trippett and Walker, 1961). To synthesize the phosphorane reagent, 96.5 g of chloroacetaldehyde (50% in water) and 1.5 l chloroform were mixed and further distilled until 1 L was obtained. A 200 mL portion of chloroform and 131 g of triphenylphosphine were then added to the residue, and the mixture was heated under reflux for 5 h and stored overnight at room temperature. The solvent was removed under vacuum and the residue recrystallized in a chloroform-ethyl acetate mixture. A 20 g portion of the resulting phosphonium salt was mixed with 100 mL of absolute ethanol and 12 g of triethylamine. The flask was stoppered and stirred for 1 h. After evaporation under vacuum, the residue was dissolved in 100 mL of dichloromethane. The solution was washed three times with 20 mL of distilled water and dried overnight on anhydrous sodium sulfate. The solvent was removed under vacuum at temperatures not exceeding 25°C and the phosphorane reagent stored over silica gel. In a round-bottomed flask containing a stirring bar were mixed 8 g of phosphorane and 250 mL benzene with the deuterated heptanal. The mixture was heated overnight under reflux in a 110°C glycerin bath. The benzene was then removed under vacuum and the red residue extracted three times with 30 mL of hexane. The pale yellow hexane solution was concentrated under vacuum and the resulting oil purified by gas chromatography at 140°C on a 1.80 m silicone SE-30

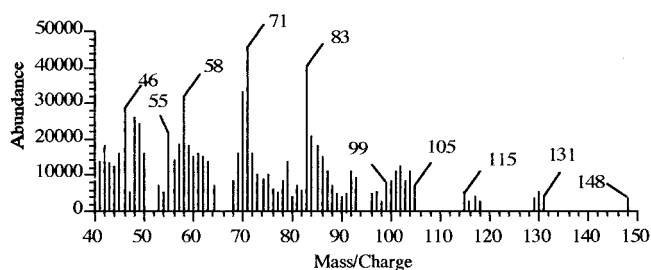


Figure 1. Mass spectrum of deuterated *trans*-2-nonenal.

column. Deuterated *trans*-2-nonenal was recovered at 25°C in dichloromethane and quantified by GC-FID with the response coefficient of the unlabeled alkenal. Its chemical purity was estimated by gas chromatography to exceed 95%. A Kovats Index of 1125 was obtained with the CP-Sil 5CB column compared to 1132 for the unlabeled compound. The mass spectrum of the deuterated *trans*-2-nonenal is shown in Figure 1.

Beer Production with Addition of Deuterated *trans*-2-Nonenal to Wort Preparation. Addition of Deuterated *trans*-2-Nonenal through Mashing. A 4.3 kg flour of an Alexis malt kilned up to 84°C was added under shaking into 13 L of water maintained at 36.5°C in the mash tun. After the mixture was mashed for 15 min at 36.5°C and 30 min at 49.5°C , 33.5 mg of deuterated *trans*-2-nonenal, transferred from dichloromethane to absolute ethanol and further dissolved in 100 mL of deionized water, was added to a concentration of 1.5 ppm as measured in an equivalent 12 °P wort. After being heated at 63.5°C for 15 min and at 76°C for 10 min, the temperature of the wort was raised to 78°C before filtration on a 2001 mash filter (Meura, Louvain-la-Neuve, Belgium). The wort was further adjusted to 12 °P with deionized water and boiled with liquid CO_2 hop extract (16 °EBU) for 90 min. After clarification for 20 min, the hot break was removed and the wort quickly cooled to 12°C . The pH was adjusted to 5.2 and the density to 12 with sulfuric acid and sterilized water, respectively. The 8 ppm oxygenated wort was fermented with a lager yeast (12.5×10^6 cell/mL at pitching) as follows: primary fermentation at 12°C for 4 days, 14°C for 2 days, and 16°C for 4 days; maturation at 10°C for 2 days, 7°C for 2 days, and 0°C for 3 days. The beer was filtered on a membrane filter and bottled.

In the second experiment, labeled *trans*-2-nonenal was monitored through boiling: 10 mg of deuterated *trans*-2-nonenal was added as described above to 18.2 kg of flour mashed in 57 L of water (final concentration = 100 ppb in a 12 °P equivalent wort).

