Volatile Components in Stored Rice [*Oryza sativa* (L.)] of Varieties with and without Lipoxygenase-3 in Seeds

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Lipoxygenase (LOX) is thought to play an important role in the formation of desirable or undesirable flavor and aroma in many plant products. In rice seeds, LOX activity is localized in the bran fraction and LOX-3 is the major isozyme component. We used gas chromatography−mass spectrometry to determine whether the degree of staleness in the flavor of stored brown rice was related to the presence of LOX-3. We found that the amount of hexanal, pentanal, and pentanol in normal raw LOX-3 rice markedly increased during storage at 35 °C. That in LOX-3-less rice increased slightly but was a third to a fifth that of normal LOX-3 rice. In cooked rice, the amount of these components from glutinous rice exceeded that in nonglutinous rice, and that in normal LOX-3 rice exceeded that in LOX-3-less rice. These results indicate that the stale flavor production in LOX-3-less rice during storage is less than that in normal LOX-3 rice.

Keywords: *Hexanal; lipoxygenase-3; lipoxygenase-3-less; rice; stale flavor; storage*

INTRODUCTION

Rice is the staple diet of more than half the world's population, primarily in East and Southeast Asia. While Japan is self-sufficient in rice, the variability of rice supply makes storage stability important in ensuring ongoing high-quality rice availability. Rice grain deterioration and the development of stale flavor during storage are serious problems that reduce stored grain quality. Many efforts have been made to stabilize brown rice storage quality. Optimum packaging materials, temperature, and atmosphere help slow oxidative deterioration (Mitsuda et al., 1972; Sowbhagya and Bhattacharya, 1976; Ory et al., 1980; Sharp and Timme, 1986). However, the high cost of land and construction in Japan complicate the building of temperature-controlled warehouses that would help ensure the success of postharvest rice treatment.

Many researchers have reported that free fatty acid (FFA) content increases during rice storage and suggest that lipid degradation is responsible for deterioration during storage (Yasumatsu and Moritaka, 1964; Yasumatsu et al., 1966; Aibara et al., 1986; Shin et al., 1986; Takano, 1993). Lipoxygenase (LOX) catalyzes the peroxidation of polyunsaturated fatty acids containing a 1,4-pentadiene structure, such as linoleic and linolenic acids, into conjugated hydroperoxy fatty acids. Hydroperoxides produced enzymatically and nonenzymatically are further transformed into volatile compounds changing or adding flavor, and LOXs are thought to play an important role in the formation of desirable or undesirable flavor and aroma in many plant products (Gardner, 1988; Hildebrand, 1989; Hatanaka, 1993). Many compounds have been identified in raw and cooked rice or bran fraction (Yasumatsu and Moritaka, 1964; Mitsuda et al., 1968; Bullard and Holguin, 1977; Legendre et al., 1978; Ory et al., 1980; Tsugita et al., 1980, 1983; Mega, 1984; Shin et al., 1986; Buttery et al., 1988). Researchers reported that some volatiles, especially hexanal, increase during storage (Yasumatsu and Moritaka, 1964; Legendre et al., 1978; Ory et al., 1980; Shin et al., 1986). Because the amount of unsaturated fatty acids decreases in stored rice, Yasumatsu and Moritaka (1964) suggested that linoleic acid in rice is oxidized gradually during storage, producing carbonyl compounds that induce rice to taste stale. Shin et al. (1986) reported that the amount of hexanal is linearly proportional to that of oxidized linoleic acid.

LOX activity in rice grain is localized in a bran-milling fraction (Shastry and Rao, 1975; Yamamoto et al., 1980). Three isozymes—LOX-1, LOX-2, and LOX-3—were found in rice embryos, and LOX-3 is the major isozyme component (Ida et al., 1983). A Thai variety, Daw Dam, was found, on the basis of immunoblot analysis and enzymatic assay for LOX-3 activity in ungerminated seeds, to lack the LOX-3 protein (Suzuki et al., 1993; Suzuki and Matsukura, 1997). Genetic analysis showed that LOX-3 absence is inherited as a single recessive trait (Suzuki, 1995; Suzuki et al., 1996a). Suzuki et al. (1996b) reported that peroxidation products of unsaturated fatty acids are lower in a Daw Dam bran fraction during storage than in rice varieties with LOX-3 in their seeds. These results suggest that the absence of LOX enzymes in rice grains alleviate oxidative deterioration.

