

Brewing Performance of Malted Lipoxygenase-1 Null Barley and Effect on the Flavor Stability of Beer

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ABSTRACT

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We examined the malting and brewing performances of a lipoxygenase-1 (LOX-1) null line of barley (*Hordeum vulgare* L.). The LOX-normal malt and the LOX-null malt were prepared from F₄ populations derived from a single cross. We could not observe any major differences in the general malt characteristics between the two malts. A brewing trial was performed using these malts. The analysis of the wort and beer revealed that the absence of LOX-1 had little effect on the general

characteristics of the wort and beer. In contrast, beer made from the LOX-null malt showed reduced levels of beer-deteriorating substances, *trans*-2-nonenal (T2N), and trihydroxyoctadecenoic acid (THOD). In the sensory evaluation, well-trained panel members recognized the significant superiority of the aged LOX-null beer in terms of staleness. These results show that the LOX-1 null barley line can be effectively used to improve the flavor stability of beer without changing the other important beer qualities.

Lipoxygenase (EC 1.13.11.12) (LOX) catalyzes the hydroperoxidation of polyunsaturated fatty acids with 1,4-*cis-cis*-penta-diene structures. In the germinating seeds of barley (*Hordeum vulgare* L.), two isozymes of LOX (LOX-1 and LOX-2) have been identified and well characterized (Yabuuchi 1976; Baxter 1982; Doderer et al 1992; Yang et al 1993; Van Mechelen et al 1999). LOX-1, which has a relatively low pI compared with LOX-2 (Yang and Schwarz 1995), predominantly produces LOX activity in the silenced seed. LOX-1 converts linoleic and linolenic acids mainly into 9-hydroperoxides (9-HPOD), whereas LOX-2 mainly produces 13-hydroperoxides (13-HPOD) (Yang et al 1993).

During brewing, two types of oxidation can cause the formation of beer-deteriorating substances (Bamforth 1999). One is a non-enzymatic oxidation process (Kaneda et al 1989; Bamforth 1993; Liégeois et al 2002). We have developed an antioxidative beer production system to reduce this type of oxidation and, in fact, the system enables us to produce beers with improved flavor stability (Kaneda et al 1999; Maeda 1999). However, we came to recognize that there is room for further improvement. This recognition led us to focus on another type of lipid oxidation, an enzymatic lipid oxidation process. LOX-1 in barley or malt is involved in this enzymatic oxidation process and forms a precursor (9-HPOD) of beer-deteriorating substances during the further reactions in the brewing process (Kobayashi et al 1993; Kuroda et al 2002, 2003). Among these beer-deteriorating substances, *trans*-2-nonenal (T2N) and trihydroxyoctadecenoic acid (THOD) have particularly attracted brewers' attention. T2N has been shown to be a major component of a negative flavor known as "cardboard" flavor or "papery" flavor in aged beer (Jamieson and Van Gheluwe 1970; Meilgaard 1975; Drost et al 1990). THOD has an adverse effect on the foam stability and flavor of beer (Bauer et al 1977; Yabuuchi and Yamashita 1979; Kaneda et al 2001; Kobayashi et al 2002). These reports suggest the involvement of barley LOX-1 in the beer quality and, in fact, brewing scientists and brewers have successfully developed process-controlling methods for improving the

flavor stability of beer by preventing enzymatic lipid-oxidation (Larsen et al 2001; Ueda et al 2001). Thus, it is of great interest for brewers and consumers to use malting barley cultivars with a low LOX-1 activity or, more preferably, without LOX-1 activity (McElroy and Jacobsen 1995; Wu et al 1997). Although we know that the LOX-1 activity is a genotypic characteristic (Baxter 1982; Schwarz and Pyle 1984; Yang and Schwarz 1995; Wu et al 1997), intensive research on the genetic variation in barley has never been reported.