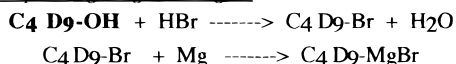
Addition of Deuterated *trans*-2-Nonenal through Boiling. A 23 L portion of an industrial filtered 12 °P wort was boiled with liquid CO_2 hop extracts (16 °EBU) for 90 min. After 40 min of boiling, 34.5 mg of deuterated *trans*-2-nonenal was transferred from dichloromethane to absolute ethanol and further dissolved in 100 mL of deionized water. The clarification, fermentation, and maturation steps were as described above.

Beer Production from Worts with Different Concentrations of Nitrogenous Compounds/Nonenal Linkages. A 20 L portion of an industrial filtered 12 °P wort was boiled with liquid CO_2 hop extract (16 °EBU) for 90 min. After 40 min of boiling, 1, 2, or 3 ppm of *trans*-2-nonenal in ethanol was added. After a 20 min clarification step, the hot break was removed and the wort quickly cooled to 18°C . The pH and density were adjusted respectively to 5.2 and 12 °P, as described above. Fermentation of the 8 ppm oxygenated wort was carried out with a top-fermentation yeast (10×10^6 cell/mL at pitching) at 18°C for 7 days, 10°C for 2 days, 7°C for 2 days, and 0°C for 3 days. The beer was filtered on a membrane filter and bottled.

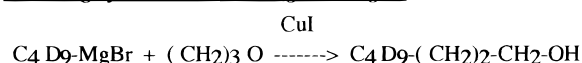
RESULTS

Since lipoxygenase activity is recognized as the major source of linoleic acid oxidation in the brewhouse, we

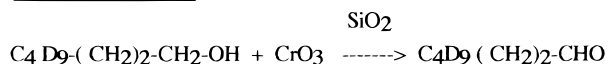
1) Transformation of deuterated butan-1-ol to the corresponding Grignard reagent



2) Adding of oxetane on the Grignard reagent



3) Alcohol oxidation



4) Wittig reaction

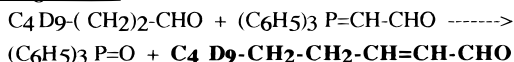


Figure 2. Principle of deuterated *trans*-2-nonenal synthesis.

Table 1. *trans*-2-Nonenal (-H) and Deuterated *trans*-2-Nonenal (-D) Contents (ppb) in Wort and Beer Samples after Addition of 1.5 ppm Deuterated Nonenal during Mashing

	<i>trans</i> -2-nonenal-H	<i>trans</i> -2-nonenal-D ^a
fresh beer	0.06	ND
beer after an accelerated aging (5 days at 40 °C)	0.19	ND
beer after a 3 month natural aging	0.27	ND

^a ND = below 0.03 ppb.

decided to add the labeled *trans*-2-nonenal after 45 min of mashing to mimic enzymatic production. The chemical synthesis of ²H-labeled *trans*-2-nonenal was designed to avoid modifying the reactivity of the alkenal (see Figure 2). Butan-1-ol-*d*₉ was chosen as source of ²H label. The corresponding Grignard reagent was transformed to heptanol, further oxidized into deuterated heptanal, so as to yield by Wittig reaction labeled *trans*-2-nonenal.

In a first experiment, mashing was carried out on a small scale (4.3 kg of flour) with addition of 1.5 ppm of deuterated *trans*-2-nonenal. Because of the small volume, we analyzed only fresh and aged beer samples. Although probably linked to nitrogenous wort compounds such as other alkenals, no labeled *trans*-2-nonenal was released into the final product during aging (concentration of labeled *trans*-2-nonenal below 0.03 ppb as shown in Table 1). The excessive level of deuterated alkenal here added allowed us to conclude that the lipoxygenase activity occurring during wort mashing does not lead to cardboard flavor in the aged beer.

