Analysis of Long-Chain Fatty Acids in Beer by HPLC–Fluorescence Detection Method

Hirotaka Kaneda,*,‡ Yukinobu Kano,‡ Minoru Kamimura,‡ Toshihiko Osawa,§ and Shunro Kawakishi§

Brewing Research Laboratories, SAPPORO Breweries Ltd., 10 Okatohme, Yaizu-Shi, Shizuoka 425, Japan, and Department of Food Science and Technology, Faculty of Agriculture, Nagoya University, Chikusa-Ku, Nagoya 464, Japan

Long-chain fatty acids (C_{12}–C_{18}) in beer were determined by the HPLC–fluorescence detection method using 9-(chloromethyl)anthracene as a labeling reagent. The detection limits were 0.2 μg/L in beer. Fatty acids of C_{14}–C_{18} in beer were reduced 10–20% during incubation at 60 °C. The degradation of unsaturated fatty acids (C18:1, C18:2) was accelerated by additions of Fe^{2+} and/or H_2O_2, while it was inhibited by EDTA. It was thought, therefore, that the degradation of these unsaturated acids was responsible for the free-radical reactions in beer. From a model experiment, it appeared that the degradation of these acids would be inhibited by polyphenols in beer.

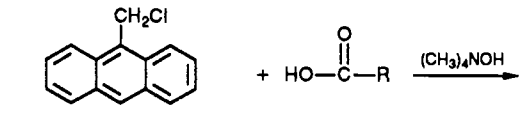
Free fatty acids in beer are important because some of them have a high flavor potential. Linoleic and linolenic acids are especially of great importance because their oxidative degradation, by means of either enzymatic or free-radical reactions, may lead to the formation of a characteristic aging flavor (Dominguez and Canales, 1974; Krauss et al., 1975; Tressl et al., 1979). The autoxidation of these unsaturated fatty acids produces several hydroperoxides (Esterbauer, 1982). These hydroperoxides are unstable and degrade into low molecular weight compounds (aldehydes, ketones, and acids) that include 2-alkenals and 2,4-alkadienals. It has been theorized that *trans*-2-nonenal is responsible for the cardboard "off-flavor" (Meilgaard, 1972; Drost et al., 1971; Jamieson et al., 1970). The threshold concentration is very low (0.11 μg/L). Wang and Siebert (1974a,b) showed that fresh beer contains 0.02–0.06 μg/L of *trans*-2-nonenal and that incubation at 38 °C causes the concentration to rise rapidly, passing the threshold level after 3–4 days, at which point the beer begins to have a papery flavor. Linolenic acid in beer degrades into other unsaturated aldehydes that have off-flavor character (Dominguez et al., 1974).

Krauss et al. (1972) and Zürcher et al. (1971) showed that long-chain fatty acids (C_{12}–C_{18}) have a relationship with beer head retention. Yabuuchi et al. (1979) reported that trihydroxyoctadecenoic acid produced from the oxidation of linoleic acid reduced beer head retention. The ratio of unsaturated (inhibitor) and saturated (promoter) acids is related to gushing problems (Sandra et al., 1973). These are sufficient reasons to be interested in the analysis of the long-chain fatty acids in beer.

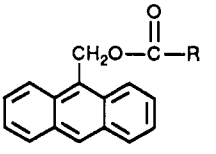
Fatty acid concentration has been determined by gas chromatographic analysis using a flame ionization detector (FID) (Klopper et al., 1975; Sandra and Verzele, 1975; Tressl et al., 1975; Tripp et al., 1968). However, the results obtained differed. Linoleic acid concentrations varied between 10 and 600 μg/L in beer. It is thought that this difference was caused not only by differences in beer types but also by the difference in precision of the GC analyses. FID can detect compounds other than fatty acids.

* Address correspondence to this author.
‡ SAPPORO Breweries Ltd.
§ Nagoya University.

Fatty acids are difficult to monitor directly at low concentrations by liquid chromatography; however, derivatization of the acids with moieties that enhance either ultraviolet absorption or fluorescence can greatly facilitate detectability (Nimura and Kinoshita, 1980; Lloyd, 1980; Lam and Grushka, 1978; Tsuchiya, 1982; Dünges, 1978). 9-Anthrylmethyl esters that formed from carboxylic acids with 9-anthradiazomethane show both high absorptivity and intense fluorescence and are readily separated on reversed-phase LC columns (Greeley, 1974). Korte (1982) showed that 9-(chloromethyl)anthracene as an alternative reagent for the preparation of 9-anthrylmethyl esters of carboxylic acids was a useful reagent for reversed-phase separations, and this analysis had a high detection sensitivity. The detection limit using fluorescence detection is 2 fmol.