Recently, we succeeded in discovering six LOX-1 null barley lines by surveying many landrace lines (Hirota et al 2004, 2005). These lines did not show any significant LOX-1 activity, and lacked the authentic LOX-1 protein. Furthermore, genetic analysis revealed that the LOX-1 null trait was governed by a single recessive gene. These findings led us to create malting barley cultivars without LOX-1.

The present study is intended to clarify the contribution of the LOX-1 null trait to the flavor and foam stabilities of beer and to examine whether or not the LOX-1 null trait influences the general characteristics of beer. Data from a brewing trial with the LOX-null malt suggested that the LOX-1 null barley line would be effectively used to improve the flavor and foam stabilities of beer without changing the other important beer qualities.

MATERIALS AND METHODS

Barley Raw Materials

A LOX-1 null mutant barley landrace (OUI003, code number SBOU2) from a collection of the Research Institute for Biore-sources (Okayama University, Japan) (Hirota et al 2004, 2005) was crossed with the Japanese landrace Taisyomugi. Individual F₂ seeds were separately propagated and maintained as a line. At F₄ generation, each line was subjected to the LOX enzyme assay and an immunological analysis, and then divided into a LOX-normal population (with LOX-1 activity) or a LOX-null population (without LOX-1 activity). The F₄ seeds belonging to the same population were bulked for micromalting. Plant materials were grown in a greenhouse or a well-controlled test field of Sapporo Breweries in Gunma prefecture, Japan.

Preparation of Malts and Malt Quality Analysis

The LOX-normal population and the LOX-null population (F₄ generation) were used for malt production. These samples achieved >95% germination at the time of malting. The malt was prepared using an automatic micromalting system (Phoenix Systems, Adelaide, Australia) as in a previous report (Ogushi et al 2002). General malt characteristics were analyzed according to the official methods

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from the European Brewery Convention (EBC 1987) for ex-steep moisture, malt yield, wort clarity, color, boiled wort color, extract (fine grind, 0.2 mm), total and soluble nitrogen, Kolbach index, apparent attenuation limit, diastatic power, viscosity, and wort β -glucan. The LOX assay was conducted as previously described (Hirota et al 2004, 2005). Briefly, a single malted grain was crushed with a hammer and then suspended in 400 μ L of cold extraction buffer (0.1M sodium acetate, pH 5.5). The homogenate was incubated for 30 min on ice with occasional vortexing, then centrifuged at 3,000 \times g for 15 min. The supernatant was used as the crude enzyme solution. The protein concentration of the crude enzyme solution was determined using the protein assay reagent (Bio-Rad) based on Bradford's method with BSA as a standard. The crude enzyme was incubated at 24°C for 5 min in a cocktail (2 mM linoleic acid, 0.05%, w/v, Tween 20, 0.1M sodium acetate, pH 5.5). The reaction was stopped by adding an equal volume of BHT solution (0.8 mM 2-6-*t*-butyl-cresol in methanol) and allowed to stand for 30 min at -20°C. The reactant was centrifuged at 3,000 \times g for 20 min and the supernatant was subjected to the hydroperoxide measurement according to Jiang et al (1991). One unit of LOX activity was defined as the amount of enzyme that produced 1 nmol of hydroperoxide (with cumene hydroperoxide as the standard)/min/1 g of malt. Negative controls of the samples were prepared by inactivating the individual crude enzymes by heat treatment at 100°C for 5 min.