To explain this result, we carried out mashing on a larger scale (18.2 kg of flour), adding 100 ppb of deuterated *trans*-2-nonenal and analyzing intermediate samples. Since measuring the nonenal potential is proposed as a means of quantifying reversible bound *trans*-2-nonenal in the wort (Collin et al., 1997), we may conclude from analysis of the spent grain that a large amount of the labeled *trans*-2-nonenal was bound to insoluble proteins (deuterated nonenal potential at pH 2: 6.9 ppb, see Table 2).

As previously observed (Collin et al., 1997), wort filtration lead to the partial extraction of the spent grain nonenal potential (0.8 before filtration and 2.7 ppb afterward). This nonenal potential due to soluble labeled

Table 2. Labeled (-D) and Unlabeled (-H) Nonenal Contents and Nonenal Potential (ppb) in Wort and Spentgrain Samples after Addition of 100 ppb of Deuterated Nonenal during Mashing

	<i>trans</i> -2-nonenal		nonenal potential	
	-H	-D	-H	-D
wort before filtration	0.66	0.85	0.7	0.8
wort after filtration	0.57	0.62	3.2	2.7
spent grain ^a (pH 2)	0.52	ND	17.9	6.9
wort before fermentation	0.30	ND	3.4	0.2

^a pH adjusted to 2 before distillation or the nonenal potential heat treatment. Results are given for an equivalent 12 °P wort including 200 g of spent grain per liter of wort.

Table 3. Labeled (-D) and Unlabeled (-H) Nonenal Contents and Nonenal Potential (ppb) in Samples Taken from the Production with Addition of Deuterated *trans*-2-Nonenal (1.5 ppm) after 40 Min of Boiling

	<i>trans</i> -2-nonenal content (ppb)			
	<i>trans</i> -2-nonenal		nonenal potential	
	-H	-D	-H	-D
wort before fermentation	1.0	15.0	4.3	15.5
fresh beer	0.20	0.22	1.6	1.3
beer after a 3 days accelerated aging at 40 °C	0.73	0.51		
beer after a 4 days accelerated aging at 40 °C	0.53	0.47		
beer after a 5 days accelerated aging at 40 °C	0.41	0.47		

nonenal was destroyed by boiling, so that the deuterated-nonenal potential was only 0.2 ppb before fermentation. Auto-oxidation during the boiling step thus probably accounts for the unlabeled nonenal potential measured in the final wort (4.0 ppb).

To confirm that new linkages between nonenal and proteins can form during the boiling step, 1.5 ppm labeled *trans*-2-nonenal was added after 40 min of boiling, allowing interactions with nitrogenous compounds for 35 min of boiling and the 15 min clarification step. Addition of a high level of *trans*-2-nonenal was necessary in order to avoid quick losses by steam distillation or oxidation. In the case of linoleic acid auto-oxidation, synthesized *trans*-2-nonenal is supposedly better protected in the lipidic environment until it binds to proteins.

As shown in Table 3, a labeled nonenal potential of 15.5 ppb was measured in the wort before fermentation. The fact that half of this nonenal potential is due to free nonenal (Drost's experiment only removes 50% of the initial *trans*-2-nonenal) means that around 8 ppb are reversibly linked to nitrogen materials. The results for fresh beer confirm that the nonenal potential can partly subsist during fermentation (1.3 ppb in fresh beer). On the other hand, free deuterated *trans*-2-nonenal was well reduced by yeast despite its high level before fermentation so that only 0.2 ppb was detected in the fresh beer. *trans*-2-Nonenal of the fresh beer is oxidized into nonenoic acid at the beginning of aging (Collin et al., 1997). Therefore, *trans*-2-nonenal found in aged beer is totally issued from precursors. As significant levels of deuterated *trans*-2-nonenal were detected in aged beers after 3, 4, or 5 days at 40 °C, we can conclude that the precursors of the cardboard flavor, which account for the nonenal potential of the clarified wort, are produced mainly during boiling.