9-(chloromethyl)anthracene



9-anthrylmethyl ester

In this paper, long-chain fatty acids (C_{12}–C_{18}) in beer were determined by HPLC using 9-(chloromethyl)anthracene as a fluorescence labeling reagent and the oxidative degradation of fatty acids by free-radical reactions was studied.

EXPERIMENTAL PROCEDURES

Beer. Bottled lager beer obtained before pasteurization and without added L-ascorbic acid was used for the study of the free-radical reactions. Beers for the determination study were obtained commercially.

Reagents. 9-(Chloromethyl)anthracene (9-CA) and tetramethylammonium hydroxide (10% in methanol) (TMA) were purchased from Tokyo Kasei Kogyo Co., Ltd. Hydrogen peroxide (31% w/v) was obtained from Mitsubishi Gas Co. (Tokyo)

0021-8561/90/1438-1363$02.50/0

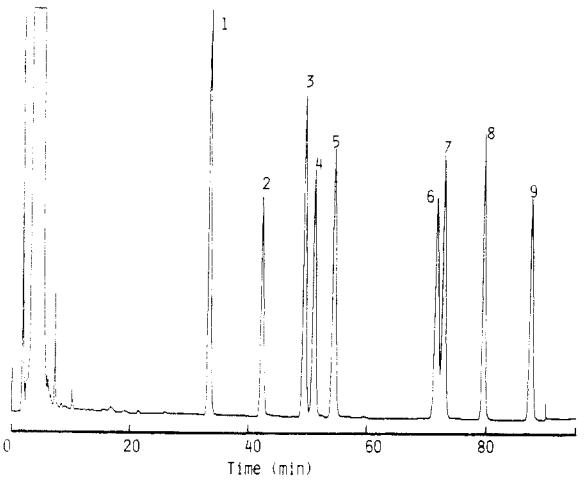


Figure 1. HPLC chromatogram of pure fatty acids. (1) Lauric acid (C12:0); (2) linolenic acid (C18:3); (3) myristic acid (C14:0); (4) palmitoleic acid (C16:1); (5) linoleic acid (C18:2); (6) palmitic acid (C16:0); (7) oleic acid (C18:1); (8) *n*-heptadecanoic acid (C17:0); (9) stearic acid (C18:0).

and stored at 4 °C. Acetonitrile 300, *N*,*N*-dimethylformamide (Lu) (DMF), and ethylenediaminetetraacetic acid, disodium salt, were obtained from Wako Pure Chemicals Industries Ltd.

Apparatus. The HPLC instrument was a JASCO 801-SC (Japan Spectroscopic Co., Ltd.) equipped with a JASCO 850-AS automatic sample injector, a JASCO 860-CO column thermostating oven, a JASCO 820-FP spectrofluorometric detector, and a Shimazu CR-3A integrator. The column was a Kaseisorb LC ODS-60-5S (4.6 × 250 mm, Tokyo Kasei Kogyo) that is packed with silica gel decorated with C_{18}.

Model System. Linoleic acid (50 ppb) and oleic acid (50 ppb) in 0.1 M acetate buffer (pH 4.3) including 4.5% (v/v) ethanol were prepared, and 2.6 mM H_2O_2–500 ppm $FeSO_4$ and/or 100 ppb catechin were added.

Treatment of Beer and Model Solution. Beer or the model solution (240 mL) was transferred into a brown bottle (headspace, 100 mL), and the bottle was sealed and incubated at 60 °C.

Sample Preparation. Beer or the model solution (200 mL) acidified with 10 mL of 6 N H_2SO_4 was transferred into a 1-L separation funnel, 100 μL of internal standard solution (100 mg/L *n*-heptadecanoic acid in methanol), 200 mL of ether–pentane solution (1:1), and 60 g of NaCl were then added, and the mixture was shaken for 30 min. The mixture was then centrifuged (5000 rpm, 5 min). The 150-mL ether–*n*-pentane (E–P) phase was washed with 150 mL of 5% $NaHCO_3$. The 140-mL E–P phase was evaporated with a rotoevaporator. The residue was dissolved in 1 mL of 6 mM TMA in DMF. The solution and 1 mL of 7.5 mM 9-CA in DMF were transferred to a test tube, and the test tube was sealed and then incubated at 75 °C for 20 min. This sample was used for the high-performance liquid chromatography (HPLC).