Pilot-Scale Brewing

Low-malt beers (Happoshu) were made from the LOX-null malt and LOX-normal malt with the same recipe according to the standard method of the Production & Technology Development Center of Sapporo Breweries. Briefly, the wort was prepared using the malt (24%, w/w, in total raw materials), corn syrup, and hops in a 50-L pilot-scale plant. Each malt (1.5 kg) was mashed alone with 15 L of mashing water according to a diagram of 50°C for 20 min, 65°C for 30 min, and 75°C for 3 min. After mashing, wort lautering was done with a lauter tun. Before boiling, the lautered wort (35 L) was mixed with 5 kg of starch syrup. Hop pellets (13 g) (87.0 BU) (EBC) was added into the wort. After boiling for 70 min, the boiled wort was cooled to 10°C. The extract content of the cooled wort was adjusted by water addition to 11.6–11.8%. The temperature of the fermentation was kept at 13°C. After transferring the fermented wort to a 30-L lagering tank under a CO₂ atmosphere, the maturation was conducted at 13°C for six days, then at 0°C for two weeks. Filtration and bottling were done using the pilot-scale equipment under anti-oxidative conditions. Samples were stored at 37°C for seven days or 14 days to force flavor deterioration. The wort and beer were analyzed for the general characteristics according to the official methods (EBC 1987). The THOD concentration in the beer was determined as previously described (Kobayashi et al 2000). To determine the T2N concentration in the beer, 8 mL of beer was transferred to a vial containing 3 g of NaCl, and after adding 20 μ L of 20 ppm 1-nonanol (Tokyo Kasei Kogyo Co., Tokyo, Japan) in ethanol, it was capped with silicon-coated caps. Aldehydes accumulated in the vial were trapped by inserting a membrane 100 μ m thick of a polydimethylsiloxane solid-phase micro-extraction fiber probe (Spelco, Sigma) into the headspace of the vial for 15 min. The probe was then introduced into the injection port and thermally desorbed for 5 min at 260°C onto a DB-1 column (liquid phase: dimethylpolysiloxane, 30 m \times 0.25 mm; film thickness: 1 μ m) (J&W Scientific). GC-MS analysis was done with Hewlett Packard HP6890/MSD5972A system. Select ions used were $m/z = 70$. The flow rate of helium gas was 1.0 mL/min. The temperature of the oven was held at 60°C for 1 min, and then raised at 5°C/min to 260°C. Calibration curves were derived by adding authentic 2(*E*)-nonenal to the sample. Concentration of aldehyde in each sample was measured by the peak area relative to that of internal standard (1-nonanol). The foam stability

of the beer was assessed as the NIBEM value using the foam stability tester type NIBEM-T (Haffmans B. V., Venlo, Holland) according to the manufacturer's instructions.

Sensory Evaluation

The sensory quality of the prepared beer sample was evaluated for two attributes: the staleness-related off-flavor and the total staleness. The off-flavor is mainly recognized as papery, whisky-like or caramel-like flavors (Shimizu et al 2002). The total staleness is defined as the overall impression of staleness. The stored beers were allowed to cool to 5°C before the sensory evaluation. The sensory evaluation was conducted for the beers stored at 37°C for 7 and 14 days by a group of 13 well-trained panelists. For each attribute, the staling degree was rated from 0 (not stale) to 4 (strongly stale) at 0.5 intervals. The means of the sensory scores in each attribute were analyzed using the paired *t*-test to determine the significant differences between the LOX-null beer and LOX-normal beer.

RESULTS

General Characteristics of Malts, Wort, and Beers

To evaluate the quality of the malt made from the LOX-1 null mutant barley, we prepared the LOX-normal and LOX-null F₄ populations derived from the same cross. We then made the malts from these bulked F₄ populations. The primary reason for using the bulked F₄ populations is that it allows the preparation of malts with similar genetic backgrounds except for the LOX-1 gene. The malts made from these barley populations were analyzed for the general malt characteristics. We did not observe any major differences in the general malt characteristics between these malts (Table I).

In contrast to the general quality, the LOX activity of the LOX-null malt was quite different from that of the LOX-normal malt. The total LOX activity of the LOX-null malt was 8.1% of that of the LOX-normal malt (Table I). The trace activity detected in the LOX-null malt (1.3 unit) would be the remaining LOX-2 activity. This is because we have confirmed the LOX-1 deficiency in the LOX-1 null barley line (OUI003) from several viewpoints: the LOX activity, the immunological reactivity (Hirota et al 2005), the genomic DNA sequence, the cDNA sequence, the transcript expression in seed, and the expression of the cDNA in *E. coli* (*unpublished data*). On the other hand, a large part of the total LOX activity detected in the LOX-normal malt (16.1 unit) is regarded as LOX-1 activity. This is because the LOX-2 in germinating seed, which has a relatively heat-labile nature compared with LOX-1, is preferentially inactivated during the heat-drying process in malting called kilning (Hugues et al 1994; Kuroda et al 2002). In fact, it has been reported that total LOX activity in malt was predominantly

