Studies on Changes in Fatty Acid Composition and Content of Endogenous Antioxidants During γ Irradiation of Rice Seeds

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Accelerated aging effects, induced by γ irradiation, were investigated on the fatty acid composition of lipids and on the content of endogenous antioxidants of four Indica and four Japonica rice seeds with and without intact hull. While the linoleic acid content of the phospholipids decreased gradually with the increase in irradiation doses, there was a corresponding increase in the linoleic acid content of the free fatty acids. Such changes were drastic, especially in the case of Japonica rice seeds irradiated without intact hull. However, the neutral lipids were found to be resistant to γ irradiation.

The α-tocopherol content was found to decrease (markedly) in rice seeds irradiated with or without hull, especially in the Japonica rice seeds. At a dose of 15 kGy only traces of α-tocopherol could be detected in Japonica and Indica rice seeds irradiated with and without intact hull. Oryzanol, a relatively weaker antioxidant, was found to be more resistant to oxidative damage than α-tocopherol. At 15 kGy, the oryzanol content ranged from 59 μg to 170 μg/g lipid in rice seeds irradiated with intact hull, while the corresponding value for rice seeds irradiated without hull was 52 μg to 153 μg/g lipid.

The overall susceptibility to oxidative damage was less in Indica rice seeds, indicating that the antioxidative defense system offered better protection in overcoming oxidative stress in Indica rice hull than in Japonica rice hull.

Storage of seeds for extended periods leads to a number of degradative changes that cause reduction in percentage germination, while those seeds that do germinate produce weak seedlings (1,2). Various factors may be responsible for aging, loss of vigor and death of seeds during long-term storage. Of these, lipid peroxidation is thought to be a primary cause for seed deterioration (3). Of all the primary constituents of rice, lipids are present at the lowest level (4-6). However, they are liable to being hydrolyzed and oxidized during storage, resulting in a change in flavor characteristics, and they may form deleterious substances that can be harmful to membrane functions and integrity (7).

The initiation of lipid peroxidation by hydroxy radicals generated during γ irradiation of biological systems has been studied by many researchers (8,9). Model experiments to induce accelerated aging indicated that the peroxide value of rice grain irradiated without intact hull increased more rapidly than that of grain irradiated with intact hull (10,11). A similar difference in increases has been observed in the electron spin resonance (ESR) signal intensities. The major natural antioxidants reported in rice seeds are α-tocopherol and oryzanol, which are concentrated mainly in the bran-germ fraction (12); a novel phenolic antioxidative defense system exists in rice hull (13). Preliminary observations on accelerated aging of rice seeds induced by γ irradiation (11) prompted us to further investigate the radiation-induced changes in the fatty acid composition of rice lipids, as well as the contents of endogenous antioxidants, α-tocopherol and oryzanol.

MATERIALS AND METHODS

Rice seeds. Eight rice cultivars (*Oryza sativa* L.), Katakutara, Century Patna, IR-8, K-184 (Indica), Koshihikari, Kusabue, Sachiwatari and Himenomochi (Japonica), were cultivated under controlled conditions at the experimental farm of Nagoya University. Immediately after harvest, the seeds were sun-dried, for about two weeks, to a moisture level of around 10%. This also influenced the break in dormancy of newly harvested rice seeds.

γ Irradiation. Two types of irradiation experiments were carried out simultaneously. In the first type, rice seeds with intact hull were irradiated with doses of 5, 10 and 15 kGy by a 4-kCi ^{60}Co γ irradiator (Japan, 676 Gy/hr), while in the other, rice seeds were dehulled first in a laboratory mill, and the brown rice samples thus obtained were used. Both sets of experiments were carried out in duplicate.

Lipid analysis. Extraction. Rice seeds irradiated with and without intact hull were first powdered in a laboratory grinder so as to pass through a 60-mesh screen. Rice flour of each sample (10 g) was extracted overnight with $CHCl_3$-MeOH (2:1, v/v; 2 × 30 ml), and the extract was filtered and evaporated in vacuo on a rotary evaporator below 40°C to get the crude lipid sample. The crude lipids were transferred to sample bottles, dried and weighed.