A second experiment confirmed this hypothesis. Indeed, when the nonenal potential of the wort was

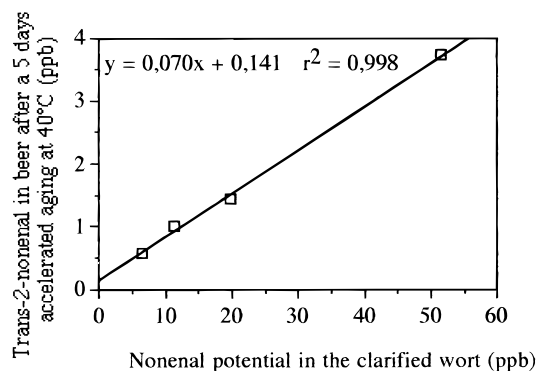


Figure 3. Relationship between the nonenal potential of the wort before fermentation and *trans*-2-nonenal level in aged beer.

artificially increased by increasing the levels of *trans*-2-nonenal through boiling, the amount of *trans*-2-nonenal found in the aged beer correlated with the amount added during boiling (see Figure 3).

We may thus assume that the reduction power of the wort will be of prime importance in achieving better control of the cardboard flavor. Collin et al. (1997), Lermusieau et al. (1999), and Drost et al. (1990) have previously shown that the oxidation level during mashing (oxygen or carbon dioxide, high or low lipoxygenase activity...) did influence the rate at which the beer ages, probably by influencing auto-oxidation in the kettle.

CONCLUSION

Unlike its enzymatically produced counterpart, *trans*-2-nonenal synthesized by linoleic acid auto-oxidation during boiling can persist in the fresh beer because it binds to nitrogenous wort compounds. This yeast-resistant *trans*-2-nonenal fraction, estimated by measuring the nonenal potential, will be released as *trans*-2-nonenal during aging, mainly when the pH is low or the temperature high. As previously suggested, a low level of oxygen during mashing, or a high malt antioxidant level, should logically decrease the cardboard flavor of aged beer. All efforts must now be made to strengthen the reduction power of the wort, as measured by ESR-spin trapping technique (Kaneda et al., 1988; Uchida et al., 1996a,b), decolorization of the DPPH radical (Kaneda et al., 1995 a,b), or chemiluminescence (Kaneda et al., 1990 a,b, 1994). Better flavor stability will require both careful selection of raw materials and technological improvements in the brewhouse.

ACKNOWLEDGMENT

The authors thank Interbrew (Leuven, Belgium) and Tepral (Strasbourg, France) for financial support.

LITERATURE CITED

- Amarnath, V.; Valentine, W. M.; Montine, T. J.; Patterson, W. H.; Amarnath, K.; Bassett, C. N.; Graham, D. G. Reactions of 4-hydroxy-2(E)-nonenal and related aldehydes with proteins studied by carbon-13 nuclear magnetic resonance spectroscopy. *Chem. Res. Toxicol.* **1998**, *11*, 317–328.
- Collin, S.; Noël, S. Réactions chimiques et enzymatiques productrices d'arômes au brassage. *Cerevisia Biotechnol.* **1994**, *19*, 25–32.
- Collin, S.; Noël, S.; Bonte, S.; Metais, N.; Bodart, E.; Peladan, F.; Dupire, S. Utilisation d' $^{18}\text{O}_2$ pour évaluer l'impact des phénomènes d'oxydation durant le brassage et le stockage de la bière (The use of $^{18}\text{O}_2$ in appraising the impact of