TABLE I
General Characteristics of LOX-Normal and LOX-Null Malts

Malt Characteristics	LOX-Normal	LOX-Null
Ex-steep moisture (%)	44.8	44.5
Malt yield (% db)	90.3	90.7
Wort clarity	2	2
Color (°EBC)	2.1	2.2
Boiled wort color	3.2	3.3
Extract (% db)	71.4	73.5
Total nitrogen (%)	2.49	2.29
Soluble nitrogen (%)	0.648	0.645
Kolbach index	26.0	28.1
Apparent attenuation limit (%)	78.8	79.0
Diastatic power (WK)	348	377
Viscosity (mP-sec)	1.87	1.89
Wort β -glucan (mg/L)	427	392
LOX activity (unit) ^a	16.1	1.3

^a Averages of eight samples. Unit was defined in text.

attributed to the LOX-1 activity (Hugues et al 1994; Yang and Schwarz 1995).

The worts and the beers were made from each of the malts and analyzed for their general characteristics. No apparent difference in general characteristics was observed regardless of the presence or absence of LOX-1 (Table II).

T2N Concentration and Sensory Evaluation of Beers

The T2N concentrations of the LOX-normal and LOX-null beers were determined after different periods of storage: 7 days at 0°C (corresponding to fresh beer); 7 days at 37°C; and 14 days at 37°C. As shown in Table III, the T2N concentrations of each beer increased during first 7 days of storage at 37°C regardless of malts used. However, the T2N level in the LOX-null beer was significantly lower than that in the LOX-normal beer by 66% after 7 days of storage and by 75% after 14 days of storage (Table III).

The sensory evaluations were conducted for the LOX-normal beer and the LOX-null beer by 13 well-trained panel members (Table IV). For every storage condition, the LOX-null beer showed a significant superiority in off-flavor and total staleness compared with the LOX-normal beer. The most common comment by the panel members was the papery off-flavor in the LOX-normal beer.

THOD Concentration and Foam Stability of Beers

The THOD concentrations were determined in the fresh LOX-normal and LOX-null beers. The THOD concentration of the LOX-null beer was reduced to 47% (1.7 mg/L) of that of the LOX-normal beer (3.6 mg/L) (Table V). The foam stability of these beers was evaluated as the NIBEM value, which measures the period of time (sec) required for a fixed decay of the foam. As we expected, the value of NIBEM for the LOX-null beer was higher than that of the LOX-normal beer by 21 sec (Table V).

TABLE II
General Characteristics of LOX-Normal and LOX-Null Worts and Beers

Characteristics	LOX-Normal	LOX-Null
Wort		
Extract (%)	11.78	11.60
Final attenuation (%)	70.7	70.9
Apparent attenuation limit (%)	86.9	86.9
pH	5.88	5.93
Color (°EBC)	2.1	2.1
Bitter unit	31.2	27.3
Total nitrogen (mg/100 mL)	24	22
Polyphenol (mg/L)	44	48
Free amino nitrogen (mg/L)	46	51
Beer		
Original gravity (%)	11.82	11.56
Final extract (%)	3.43	3.38
Final attenuation (%)	71.0	70.7
Apparent extract (%)	1.44	1.45
Apparent attenuation limit (%)	87.8	87.4
Alcohol (w/w %)	4.33	4.21
pH	3.51	3.28
Pressure (20°C) (kg/cm ²)	2.35	2.55
Color (°EBC)	1.5	1.7
Total nitrogen (mg/100 mL)	16	19
Polyphenol (mg/L)	45	43
Free amino nitrogen (mg/L)	10	12

TABLE III
Trans-2-Nonenal Concentrations of Stored LOX-Normal and LOX-Null Beers^a

Storage Condition	LOX-Normal	LOX-Null
7 days at 0°C	0.02 ± 0.0024	0.01 ± 0.0007
7 days at 37°C	0.35 ± 0.0023	0.12 ± 0.0026
14 days at 37°C	0.36 ± 0.0115	0.09 ± 0.0053

^a Averages of three determinations (µg/L) ± one standard deviation.