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This study was undertaken to determine whether an amount of the stale flavor in stored brown rice was related to the presence of LOX-3 in its seed. To do so, we compared two types of rice sans LOX-3 and two types of rice with normal LOX-3 activity.

EXPERIMENTAL PROCEDURES

Rice Samples. We used four rice [*Oryza sativa* (L.)] varieties: Daw Dam, a Thai variety lacking LOX-3 in its seeds and having a glutinous endosperm (Suzuki et al., 1993, 1996a); CI-115, a pure line selected from a Chinese variety, Chongtui, lacking LOX-3 in its seeds and having a nonglutinous endosperm (see Results); Koganemochi, the most popular glutinous variety in Japan; and Koshihikari, the most popular nonglutinous variety in Japan. Koganemochi and Koshihikari have normal LOX-3 activity. Samples were collected from an experimental field managed by the National Agriculture Research Center, Japan, in 1997. Harvested rice was dehulled with a Satake experimental dehuller (Hiroshima, Japan), and its moisture content was adjusted to 14.0−14.5%; 50 g of each brown rice variety was stored in a polyethylene bag at 4 or 35 °C for 2, 4, or 8 weeks. After storage, rice was kept below −40 °C until analysis.

Gas Chromatography of Fatty Acid Methyl Esters. A 5 g sample of the brown rice stored for the stated period was homogenized with 25 mL of hexane containing 0.05% butylhydroxytoluene in a Polytron (Kinematica, Switzerland). After the homogenate was centrifuged, the pellet was homogenized twice with another 25 mL of hexane. Extracts were combined, concentrated on a rotary evaporator at below 40 °C, and dissolved in 1 mL of heptane as total lipids. After 0.5 μg of methyl heptadecanoate (Serdary Research Laboratories, Inc.) was added as an internal standard to the aliquot of the total lipid fraction, FFA in the fraction was methylated with ethereal diazomethane (Fujino, 1987a). The sample was dried, dissolved with heptane, and injected onto a Hewlett-Packard (HP) Model 6890A gas chromatograph (GC) (Cheadle Heath, U.K.) coupled with a flame-ionization detector and a capillary column (CP−WAX 52CB; 30 m × 0.25 mm i.d., 0.25 μm film thickness, Chrompack, Netherlands). The column temperature was programmed from 90 to 220 °C (25 °C/min), holding the initial temperature for 1 min and the final temperature for 15 min. The injection temperature was 270 °C and detector temperature was 250 °C.

To determine fatty acid composition of total lipid in the four rice varieties, an aliquot of the total lipid fraction was transesterified with hydrochloride methanol for 2 h at 80 °C (Fujino, 1987b). Methyl heptadecanoate was added as an internal standard and fatty acid methyl esters were analyzed on a GC as stated above.

Volatile Components from Raw Brown Rice. Volatile components from raw brown rice were analyzed by the method of Sakui et al. (1996) with slight modifications. The stored brown rice (10 g) was weighed into a 20 mL vial, which was then sealed with a septum secured by an aluminum cap. After the vial was heated at 80 °C for 20 min in a headspace sampler magazine (HP Model 7694A), a 3 mL gas sample was removed from the headspace of the vial and placed onto a GC (HP Model 6890A) equipped with a HP Model 5972A mass spectrometer (MS). Analysis conditions were as follows: a capillary column (HP−Wax), 60 m long × 0.25 mm i.d. fused silica, wall coated with cross-linked polyethylene glycol (0.25 μm film thickness); oven temperature, held at 35 °C for 2 min, increased in 3-step linear gradations from 35 to 240 °C (4 °C/min from 35 to 160 °C, 5 °C/min from 160 to 180 °C, and 10 °C/min from 180 to 240 °C), and held at 240 °C for 3 min; injection temperature, 200 °C; injection, split (1/5 ratio) injection; carrier gas, helium; flow rate, 1 mL/min. To identify volatile components from brown rice, individual volatile components were verified by comparing both GC retention times and their mass spectra with those of standards. An internal standard was not added to these GC−MS assays because of its adhesion to rice grains. Standard samples (aldehydes and alcohols mixture) were assayed every 5 vials to verify instrument sensitivity.