Chromatography. Chromatographic conditions used for the analytical method were as follows: fluorescence detection, λ_{ex} 365 nm and λ_{em} 412 nm; injection volume, 50 μL; flow rate, 1.1 mL/min; column temperature, 40 °C; chart speed, 2 mm/min; eluting solution A, 90% acetonitrile; eluting solution B, 100% acetonitrile. The eluting program followed this sequence: (1) 0–5 min, isocratic, 100% A; (2) 5.01–45 min, gradient, 0–50% B; (3) 45.01–65 min, isocratic, 50% A and 50% B; (4) 65.01–70 min, gradient, 50–100% B; (5) 70.01–85 min, isocratic 100% B; (6) 85.01 min, isocratic, 100% A. Peak patterns of the pure fatty acids (C_{12}–C_{18}) are shown in Figure 1.

RESULTS AND DISCUSSION

Determination of Fatty Acids in Beer. The general procedure for the derivatization of carboxylic acids with 9-CA was reported by Korte (1982). It was thought, however, that this procedure might not be the optimum reaction condition in beer, which has compounds other than fatty acids. The reaction conditions with beer samples for various concentrations of 9-CA and TMA and reaction times were therefore studied by comparison of the total peak height of the C_{12}–C_{18} fatty acids (see Figures 2–4). From the results, the following conditions for the derivatization of 9-anthrylmethyl esters and preparation of the HPLC samples were chosen; a sample was incubated with 7.5 mM CA and 6 mM TMA at 75 °C for 20 min. The peak pattern of the fatty acids (C_{12}–C_{18}) in beer is shown in Figure 5. *N*-Caprylic (C8:0), pelargonic (C9:0), *n*-capric (C10:0), and 9-decenoic acid (10:1) can be determined by this analysis, although the data are not shown. The calibration graphs prepared by the standard addition method using *n*-heptadecanoic acid (C17:0) as an internal standard were linear over the range 0–50 μg/L added fatty acids (Figure 6). The detection and determination limits of fatty acids in beer were 0.2 and 0.4 μg/L, respectively. This method has higher sensitivity than that for the typical GC method (10 μg/L).

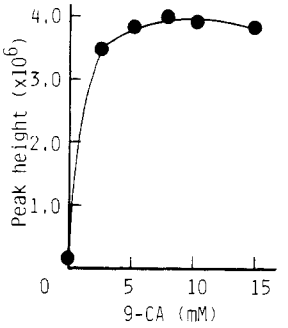


Figure 2. Effect of 9-CA concentration on peak height of fatty acids in beer. Peak height is the sum of peak heights of C_{12}–C_{18} in beer.

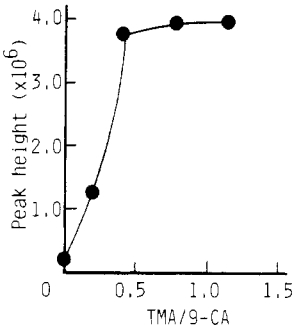


Figure 3. Effect of TMA concentration on peak height of fatty acids in beer. Peak height is the sum of peak heights of C_{12}–C_{18} in beer.

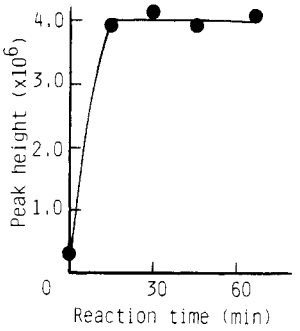


Figure 4. Effect of reaction time on peak height of fatty acids in beer. Peak height is the sum of peak heights of C_{12}–C_{18} in beer.

Analytical results for 10 commercial beers are summarized in Table I. The concentrations of the long-chain fatty acids were very different in the various beers, and the differences were especially notable for lauric acid

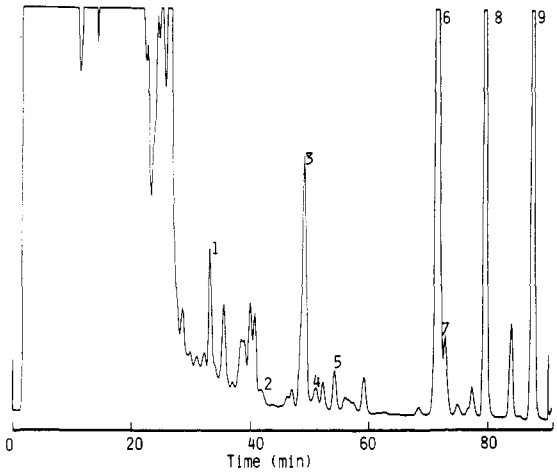


Figure 5. HPLC chromatogram of fatty acids in beer. (1) Lauric acid (C12:0); (2) linolenic acid (C18:3); (3) myristic acid (C14:0); (4) palmitoleic acid (C16:1); (5) linoleic acid (C18:2); (6) palmitic acid (C16:0); (7) oleic acid (C18:1); (8) *n*-heptadecanoic acid (C17:0); (9) stearic acid (C18:0).