DISCUSSION

The staleness-related off-flavors are perceived for beers that are one- to three-months old. Lager-type beers especially are more susceptible to these off-flavors (Drost et al 1990). It has been recognized as the intrinsic nature of beer that it gradually loses the original fresh flavor, and brewing scientists have been trying to find the solution to this problem for years. Many substances and pathways have been suggested to be involved in the staling reaction of beer (Bamforth 1999). The staling reaction during storage can be expediently translated into a simplified mechanism. That is, oxidative (Kaneda et al 1999) or nonoxidative (Noël and Collin 1995) conversions of precursors to stale flavor compounds during beer storage. Based on the simplified mechanism, three measures against the staling reaction are possible: reducing the precursors of the stale flavor, suppressing the conversion rate of the precursors to the stale flavor compounds, and masking the stale flavor. The reduction or removal of the precursors are regarded as direct measures to control the stale flavor, although it has been difficult to accomplish this. Instead, many practical efforts have been devoted to the suppression of the conversion rate of responsible components. For example, at a modern production site for beer, the oxygen is removed from beer to its minimum level, and the antioxidant derived from the malt and hops is kept in the finished beer as much as possible under the practical control of an anti-oxidative brewing process, which realized the freshness of beer for a longer period (Kaneda et al 1999; Maeda 1999; Takashio and Shinotsuka 2001).

In spite of these efforts, the cardboard flavor or papery flavor of stale beer is still one of the major problems in the brewing industries. The formation of T2N is considered to be the main cause of these off-flavors (Drost et al 1990). Barley seed LOX-1 catalyzes the formation of a precursor (9-HPOD) of T2N through oxidation of unsaturated fatty acids such as linoleic acid during mashing (Kuroda et al 2003). Based on these facts, various methods have been proposed to reduce the precursor by suppressing the LOX activity during the malting and mashing processes (Drost et al 1990; Wu et al 1997; Larsen et al 2001; Ueda et al 2001). In principle, the use of barley without the LOX-1 activity is regarded as the simplest method to reduce these off-flavors in beer. In the present report, we demonstrated the effectiveness of the LOX-1 null barley we discovered in controlling the staleness-related off-flavor.

We showed that the T2N concentration in the LOX-null beer stored at 37°C was 0.09–0.12 µg/L, whereas that in the LOX-normal beer was 0.35–0.36 µg/L (Table III). The sensory threshold

TABLE IV
Sensory Evaluations of LOX-Normal and LOX-Null Beers^a

Storage Conditions and Attributes	LOX-Normal	LOX-Null
7 days at 37°C		
Off-flavor ^b	2.2 ± 0.63	1.7 ± 0.53
Total staleness ^c	2.3 ± 0.63	1.8 ± 0.52
14 days at 37°C		
Off-flavor ^c	2.7 ± 0.52	2.0 ± 0.69
Total staleness ^c	2.6 ± 0.55	2.0 ± 0.65

^a Values reported as mean of 13 sensory scores ± one standard deviation.

^b Significantly different at the 5% probability level (paired *t*-test).

^c Significantly different at the 1% probability level (paired *t*-test).