Figure 1. Immunological analysis of LOX-3 content in a rice seed. LOX-3 content in Koshihikari (lane 1), Daw Dam (lane 2), CI-115 (lane 3), and Koganemochi (lane 4) rice is shown.

Volatile Components from Cooked Rice. A typical Japanese cooking procedure was conducted with a 20 mL vial containing raw brown rice (6 g) and 6 mL of distilled water. The vial was sealed with a septum secured by an aluminum cap and then autoclaved at 110 °C for 20 min. After the vial was heated at 80 °C for 20 min in a headspace magazine, headspace volatiles were analyzed as stated above.

Lipoxygenase Content. The production of mAbs specific to LOX-3 has been described elsewhere (Suzuki et al., 1992). A manually selected rice embryo was homogenized individually with 40 μL of 62.5 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) (pH 6.8), containing 2% sodium dodecyl sulfate (SDS), 5% 2-mercaptoethanol, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mM monoiodoacetic acid, 0.08% bromophenol blue, and 10% glycerol. SDS−polyacrylamide gel electrophoresis and immunoblotting were conducted as described previously (Suzuki et al., 1993). Protein bands bound to mAbs on a polyvinylidene difluoride membrane were detected by peroxidase reaction with 3,3′-diaminobenzidine as the substrate.

RESULTS

Lipoxygenase Content. Suzuki et al. (1992, 1993) surveyed the rice germplasm collection and found one accession, Daw Dam, which lacks LOX-3 activity and the protein in ungerminated seed. A Thai upland rice, Daw Dam, belongs to Javanica rice (Suzuki, 1995), and an area including Nepal, Bhutan, Assam, Myanmar, Vietnam, and Yunnan, China was widest in the variation of esterase zymogram patterns (Nakagahra et al., 1975). We surveyed the germplasm collection in this area for other LOX-3-less varieties and found several varieties. One, CI-115, was a pure line from a Yunnan upland variety, Chongtui, and had a nonglutinous endosperm (Figure 1).

Because our preliminary experiment showed that profiles of volatile production differed between glutinous and nonglutinous varieties, we used four varieties for this study: two glutinous varieties, LOX-3-less (Daw Dam) and normal LOX-3 activity (Koganemochi), and two nonglutinous varieties, LOX-3-less (CI-115) and normal LOX-3 activity (Koshihikari). We compared LOX-3 content in these four varieties by immunoblotting (Figure 1). A LOX-3 band was detected in an extract from an embryo of Koganemochi and Koshihikari but not in that of Daw Dam and CI-115.

Content and Composition of Fatty Acids. Fat acidity is one of the most important indices of changes in rice quality during storage and FFA content increases

Table 1. Linoleate and Total Free Fatty Acid Content[a] of Rice Grains from Varieties with LOX-3 in Seeds (Koganemochi and Koshihikari) and without LOX-3 (Daw Dam and CI-115)

weeks	°C	Daw Dam	Koganemochi	CI-115	Koshihikari
			Linoleate		
0		246 ± 3	228 ± 0	99 ± 1	111 ± 1
2	4	246 ± 3	247 ± 1	95 ± 0	90 ± 1
2	35	262 ± 3	237 ± 1	114 ± 1	106 ± 1
4	4	328 ± 9	273 ± 4	105 ± 2	122 ± 1
4	35	264 ± 3	244 ± 2	123 ± 1	157 ± 2
8	4	269 ± 2	254 ± 0	144 ± 2	172 ± 2
8	35	285 ± 1	222 ± 1	163 ± 2	170 ± 2
			Total FFA		
0		484 ± 1	530 ± 4	253 ± 0	275 ± 3
2	4	486 ± 3	572 ± 8	250 ± 3	219 ± 5
2	35	524 ± 6	561 ± 4	298 ± 1	267 ± 2
4	4	673 ± 15	673 ± 6	279 ± 5	309 ± 4
4	35	564 ± 8	607 ± 5	329 ± 5	386 ± 5
8	4	616 ± 2	657 ± 4	390 ± 8	426 ± 6
8	35	659 ± 3	632 ± 2	454 ± 5	454 ± 5

[a] Given in micrograms per gram of brown rice.

during storage (Takano, 1993). Total FFA and linoleic acid in all varieties increased with increasing storage at both storage temperatures (Table 1). Amounts in glutinous Daw Dam and Koganemochi were higher than those in nonglutinous CI-115 and Koshihikari. When amounts were compared for glutinous and nonglutinous varieties, that in LOX-3-less varieties was almost the same as in normal LOX-3 varieties.