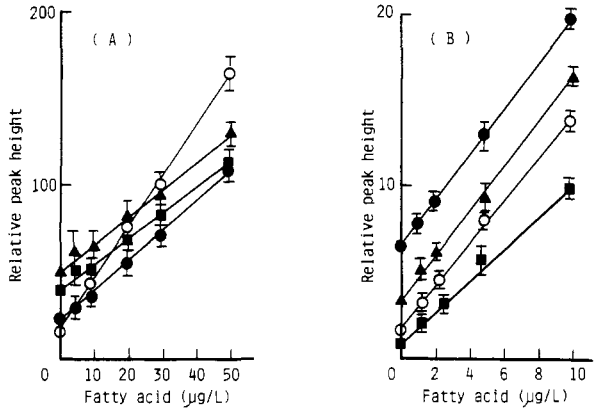


Figure 6. Determination of fatty acids in beer by the standard addition method. (A) Saturated fatty acids; (B) unsaturated fatty acids. (○) Lauric acid (C12:0) or palmitoleic acid (C16:1); (●) myristic acid (C14:0) or oleic acid (C18:1); (▲) palmitic acid (C16:0) or linoleic acid (C18:2); (■) stearic acid (C18:0) or linolenic acid (C18:3). The relative peak height was calculated with respect to the peak height of the internal standard (C17:0). Each data point is the average of three replicates, and the vertical line represents standard deviation.

(C12:0) and stearic acid (C18:0). The concentrations of unsaturated fatty acids in all types of beer were low. The total unsaturated fatty acids were only from 2 to 20 μg/L. From the experiments, it was made clear that the HPLC fluorescence method is useful for the analysis of long-chain fatty acids.

Free-Radical Reactions of Fatty Acids. In previous papers (Kaneda et al., 1988a,b), we detected free radicals that are generated during the incubation of beer and concluded that these radicals are produced by the reactions of hydrogen peroxide generated during beer oxidation and small amounts of metals. The free radicals attack several beer components as well as isohumulones, which are the main bitter substances in beer, and produce many kinds of aldehydes and ketones.

In this study, vials with a headspace greater than that of actual commercial beers was used. In Table II, lauric acid (C12:0) increased during the incubation of beer at 60 °C. Other saturated fatty acids (C_{14}–C_{18}) decreased about 10–20% in 5 days. Oleic and linoleic acids in beer decreased about 15% during the storage at 60 °C for 5 days. When a sensory test was carried out, a very strong stale flavor was observed in the treated beer. However, the oxidative degradation of these unsaturated fatty acids seems to be at a lower level in actual commercial beers, because they have much less oxygen and are stored at lower temperatures.

The decrease in unsaturated fatty acids (C18:1, C18:2) was accelerated by the addition of Fe^{2+} or H_2O_2 and more so by the combination of them. It was inhibited by the addition of EDTA. Saturated fatty acids (C_{12}–C_{18}) were not significantly affected by the addition of Fe^{2+}, H_2O_2, or EDTA. The concentrations of palmitoleic acid (C16:1) and linolenic acid (C18:3) were only at trace levels in fresh beer and could not be compared. It seems, therefore, that unsaturated fatty acids are degraded by free-radical reactions in beer. This degradation could produce the aldehydes, ketones, and acids that cause the characteristic aging flavors. However, the level of degradation was not great. It was thought that beer might have a system which protects against the oxidative degradation of unsaturated fatty acids.

Model System Experiment. Linoleic and oleic acid concentrations decreased 20–25% after incubation at 60 °C for 5 days in the model system (Figure 7). The decrease in these unsaturated fatty acids was greatly accelerated, and these acids were over 90% degraded, when Fe^{2+} and H_2O_2 were added. These results show that these fatty acids can be degraded by free-radical reactions in a medium of 4.5% ethanol and pH 4.3. Many kinds of lower molecular weight fatty acids were produced in this oxidation system (data not shown).