TABLE V
Foam Stability and THOD Concentration of LOX-Normal and LOX-Null Beers

	LOX-Normal	LOX-Null
NIBEM value (sec)	239	260
THOD concentration (mg/L) ^a	3.6	1.7

^a Averages of three determinations.

of T2N is reported to be $\approx 0.11 \mu\text{g/L}$ (Meilgaard 1975). Therefore, the T2N concentration of the LOX-null beer was suppressed to just around its threshold level even after storage at 37°C for 14 days, which corresponds to storage at room temperature for more than two months. This suggests that beer made from the LOX-1 null barley would not attain the T2N threshold level under normal storage conditions. Based on the T2N threshold level and the T2N concentration of the LOX-null beer, it was expected that the LOX-null beer produces less off-flavor than the LOX-normal beer. In fact, the sensory evaluation revealed that the stored LOX-null beer showed a statistically significant superiority in terms of off-flavor and total staleness compared with the LOX-normal beer (Table IV). So far, we have obtained similar tendencies (a statistically significant sensory difference) of the LOX-null beers in other brewing experiments using different recipes and different barley populations (*unpublished results*). In this sensory evaluation, the most common comment by the panel members was the papery off-flavor of the LOX-normal beers stored at 37°C. These results are consistent with the relationship between the T2N concentration (or nonenal potential) and stale flavor reported by Drost et al (1990).

The findings we obtained for the flavor-stability of the LOX-null beer suggest a few practical advantages when using the LOX-1 null barley in brewing. The first advantage expected is that the use of this barley can be easily applied to various kinds of brewing facilities to improve the flavor-stability of their beers without changing any processes. Brewing with this barley will become the easiest way to improve the flavor-stability of beers, especially for the breweries without access to modern facilities. The second advantage is that the use of this barley requires no special processes for just reducing LOX-1 activity during malting and mashing. This advantage may enable us to reduce the cost and energy needed for inactivating the LOX-1. The third advantage is that the use of this barley can reduce the development of the cardboard flavor during the long-distance transportation of the beers, which is enhanced by the high temperature and vibration during transportation. This advantage may make it possible to provide worldwide consumers with the beer that has a flavor closer to that of the original.

Another effect of the LOX-1 null barley on the beer quality concerns the improvement of the foam stability. Previously, we clarified the involvement of LOX-1 and other factors in the formation of THOD (Kuroda et al 2002), which is known to have an adverse effect on foam stability (Kobayashi et al 2002). In the present study, we confirmed that the LOX-null beer showed a reduced level of THOD and an improved foam stability compared with the LOX-normal beer (Table V). These results are consistent with the previous reports and clarified the contribution of LOX-1 to the THOD formation in brewing. The remaining THOD in the LOX-null beer indicates the existence of pathways other than the LOX-1 cascade pathway that produces THOD during mashing. One possible pathway is the hydrolysis of the esterified THOD precursors (Wackerbauer and Meyna 2002).

From the viewpoint of barley breeding, it is important to know the negative effects of the LOX-1 null trait. We analyzed the general characteristics of the malt, wort, and beer made from two F₄ populations derived from a single cross between the LOX-1 null mutant line OUI003 (SBOU2) and a wild-type line. We did not observe any major differences in these qualities between these populations (Tables I and II). These results indicate that the absence of LOX-1 has no apparent effect on these characteristics. This is a prerequisite for creating new barley cultivars with the LOX-1 null trait.

CONCLUSIONS

The present study clarified the advantages of the LOX-1 null barley as a brewing raw material. The flavor and foam stabilities of the beer were improved by using the LOX-1 null barley. We

could not observe any major adverse effect on the general characteristics of the malt, wort, and beer. Therefore, we believe that the LOX-1 null barley can be successfully used to improve the flavor and foam stabilities of beer in breweries. We are now accelerating our breeding programs for the introduction of the LOX-1 null trait into advanced malting barley cultivars.