A comparison of fatty acid composition in three rice varieties—Daw Dam, Koganemochi, and Koshihikari—showed that total lipids consisted of 36−38% oleic acid, 37−40% linoleic acid, 17−20% palmitic acid, and small amounts (1−3%) of stearic acid and linolenic acid (Table 2). These results agree well with those from other reports (Aibara et al., 1986; Taira et al., 1988; Suzuki et al., 1996b). CI-115 composition differed strangely from other varieties: 7−8% higher in oleic acid and 4−6% lower in linoleic acid. Compositional differences in rice varieties between storage periods (before and after 8 weeks) were not found.

Changes in Volatile Components in Raw Brown Rice during Storage. To detail volatile components in brown rice, a gas sample released from rice after heating at 80 °C or accumulated in the headspace of a vial was subjected to headspace GC−MS. Preliminary study showed that volatile components from less than 10 g of raw brown rice or 6 g of cooked rice were effectively analyzed by direct injection to the GC−MS without prior volatile enrichment or grain grinding. Volatile components in the headspace of brown rice stored at 4 °C and at 35 °C for 0, 2, 4, and 8 weeks were determined by GC−MS. Typical gas chromatographic profiles of volatile components of raw brown rice (Daw Dam and Koshihikari) stored at 35 °C for 8 weeks are shown in Figure 2. A comparison of curves indicated a notable decrease in acetaldehyde, acetone, methanol, and ethanol and a notable increase in pentanal, hexanal, and pentanol from original samples (panels A and B) compared to two 8-week samples (panels C and D). We plotted net areas of major components to get a more concise picture of changes in the profiles of volatile components from the different varieties with storage time. The amount of hexanal in all varieties stored at 4 °C scarcely changed throughout storage (Figure 3). That of hexanal in normal LOX-3 Koganemochi and Koshihikari stored at 35 °C, however, was almost unchanged during the first 2 weeks of storage and increased markedly thereafter, resulting in about a 3−5-fold increase over that before storage. In contrast, that in LOX-3-free Daw Dam and CI-115 stored at 35 °C showed almost no change during the first 2 weeks of storage, and increased only slightly afterward. That in Koganemochi stored 8 weeks at 35 °C was 2.5−3.3-fold higher, and in Koshihikari, 5.5−7.2-hold higher than in Daw Dam and CI-115. Similar profiles for amounts of pentanal and pentanol during storage were also obtained (Figure 3). In contrast to the time course in these components, the amount of acetaldehyde decreased gradually during storage at 4 °C and rapidly at 35 °C, as did acetone, methanol, and ethanol (data not shown).

Changes in Volatile Components in Cooked Brown Rice during Storage. We analyzed changes in volatile components of cooked brown rice stored for 8 weeks. Time course profiles of hexanal, pentanal, and pentanol content during storage differed for glutinous and nonglutinous rice (Figure 4). The amount of these components in glutinous Daw Dam and Koganemochi were notably higher throughout storage than in nonglutinous CI-115 and Koshihikari, regardless of whether LOX-3 was contained in the seeds. That in all rice stored at 35 °C increased constantly throughout storage without a time lag, in contrast to raw rice. Comparing glutinous and nonglutinous, the amount in normal LOX-3 rice was higher throughout storage than that in LOX-3-less rice varieties. In glutinous varieties, the amount of hexanal in Koganemochi was higher than that in Daw Dam; in nonglutinous varieties, that in Koshihikari was higher than that in CI-115 (Figure 4). Pentanal and pentanol during storage showed similar profiles. Thus, the amount of hexanal, pentanal, and pentanol in LOX-3-less varieties was lower than that in normal LOX-3 varieties.