The degradation of linoleic and oleic acids was significantly inhibited by the addition of 100 mg/L catechin. It was thought that catechin would act as a radical scavenger and protect unsaturated fatty acids from oxidative degradation. It is well-known that beer has hundreds of milligrams per liter of polyphenols other than catechin (McMurrough and Henningan, 1984; Ohtsu and Hashimoto, 1982). It was therefore expected that the oxidative degradation of unsaturated fatty acids in packaged beer could be inhibited by polyphenols.

It is well-known that polyphenols in beer interact with proteins and cause a haze during the storage of beer (Asano et al., 1982; Gramshaw, 1970). The role of polyphenols in beer flavor stability has not, however, been fully elucidated. Harris and Ricketts (1959a,b) and McFarlane et al. (1963a,b) showed that the use of polyamide resins such as Polyclar AT or Nylon 66 may impart to beer an enhanced flavor stability. These authors explained that this was due to the removal of the oxidation products of anthocyanogens and catechins, which affect beer flavor and color. Recently, a number of researchers (Baxter et al., 1987; Delcour et al., 1984; 1985a,b; Erdal, 1986) have tried brewing using anthocyanogen-free malts to reduce the level of polyphenols in beer and to increase colloidal stability. However, enhanced flavor stability could not be shown.

There are other antioxidants in beer, as well as Maillard reaction products (Moll et al., 1986) and sulfur compounds. Not only polyphenols but also these antioxidants in beer are thought to play the most important role during beer aging.

CONCLUSIONS

Free-radical reactions in beer are responsible for the degradation of unsaturated fatty acids. However, the level of degradation products of unsaturated fatty acids is very low, because packaged beer contains the low levels of unsaturated fatty acids and oxygen and antioxidants in beer, as well as polyphenols, that can inhibit these radical reactions. Moreover, the oxidation of unsaturated fatty acids

Table I. Long-Chain Fatty Acids in Several Types of Beers

beer	saturated fatty acid,[a] μg/L				unsaturated fatty acid,[a] μg/L			
	C12:0	C14:0	C16:0	C18:0	C16:1	C18:1	C18:2	C18:3
Japanese lager								
brand A	5.5 ± 0.8	15.0 ± 2.4	30.7 ± 5.7	20.7 ± 3.3	1.0 ± 0.3	5.2 ± 0.8	3.2 ± 0.8	0.8 ± 0.3
brand B	9.1 ± 1.2	15.4 ± 2.4	37.8 ± 5.0	29.6 ± 3.5	1.1 ± 0.3	0.9 ± 0.3	1.8 ± 0.5	0.6 ± 0.3
brand C	7.2 ± 1.0	17.3 ± 2.0	40.3 ± 6.1	37.5 ± 4.5	1.0 ± 0.3	3.4 ± 0.8	1.4 ± 0.5	tr[b]
brand D	5.3 ± 0.8	13.1 ± 2.3	35.8 ± 7.0	18.1 ± 2.5	tr[b]	tr[b]	1.6 ± 0.3	0.7 ± 0.3
Japanese all malt	18.2 ± 3.0	16.9 ± 2.1	50.7 ± 9.9	28.9 ± 3.0	2.0 ± 0.4	0.7 ± 0.3	5.2 ± 0.9	2.5 ± 0.8
Japanese Pilsner	8.2 ± 2.0	8.0 ± 1.8	25.3 ± 5.5	21.9 ± 3.0	1.7 ± 0.4	10.0 ± 1.5	5.4 ± 0.6	1.7 ± 0.8
American light								
brand A	47.8 ± 6.8	10.0 ± 1.7	29.6 ± 5.3	9.4 ± 1.0	1.2 ± 0.3	4.2 ± 0.6	2.5 ± 0.3	0.8 ± 0.3
brand B	66.9 ± 9.9	13.0 ± 1.8	27.4 ± 4.9	17.9 ± 2.0	0.9 ± 0.3	11.2 ± 1.0	7.1 ± 0.9	3.0 ± 0.7
European								
brand A	47.0 ± 7.1	8.0 ± 1.2	15.2 ± 1.2	1.1 ± 0.3	1.9 ± 0.5	3.7 ± 0.7	2.9 ± 0.3	1.6 ± 0.4
brand B	14.4 ± 1.2	8.0 ± 1.8	11.6 ± 2.0	1.0 ± 0.5	1.0 ± 0.8	3.4 ± 1.0	2.2 ± 0.5	1.2 ± 0.3

[a] The data represent the means of three determinations. [b] tr, trace.