- oxidation processes during mashing and beer storage). *European Brewery Convention*; IRL Press, Oxford University Press: Oxford, U.K., 1997; pp 535–544.
- Drost, B. W.; Van den Berg, R.; Freijee, F. J. M.; van der Velde, E. G.; Hollemans, M. Flavor stability. *J. Am. Soc. Brew. Chem.* **1990**, *48*, 124–131.
- Garcia del Vado, M. A.; Echevarria, G. R.; Garcia Blanco, F.; Santos Blanco, J. G.; Vallejo, J. L.; Garcia de Paz, J. L. On the stability of the Schiff bases of Pyridoxal 5'-phosphate with polypeptides containing L-lysine. *Helv. Chim. Acta* **1991**, *74*, 1749–1756.
- Grigsby, J. H.; Palamand, S. R.; Hardwick, W. A. Further studies on the staling of beer. *Proc. Am. Soc. Brew. Chem.* **1974**, 64–69.
- Houston, C. T.; Baker, A. J.; Novotny, M. V.; Reilly, J. P. Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry as a tool to probe the reactions of *trans*-hex-2-enal with proteins. *J. Mass Spectrom.* **1997**, *32*, 662–665.
- Hudlicky, M. Oxidations in organic chemistry; Monograph; American Chemical Society: Washington, DC, 1990; p 186.
- Huynh, C.; Derguini-Boumechal F.; Linstrumelle G. Copper-catalysed reactions of grignard reagents with epoxides and oxetane. *Tetrahedron Lett.* **1979**, *17*, 1503–1506.
- Jamieson, A. M.; Van Gheluwe, J. E. A. Identification of a compound responsible for cardboard flavour in beer. *Proc. Am. Soc. Brew. Chem.* **1970**, 192–197.
- Kaneda, H.; Kano, Y.; Kamimura, M.; Osawa, T.; Kawakishi, S. Detection of chemiluminescence produced during beer oxidation. *J. Food Sci.* **1990**, *55*, 881–882.
- Kaneda, H.; Kano, Y.; Kamimura, M.; Osawa, T.; Kawakishi, S. Evaluation of beer deterioration by chemiluminescence. *J. Food Sci.* **1990**, *55*, 1361–1364.
- Kaneda, H.; Kano, Y.; Osawa, T.; Ramarathnam, N.; Kawakishi, S.; Kamada, K. Detection of free radicals in beer oxidation. *J. Food Sci.* **1988**, *53*, 885–888.
- Kaneda, H.; Kobayashi, N.; Furusho, S.; Sahara, H.; Koshino, S. Chemical evaluation of beer flavor stability. *MBAA Tech. Quart.* **1995**, *32*, 76–80.
- Kaneda, H.; Kobayashi, N.; Furusho, S.; Sahara, H.; Koshino, S. Reducing activity and flavor stability of beer. *MBAA Tech. Quart.* **1995**, *32*, 90–94.
- Kaneda, H.; Kobayashi, N.; Tsuchiya, Y.; Munekata, M.; Koshino, S. Some applications of chemiluminescence analysis to brewing. *J. Am. Soc. Brew. Chem.* **1994**, 163–168.
- Kautiainen, A. Determination of hemoglobin adducts from aldehydes formed during lipid peroxidation in vitro. *Chem. Biol. Interact.* **1992**, *83*, 55–63.
- Lermusieau, G.; Noël, S.; Liégeois, C.; Collin, S. A non-oxidative pathway for the development of *trans*-2-nonenal in beer. *J. Am. Soc. Brew. Chem.* **1999**, 29–33.
- Narziss, L. Beer stability. *Brauwelt Int.* **1987**, *1*, 46–50.
- Noël, S.; Collin, S. *trans*-2-Nonenal degradation products during mashing. *European Brewery Convention*; IRL Press, Oxford University Press: Oxford, U.K., 1995; pp 483–490.
- Trippett, S.; Walker, D. M. The phosphobetaines: preparation and properties. *J. Chem. Soc.* **1961**, *246*, 1266–1272.
- Uchida, M.; Ono, M. Improvement for oxidative flavor stability of beer – Role of OH-radical in beer oxidation. *J. Am. Soc. Brew. Chem.* **1996a**, 198–204.
- Uchida, M.; Suga, S.; Ono, M. Improvement for oxidative flavor stability of beer – Rapid prediction method for beer flavor stability by electron spin resonance spectroscopy. *J. Am. Soc. Brew. Chem.* **1996b**, 205–211.
- Vogel, A. I. *Practical organic chemistry*, 3rd ed.; Longmans: Essex, 1962.
- Zidek, L.; Dolezel, P.; Chmelik, J.; Baker, A. G.; Novotny, M. Modification of horse heart cytochrome *c* with *trans*-2-hexenal. *Chem. Res. Toxicol.* **1997**, *10*, 702–710.

Received for review February 26, 1999. Revised manuscript received June 14, 1999. Accepted July 7, 1999.

JF990211R