DISCUSSION

We reported previously that unsaturated fatty acids peroxidize at lower levels in Daw Dam than in varieties with normal LOX-3 activity in seeds (Suzuki et al. 1996b). However, these experiments were done with a bran fraction, not a brown rice. After rice milling, triacylglycerols in rice bran decompose rapidly because of high lipolytic activity in the rice bran fraction (Takano, 1993), releasing FFAs, including unsaturated fatty acids (LOX substrates). We thus had to clarify whether the oxidative stability of lipids in Daw Dam is present both in stored brown rice and in bran fractions. From these reasons, we studied whether stale flavor in brown rice during storage was related to LOX-3 in its seeds. To do so, we compared Daw Dam and CI-115 rice, which lack LOX-3 activity in their seeds, with Koganemochi and Koshihikari rice, which have normal LOX-3 activity (Suzuki et al., 1993, 1996b; Figure 1). A comparison of FFA contents and volatile components in stored rice showed some differences among varieties and in storage effects at two different temperatures (Tables 1 and 2 and Figures 2−4).

The quality of rice, especially flavor, is easily deteriorated by oxidation during storage. The trigger in this deterioration of rice flavor is a change in the spherosome in aleurone cells (Takano, 1993). When the spherosome membrane is broken by physical damage during harvest, transport, and storage, neutral lipids are hydrolyzed by lipase, increasing FFAs. Free unsaturated fatty

Table 2. Fatty Acid Profile (%) of Rice Grains of Varieties with LOX-3 in Seeds (Koganemochi and Koshihikari) and without LOX-3 (Daw Dam and CI-115)

	Daw Dam			Koganemochi			CI-115			Koshihikari		
	0 week	8 weeks		0 week	8 weeks		0 week	8 weeks		0 week	8 weeks	
		4 °C	35 °C		4 °C	35 °C		4 °C	35 °C		4 °C	35 °C
palmitate	18.9	18.2	18.3	22.3	21.4	21.5	17.1	16.5	16.5	19.4	19.2	19.3
stearate	1.7	1.8	1.8	1.6	1.7	1.7	2.6	2.8	2.6	1.8	1.9	1.8
oleate	38.1	39.0	39.2	36.5	37.0	37.1	45.0	45.5	45.5	38.0	38.8	38.5
linoleate	39.7	39.4	39.0	37.7	37.9	37.7	33.4	33.3	33.4	38.8	38.1	38.4
linolenate	1.2	1.2	1.1	1.4	1.4	1.3	1.3	1.2	1.2	1.5	1.4	1.4
arachidate	0.4	0.5	0.5	0.5	0.6	0.6	0.6	0.8	0.8	0.6	0.6	0.6