Table II. Effect of Free Radicals on Long-Chain Fatty Acids in Beer

incubation[a] time (days)	treatment (additions)	saturated fatty acid				unsaturated fatty acid	
		C12:0	C14:0	C16:0	C18:0	C18:1	C18:2
0	no additions	100[b]	100[b]	100[b]	100[b]	100[b]	100[b]
2	no additions	117 ± 10	73 ± 7	90 ± 10	84 ± 15	90 ± 5	88 ± 7
2	5 mM EDTA	118 ± 8	84 ± 10	102 ± 10	98 ± 15	100 ± 5	102 ± 7
2	2.6 mM H_2O_2	113 ± 11	78 ± 15	93 ± 7	85 ± 7	79 ± 10	86 ± 6
2	500 μg/L $FeSO_4$	132 ± 23	88 ± 17	90 ± 15	84 ± 8	89 ± 4	90 ± 5
2	2.6 mM H_2O_2 + 500 μg/L $FeSO_4$	128 ± 18	87 ± 20	100 ± 7	93 ± 11	72 ± 10	75 ± 10
5	no additions	128 ± 8	68 ± 10	78 ± 10	87 ± 4	85 ± 6	84 ± 5
5	5 mM EDTA	132 ± 15	68 ± 7	89 ± 5	92 ± 10	100 ± 7	99 ± 7
5	2.6 mM H_2O_2	134 ± 20	70 ± 10	83 ± 4	84 ± 8	73 ± 6	77 ± 4
5	500 μg/L $FeSO_4$	138 ± 21	71 ± 15	80 ± 10	80 ± 8	75 ± 7	78 ± 6
5	2.6 mM H_2O_2 + 500 μg/L $FeSO_4$	140 ± 15	62 ± 20	79 ± 9	79 ± 7	65 ± 8	70 ± 3

[a] Beer was incubated at 60 °C. [b] Each fatty acid concentration was calculated with respect to that of beer with an incubation time of 0 days, no additions. The data represent the means of three determinations.

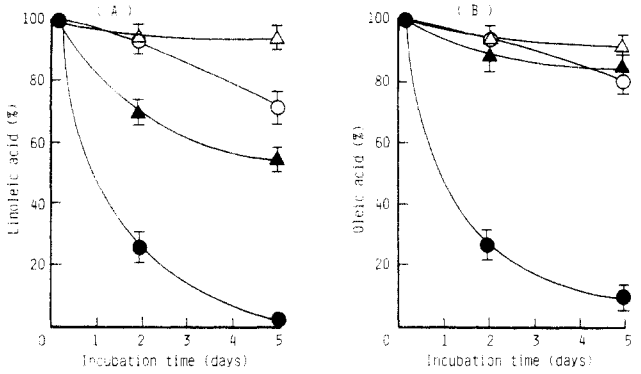


Figure 7. Effect of catechin on free-radical reactions of unsaturated fatty acids. (A) Linoleic acid; (B) oleic acid. (○) Control (no additions); (●) addition of 2.6 mM H_2O_2 and 500 μg/L Fe^{2+}; (△) addition of 100 mg/L catechin; (▲) addition of 2.6 mM H_2O_2, 500 μg/L Fe^{2+}, and 100 mg/L catechin. Each data point is the average of three replicates, and the vertical line represents standard deviation.

does not produce only unsaturated aldehydes such as 2-alkenals and 2,4-alkadienals (Esterbauer, 1982). Alkenals are main products. In this model study, it was confirmed that many kinds of fatty acids were produced from oleic and linoleic acids. It was therefore concluded that the degradation of unsaturated fatty acids could not significantly contribute to the formation of the off-flavor in packaged beer.

ACKNOWLEDGMENT

We thank M. Kobayashi, Brewing Research Laboratories, SAPPORO Breweries Ltd., and Dr. K. Kamada, a manager of the Nagoya factory, SAPPORO Breweries, Ltd., for their valuable advice.

LITERATURE CITED

Asano, K.; Shinagawa, K.; Hashimoto, N. Characterization of haze-forming proteins of beer and their roles in chill haze formation. *J. Am. Soc. Brew. Chem.* **1982**, *40*, 147–154.

Baxter, E. D.; Morris, T. M.; Picksley, M. A. Low Proanthocyanidin malts for production of chilled and filtered or fined beer. *J. Inst. Brew.* **1987**, *93*, 387–391.