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LITERATURE CITED

- Bamforth, C. W. 1999. The science and understanding of the flavour stability of beer: A critical assessment. *Brauwelt Int.* 17:98-110.
- Bamforth, C. W., Muller, R. E., and Walker, M. D. 1993. Oxygen and oxygen radicals in malting and brewing: A review. *J. Am. Soc. Brew. Chem.* 51:79-88.
- Bauer, C., Grosch, W., Weiser, H., and Jugel, H. 1977. Enzymatic oxidation of linoleic acid: Formation of bitter tasting fatty acids. *Z. Lebensm. Unters. Forsh.* 164:171-176.
- Baxter, E. D. 1982. Lipoxydase in malting and mashing. *J. Inst. Brew.* 88:390-396.
- Doderer, A., Kokkelink, I., Van Der Veen, S., Valk, B., Schram, A. W., and Douma, A. C. 1992. Purification and characterization of two lipoxygenase isozymes from germinating barley. *Biochem. Biophys. Acta* 1120:97-104.
- Drost, B. W., Van den Berg, R., Freijee, F. J. M., Van der Velde, E. G., and Holleman, S. M. 1990. Flavor stability. *J. Am. Soc. Brew. Chem.* 48:124-131.
- EBC. 1987. *Analytica*, 4th Ed. European Brewery Convention. Brauerei und Getraenke Rundschau: Zurich.
- Hirota, N., Kaneko, T., Kuroda, H., Ito, K., and Takeda, K. 2004. Genetic variation of barley seed lipoxygenase-1: Null mutants. Pages 69-73 in: *Proc. Int. Barley Genetics Symposium, 9th (II)*. I. J. Spunar and J. Janikova, eds. Agric. Res. Inst. Kromeriz Ltd.: Czech Republic.
- Hirota, N., Kaneko, T., Kuroda, H., Kaneda, H., Takashio, M., Ito, K., and Takeda, K. 2005. Characterization of lipoxygenase-1 null mutants in barley. *Theor. Appl. Genet.* 111:1580-1584.
- Hugues, M., Boivin, P., Gaillard, F., Nicolas, J., Thiry, J., and Andrichard-Forget, F. 1994. Two lipoxygenase from germinating barley—Heat and kilning stability. *J. Food Sci.* 59:885-889.
- Jamieson, A. M., and Van Gheluwe, J. E. A. 1970. Identification of a compound responsible for cardboard flavor in beer. Pages 192-197 in: *Proc. Am. Soc. Brew. Chem.: St. Paul, MN*.
- Jiang, Z. Y., Woollard, A. C. S., and Wollff, S. P. 1991. Lipid hydroperoxide measurement of Fe²⁺ in the presence of Xylenol Orange. Comparison with the TBA assay and an iodometric method. *Lipids* 26:853-856.
- Kaneda, H., Kano, Y., Osawa, T., Kawakishi, S., and Kamada, K. 1989. The role of free radicals in beer oxidation. *J. Am. Soc. Brew. Chem.* 47:49-53.
- Kaneda, H., Kobayashi, N., Takashio, M., Tamaki, T., and Shinotsuka, K. 1999. Beer staling mechanism. *Tech. Q. MBAA* 36:41-47.
- Kaneda, H., Takashio, M., Shinotsuka, K., and Okahata, Y. 2001. Adsorption and desorption of beer components from a lipid membrane related to sensory evaluation. *J. Biosci. Bioeng.* 92:221-226.
- Kobayashi, N., Kaneda, H., Kano, Y., and Koshino, S. 1993. The production of linoleic acid hydroperoxides during mashing. *J. Ferment. Bioeng.* 76:371-375.
- Kobayashi, N., Kaneda, H., Kuroda, H., Kobayashi, M., Kurihara, T., Watari, J., and Shinotsuka, K. 2000. Simultaneous determination of mono-, di-, and trihydroxyoctadecenoic acids in beer and wort. *J. Inst. Brew.* 106:107-110.
- Kobayashi, N., Segawa, S., Umemoto, S., Kuroda, H., Kaneda, H., Mitani, Y., Watari, J., and Takashio, M. 2002. A new method for evaluating foam-damaging effect by fatty acid. *J. Am. Soc. Brew. Chem.* 60:37-41.
- Kuroda, H., Kobayashi, N., Kaneda, H., Watari, J., and Takashio, M. 2002. Characterization of factors that transform linoleic acid into di- and trihydroxyoctadecanoic acid in mash. *J. Biosci. Bioeng.* 93:73-77.