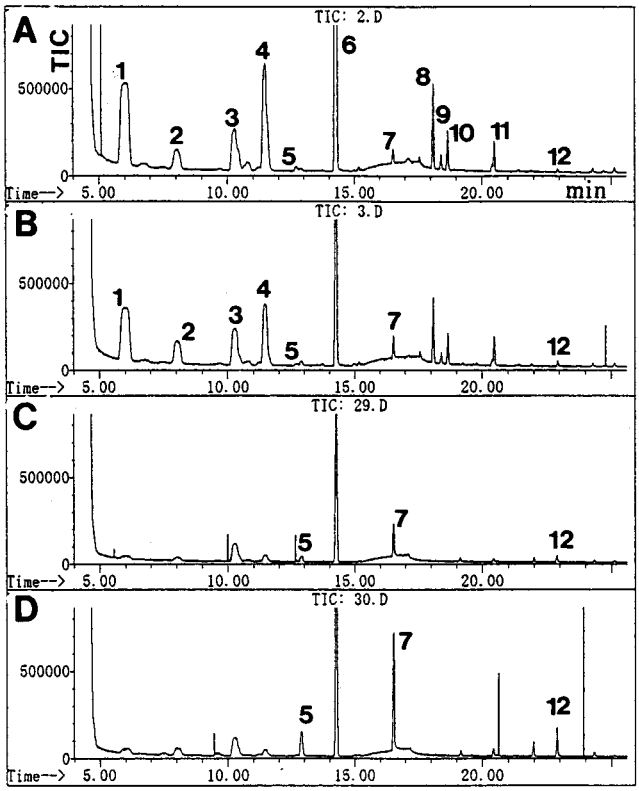

Figure 2. Typical gas chromatographic profiles of headspace volatiles in raw brown rice seeds with and without LOX-3 during 8 weeks of storage. (A) Daw Dam raw brown rice (LOX-3-less) before storage; (B) Koshihikari raw brown rice (normal LOX-3) before storage; (C) Daw Dam raw brown rice stored at 35 °C for 8 weeks; (D) Koshihikari raw brown rice stored at 35 °C for 8 weeks. (1) Acetaldehyde; (2) acetone; (3) methanol; (4) ethanol; (5) pentanal; (6) chloroform; (7) hexanal; (8−11) unknown; (12) pentanol.

acids thus produced are further converted to low-molecular-weight volatile products via hydroperoxides by the action of LOXs and subsequent autoxidation and decomposition. In this report, FFA in brown rice increased at both temperatures studied in all varieties (Table 1). Free linoleic acid in Daw Dam and CI-115 remained at the same level as other varieties during storage (Tables 1 and 2), indicating that the amount of substrates for LOX-3 in Daw Dam and CI-115 was comparable to that in other varieties.

We showed the application of direct, rapid, simple GC−MS for detecting and characterizing flavor-producing compounds in raw and cooked rice. Many analytical reports have covered volatile components in cooked rice (Yasumatsu and Moritaka, 1964; Tsugita et al., 1980, 1983; Buttery et al., 1988), but little research has been done on volatiles associated with raw rice (Bullard and Holguin, 1977; Legendre et al., 1978). In later studies, ground brown rice or bran fractions were analyzed for

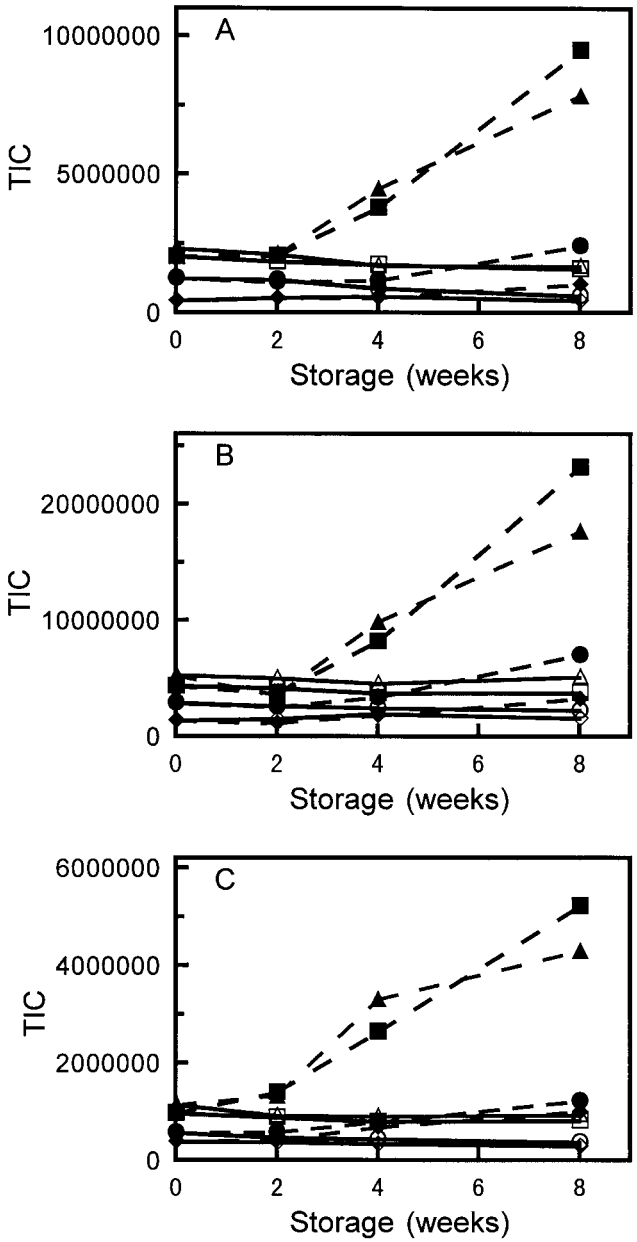

Figure 3. Changes in major components—pentanal, hexanal, and pentanol—in headspace vapor of raw brown rice seeds with and without LOX-3. (A) Pentanal; (B) hexanal; (C) pentanol. (○, ●) Daw Dam; (□, ■) Koshihikari; (△, ▲) Koganemochi; (◇, ◆) CI-115; (○, □, △, ◇) stored at 4 °C; (●, ■, ▲, ◆) stored at 35 °C. TIC, total ion chromatography.