Carrington, R.; Collett, R.; Dunkin, I.; Halek, G. Gushing promoters and suppressants in beer and hops. *J. Inst. Brew.* **1972**, *78*, 243–254.

Delcour, J. A.; Vandenberghe, M. M.; Dondeyne, P.; Schrevens, E. L.; Wijnhoven, J. Flavor and haze stability differences in unhopped and hopped all-malt Pilsner beers brewed with proanthocyanidin-free and with regular malt. *J. Inst. Brew.* **1984**, *90*, 67–72.

Delcour, J. A.; Schoeters, M. M.; Dondeyne, P.; Schrevens, E. L.; Wijnhoven, J. Flavor and haze stability difference due to hop tannins in all-malt Pilsner beers brewed with proanthocyanidin-free and with regular malt. *J. Inst. Brew.* **1985a**, *91*, 302–305.

Delcour, J. A.; Schoeters, M. M.; Meysman, E. W.; Dondeyne, P.; Schrevens, E. L.; Wijnhoven, J. Flavor and haze stability differences due to hop tannins in all-malt Pilsner beers brewed with proanthocyanidin-free malt. *J. Inst. Brew.* **1985b**, *91*, 88–92.

Dominguez, X. A.; Canales, A. M. Oxidation of beer. A rational mechanism for the degradation of unsaturated fatty acids and formation of unsaturated aldehydes. *Brew. Dig.* **1974**, July, 40–47.

Drost, B. W.; Van Eerde, P.; Hoekstra, S. F.; Strating, J. Fatty acids and staling of beer. *Proc. Congr.—Eur. Brew. Conv.* **1971**, *13*, 451–458.

Dünges, W. High-performance liquid chromatographic separation of esters of 4-hydroxymethyl-7-methoxy-coumarin. *J. Chromatogr.* **1978**, *145*, 483–488.

Erdal, K. Proanthocyanidine-free barley. Malting and brewing. *J. Inst. Brew.* **1986**, *92*, 220–224.

Esterbauer, H. Aldehydic products of lipid peroxidation. In *Free Radicals, Lipid Peroxidation, and Cancer*; Mcbrien, D. C. H., Slafer, T. F., Eds.; Academic Press: New York, 1982, pp 101–128.

Gramshaw, J. W. Beer polyphenols and the chemical basis of haze formation. Part II. Changes in polyphenols during the brewing and storage of beer—the composition of haze. *Tech. Q.—Master Brew. Assoc. Am.* **1970**, *28*, 122–133.

Greeley, R. H. Rapid esterification for gas chromatography. *J. Chromatogr.* **1974**, *88*, 229–233.

Harris, G.; Ricketts, R. W. Studies on non-biological hazes of beers VII. The use of polyamide resins for removing the haze-forming polyphenols from beer. *J. Inst. Brew.* **1959a**, *65*, 256–259.

Harris, G.; Ricketts, R. W. Studies on non-biological hazes of beers IX. Effect of Nylon 66 in delaying formation of haze. *J. Inst. Brew.* **1959b**, *65*, 418–422.

Jamieson, A. M.; Chen, E. C.; Van Gheluwe, J. E. A. Identification of a compound responsible for cardboard flavor in beer. *Proc. Am. Soc. Brew. Chem.* **1970**, 192–197.

Kaneda, H.; Kano, Y.; Osawa, T.; Ramarathnam, N.; Kawakishi, S.; Kamada, K. Detection of free radicals in beer oxidation. *J. Food Sci.* **1988a**, *53*, 885–888.

Kaneda, H.; Kano, Y.; Osawa, T.; Kawakishi, S.; Kamada, K. The role of free radicals in beer oxidation. *J. Am. Soc. Brew. Chem.* **1989b**, *47*, 49–53.

Klopper, W. K.; Tuning, B.; Vermeire, H. A. Free fatty acids in wort and beer. *Proc. Congr.—Eur. Brew. Conv.* **1975**, *15*, 659–667.

Korte, W. D. 9-(Chloromethyl)anthracene: A useful derivatizing reagent for enhanced ultraviolet and fluorescence detection of carboxylic acids with liquid chromatography. *J. Chromatogr.* **1982**, *243*, 153–157.

Krauss, G.; Zürcher, H.; Holstein, H. The foam-destroying effect of some malt lipids, their fate during the malting and brewing process. *Monatsschr. Brau.* **1972**, *25*, 113–123.

Krauss, G.; Forch, M.; Holstein, H. On the fate of some malt lipids during fermentation and storage. *Monatsschr. Brau.* **1975**, *28*, 229–237.