- Kuroda, H., Furusyo, S., Maeba, H., and Takashio, M. 2003. Characterization of factors involved in the production of 2(E)-nonenal during mashing. *Biosci. Biotechnol. Biochem.* 67:691-697.
- Larsen, O. V., Aastrup, S., Nielsen, H., and Lillelund, A. C. 2001. Improvement of flavour stability by reduction of trans-2-nonenal—A case study. *Proc. 28th EBC Congress* 56:1-7
- Liégeois, C., Meurens, N., Badot, C., and Collins, S. 2002. Release of deuterated (E)-2-nonenal during beer aging from labeled precursors synthesized before boiling. *J. Agric. Food Chem.* 50:7634-7638.
- Maeda, K. 1999. Preventive production of beer against oxidation. *Tech. Q. MBAA* 36:55-59.
- McElroy, D., and Jacobsen, J. 1995. What's brewing in barley biotechnology? *Bio/Technol.* 13:245-249.
- Meilgaard, M. C. 1975. Flavor chemistry of beer. II. Flavor and threshold of 239 aroma volatiles. *Tech. Q. MBAA* 12:151-168.
- Noël, S., and Collin, S. 1995. *trans*-2-Nonenal degradation products during mashing. *Proc. 25th EBC Congress*, pages 483-490. Oxford University Press: UK.
- Ogushi, K., Barr, A. R., Takahashi, S., Asakura, T., Takoi, K., and Ito, K. 2002. Lofty Nijo: A high quality malting barley variety released from an Australian-Japanese collaboration. *J. Inst. Brew.* 108:13-18.
- Schwarz, P. B., and Pyle, R. E. 1984. Lipoxygenase and hydroperoxide isomerase activity of malting barley. *J. Inst. Brew.* 42:47-53.
- Shimizu, C., Ohno, M., Araki, S., Furusyo, S., Watari, J., and Takashio, M. 2002. Effect of reduction of carbonyl compounds by yeast on flavor stability of Happosyu. *J. Am. Soc. Brew. Chem.* 60:122-129.
- Takashio, M., and Shinotsuka, K. 2001. Continuing progress with the anti-oxidative beer production system. *Tech. Q. MBAA* 38:41-45.
- Ueda, T., Sasaki, K., Inomoto, K., Kono, K., Kagami, N., Shibata, K., and Eto, M. 2001. Development of novel malt evaluation method for improving beer flavor stability. *Proc. EBC Congress*, 28th. 55:1-9
- Van Mechelen, J. R., Schuurink, R. C., Smits, M., Graner, A., Douma, A. C., Sedee, N. J. A., Schmitt, N. F., and Valk, B. E. 1999. Molecular characterization of two lipoxygenases from barley. *Plant Mol. Biol.* 39:1283-1289.
- Wackerbauer, K., and Meyna, S. 2002. Free and triglyceride-bonded hydroxyl fatty acids in barley and malt. I. Influence of variety, region of growth and harvest year. *Monatsschr. Brau.* 55: 52-57.
- Wu, Y., Schwarz, P. B., Doehlert, D. C., Dahleen, L. S., and Horsley, R. D. 1997. Rapid separation and genotypic variation of barley (*Hordeum vulgare* L.) lipoxygenase isozymes. *J. Cereal Sci.* 25:49-56.
- Yabuuchi, S. 1976. Occurrence of a new lipoxygenase in germinating barley embryo. *Agric. Biol. Chem.* 40:1987-1992.
- Yabuuchi, S., and Yamashita, H. 1979. Gas chromatographic determination of trihydroxyoctadecanoic acid in beer. *J. Inst. Brew.* 85:216-218.
- Yang, G., and Schwarz, P. B. 1995. Activity of lipoxygenase isozymes during malting and mashing. *J. Am. Soc. Brew. Chem.* 53:45-49.
- Yang, G., Schwarz, P. B., and Vick, B. A. 1993. Purification and characterization of lipoxygenase isozymes in germinating barley. *Cereal Chem.* 70:589-595.

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