these volatile components. Although the volatiles we detected were the major components and less than 10 components (Figure 2), detection used less than 10 g of sample without prior enrichment of volatiles, as in Tenax adsorption, and grinding, enabling us to quali-

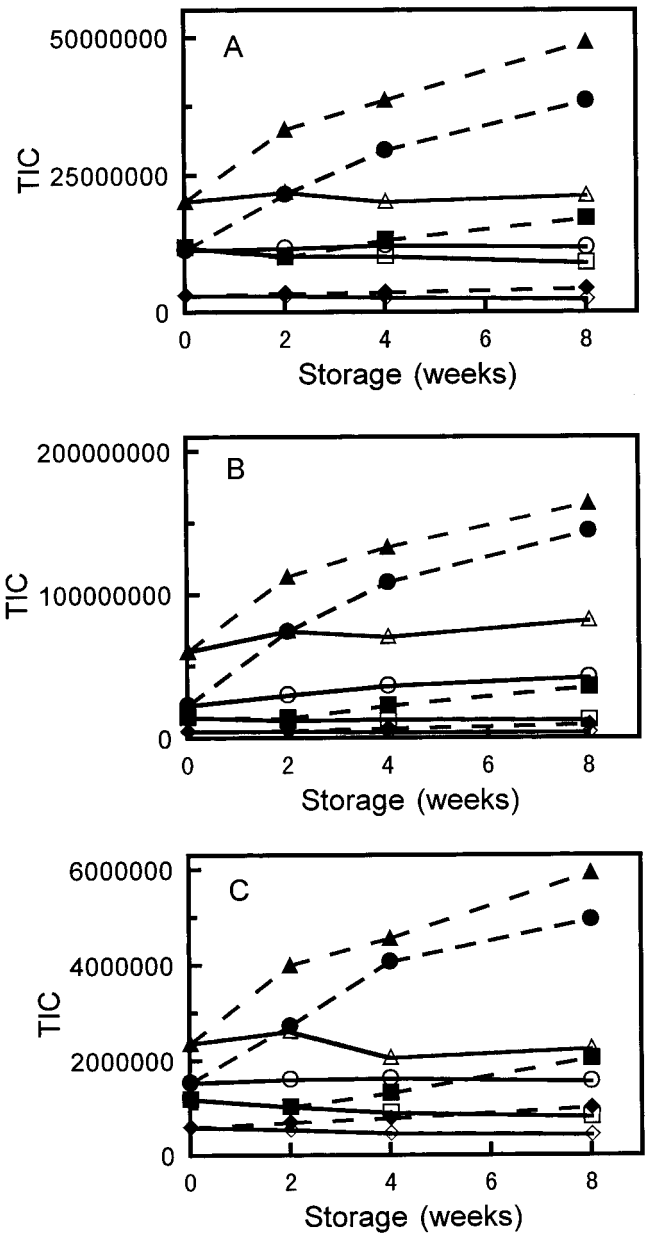

Figure 4. Changes in major components—pentanal, hexanal, and pentanol—in headspace vapor of cooked brown rice seeds with and without LOX-3. (A) Pentanal; (B) hexanal; (C) pentanol. Symbols are as described for Figure 3.

tatively compare volatile components from rice with and without LOX-3 in seeds. This simple method is potentially useful in evaluating rice breeding programs, in which only a small amount of grains is available.