Lam, S.; Grushka, E. Labeling of fatty acids with 4-bromomethyl-7-methoxycoumarin via crown ether catalyst for fluorimetric detection in high-performance liquid chromatography. *J. Chromatogr.* **1978**, *158*, 207–214.

Lloyd, J. B. F. Phenanthrimidazoles as fluorescent derivatives in the analysis of fatty acids by high-performance liquid chromatography. *J. Chromatogr.* **1980**, *189*, 359–373.

McFarlane, W. D.; Thompson, K. D.; Garratt, R. H. The retention of clarity, color, and flavor in beer through control of tannin content. *Proc. Am. Soc. Brew. Chem.* **1963a**, 98–103.

McFarlane, W. D.; Sword, P. F.; Blinoff, G. Proc. Catechintannins in brewing. *Eur. Brew. Conv.* **1963b**, 174–181.

McMurrough, I.; Henningan, P. Tanning properties of flavanols in barley and hops measured by reaction with cinchonine sulphase in relation to haze formation in beer. *J. Inst. Brew.* **1984**, *90*, 24–32.

Meilgaard, M. Stale flavor carbonyls in brewing. *Brew. Dig.* **1972**, April, 48–57.

Moll, N.; Moll, M. The shelf life of foods and beverages. *Proceedings of the 4th International Flavor Conference, Rhodes, Greece*, 1985; Charalambous, G., Ed.; Elsevier: Amsterdam, 1986; pp 97–140.

Nimura, N.; Kinoshita, T. Fluorescent labeling of fatty acids with 9-anthryldiazomethane (ADAM) for high performance liquid chromatography. *Anal. Lett.* **1980**, *13*, 191–202.

Ohtsu, K.; Hashimoto, N. Determination of catechin and proanthocyanidins in wort and beer by high pressure liquid chromatography. *Rep. Res. Lab. Kirin Brew. Co.* **1982**, *25*, 47–53.

Sandra, P.; Verzele, M. Analysis of long-chain fatty acids in beer. *J. Inst. Brew.* **1975**, *81*, 302–306.

Sandra, P.; Claus, H.; Verzele, M. Fatty acids in isomerized hop extracts and gushing. *J. Inst. Brew.* **1973**, *79*, 142–147.

Tressl, R.; Kossa, T.; Renner, R.; Koppler, H. Gas chromatographic-mass spectrometic investigation of volatile compounds of hops, wort and beer and their formation. I. Organic acids in wort and beer. *Monatsschr. Brau.* **1975**, *28*, 109–118.

Tressl, R.; Bahri, D.; Silwar, R. Formation of aldehydes oxidation of lipids and their importance as "off-flavor" components in beer. *Proc. Congr.—Eur. Brew. Conv.* **1979**, *17*, 27–41.

Tripp, R. C.; Timm, B.; Iyer, M.; Richardson, T.; Amundson, C. H. Free fatty acids and monocarbonyls in beer. *Proc. Am. Soc. Brew. Chem.* **1968**, *26*, 65–74.

Tsuchiya, H. High-performance liquid chromatography of carboxylic acids using 4-bromomethyl-7-acetoxycoumarin as fluorescence reagent. *J. Chromatogr.* **1982**, *234*, 121–130.

Wang, P. S.; Siebert, K. J. The influence of various storage conditions on the formation of 2-nonenal in bottled beer. *Proc. Am. Soc. Brew. Chem.* **1974a**, *32*, 47–49.

Wang, P. S.; Siebert, K. J. Determination of *trans*-2-nonenal in beer. *Tech. Q.—Master Brew. Assoc. Am.* **1974b**, *11*, 110–117.

Yabuuchi, S.; Yamashita, H. Gas chromatographic determination of trihydroxyoctadecenoic acids in beer. *J. Inst. Brew.* **1979**, *85*, 216–218.

Zürcher, C.; Krauss, G. Free low molecular weight fatty acids and their ethyl esters as beer aroma compounds and foam inhibitors. *Monatsschr. Brau.* **1971**, *24*, 230–237.

Received for review March 9, 1989. Revised manuscript received November 13, 1989. Accepted December 12, 1989.

Registry No. Lauric acid, 143-07-7; linolenic acid, 463-40-1; myristic acid, 544-63-8; palmitoleic acid, 373-49-9; linoleic acid, 60-33-3; palmitic acid, 57-10-3; oleic acid, 112-80-1; heptadecanoic acid, 506-12-7; stearic acid, 57-11-4.