Rice after storage of more than 1 year after harvest is called "old rice" or "long-stored rice" in Japan, and the resulting stale flavor regardless of cooking is not usually considered desirable. As stated, many researchers reported that hexanal and some aldehydes and/or alcohols are major volatile components in rice grains preservation (Yasumatsu et al., 1966; Legendre et al., 1978; Ory et al., 1980; Tsugita et al., 1983; Shin et al., 1986). We also showed the major volatile components during normal LOX-3 rice storage to be hexanal, heptanal, and pentanol (Figures 2−4). Interestingly, the amount of these components in LOX-3-less rice was lower than that in normal LOX-3 rice (Figures 2−4). These results suggest that LOX-3 participates in producing volatile components in stored rice and that the development of stale flavor in LOX-3-less rice stored at elevated temperature is less than that in normal LOX-3 rice.

The precise degradation mechanisms of hydroperoxides to hexanal in rice grains remain ambiguous. As reported previously, LOX-3 is the major isozyme component in rice grain (Ida et al., 1983; Ohta et al., 1986). LOX-3 specifically produces 9-D-hydroperoxy-10,12(*E*,*Z*)-octadecadienoic acid (9-LOOH) when linoleic acid is used as a substrate (Yamamoto et al., 1980; Ida et al., 1983; Ohta et al., 1986), not 13-L-hydroperoxy-9,11(*Z*,*E*)-octadecadienoic acid (13-LOOH), a precursor of hexanal in many higher plants (Gardner, 1988; Hatanaka, 1993). β-Scission of 9-LOOH leads to 2,4-decadienal, and hexanal is preferentially formed at moderate temperatures by selective oxidation of 2,4-decadienal (Grosch, 1987). Major volatiles of linoleic acid autoxidation and 9- and 13-LOOH decomposition are identical (Grosch, 1987). These results suggest that the degradation mechanisms of lipid hydroperoxide in rice differ from those in other plants and that hexanal is produced nonenzymatically or by an unknown pathway from linoleic acids via 9-LOOH.

In addition to the effect of LOX-3 presence or absence on volatile production, we should account for an endosperm effect—glutinous and nonglutinous—and a genetic background effect. Glutinous rice grains are generally accepted to have "characteristic glutinous flavor" different from that of nonglutinous endosperm, and components causing such a flavor are still not clear. In cooked rice, the amount of volatile components in glutinous varieties was significantly higher than in nonglutinous varieties throughout storage regardless of LOX-3 (Figure 4). The genetic background of the rice varieties we used differs too greatly—tropical Japonica (Javanica) rice (Daw Dam and CI-115) and temperate Japonica rice (Koganemochi and Koshihikari) (Suzuki, 1995; Suzuki et al., 1996a). Thus, it is necessary to exclude endosperm differences (glutinous and nonglutinous) and genetic background effects on the production of stale flavor in rice grains. With the same genetic background, it is necessary to accurately assess the relationship between LOX-3 activity and the production of stale flavor. For these reasons, development of near-isogenic lines obtained by selecting and selfing heterozygotes from a F_2 generation would be useful (Yang et al. 1995). We have been developing a series of near-isogenic lines of glutinous LOX-3-less, glutinous normal LOX-3, nonglutinous LOX-3-less, and nonglutinous normal LOX-3 lines, derived from the F_1 hybrid of Daw Dam/Nipponbare (Suzuki et al., 1996a). The heterozygotes of F_6 lines—LOX-3 (LOX-3/lox-3) and endosperm character (Wx/wx)—are currently obtained. Use of near-isogenic lines would enable simultaneous comparison of LOX-3 presence/absence and endosperm glutinous/nonglutinous character in the effect on stale flavor on the same genetic background.

As stated above, our results suggest that the development of stale flavor in LOX-3-less rice stored at elevated temperature is less than that from normal LOX-3 rice. Because external factors favoring enzymatic peroxidation of unsaturated fatty acids cannot be easily controlled, the genetic elimination of LOXs from seeds would be useful for maintaining high-quality rice during storage. The introduction of LOX-3-less character in mature seeds into varieties with superior agronomic backgrounds is thus progressing with breeding groups.

ABBREVIATIONS USED

FFA, free fatty acid; GC, gas chromatography; LOX, lipoxygenase; 9-LOOH, 9-D-hydroperoxy-10,12(*E*,*Z*)-octadecadienoic acid; 13-LOOH, 13-L-hydroperoxy-9,11-(*Z*,*E*)-octadecadienoic acid; mAb, monoclonal antibody; MS, mass spectrometer; SDS, sodium dodecyl sulfate.

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