Fractionation. Fractionation of the crude lipids into free fatty acids (FFA), neutral lipids (NL) and phospholipids (PL) was carried out according to the method of Lee and Mattick (14). The crude lipids were first dissolved in acetone (20 ml) and filtered to give the soluble fraction of FFA and NL. The insoluble PL was dissolved in 20 ml $CHCl_3$-MeOH (2:1, v/v) and filtered, and the filtrate was evaporated to dryness in vacuo, on a rotary evaporator below 40°C. The residue of PL was transferred into sample bottles, dried and weighed.

The soluble mixture of FFA and NL was evaporated to dryness and transferred to a separatory funnel, treated with a mixture of diethyl ether:*n*-hexane (1:1, 40 ml) and 0.5% Na_2CO_3 (2 × 5 ml). The contents were shaken thoroughly and allowed to stand for 15 min. The aqueous layer was separated from the ether layer, neutralized with five ml of 10% H_2SO_4 and re-extracted with diethyl ether (2 × 25 ml). The ether extracts were pooled, washed with distilled water several times until free from acid, dried over anhydrous

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Na_2SO_4, and evaporated to dryness on a rotary evaporator to give the pure FFA mixture. The FFA samples were transferred into sample bottles, dried and weighed. The organic layer left after separation of the FFA was washed with distilled water (2 × 25 ml), dried over anhydrous Na_2SO_4, and evaporated on a rotary evaporator to give the NL. The NL samples were transferred into sample bottles, dried and weighed.

Preparation of methyl esters. The dried samples of FFA, NL and PL were transesterified by refluxing with 25 ml of 5% HCl in dry MeOH for six hr at 90°C, according to the method of Adoracion and Juliano (15). After cooling, the contents of the flask were extracted with diethyl ether, dried over anhydrous Na_2SO_4, and evaporated to dryness on a rotary evaporator to yield the corresponding fatty acid methyl esters (FAME).

Analysis of methyl esters. Analysis of the FAME, in duplicate, was carried out by gas chromatography (Hitachi 263-30 GC) with a Carbowax 20M capillary column (25 m × 0.25 mm) and the column temperature maintained isothermally at 170°C, while the injection and detector oven temperatures were maintained at 250°C. The flow of the carrier gas, nitrogen, was maintained at 30 ml/min. The percentage composition of the major fatty acids, palmitic acid (16:0), oleic acid (18:1) and linoleic acid (18:2), was determined by using an integrator-recorder (Hitachi D-2000 Chromato-Integrator).

Quantification of α-tocopherol and oryzanol. Changes in the contents of the endogenous rice seed antioxidants, α-tocopherol and oryzanol, in crude rice lipid of seeds irradiated with and without intact hull were determined by high performance liquid chromatography (HPLC) (Waters Associates, Milford, Massachusetts) on a Develosil SI 60-5 column (8 × 250 mm), and monitored with a Jasco Uvidec-100-III UV spectrophotometer (Japan Spectroscopic Co. Ltd, Hachioji, Tokyo, Japan). The respective mobile phases and corresponding detection wavelengths were α-tocopherol; *n*-hexane: EtOH:dioxane (95.8:0.2:4)—298 nm, and oryzanol; *n*-hexane: EtOAc:acetic acid (50:50:1)—320 nm. The amounts of α-tocopherol and oryzanol were determined, in triplicate, by comparing the peak heights of external standards which were injected after each analysis.

RESULTS AND DISCUSSION

Effect of γ irradiation on the fatty acid composition of lipids in rice seeds irradiated with and without intact hull. The fatty acid composition of different fractions of rice lipids from seeds used in the irradiation experiments is given in Table 1. The oleic and linoleic acid contents in the free fatty acid (FFA) fraction from Indica rice seeds ranged from 25.2 to 29.3% and 34.5 to 43.2%, respectively, while the corresponding values for Japonica rice seeds ranged from 20.8 to 25.2% and 27.1 to 32.5%. NL of Japonica rice seeds had higher levels of linoleic acid than the Indica rice seeds, while the oleic acid contents did not differ appreciably among Indica and Japonica rice cultivars. In contrast, the PL fraction of Japonica rice seed lipids showed higher levels of linoleic acid, the levels being in the range of 38.7 to 42.2%, while the corresponding levels in Indica rice seeds ranged from 34.3 to 36.7%.

Changes in the fatty acid composition of rice seeds irradiated with and without intact hull are shown in Tables 2, 3 and 4. While the contents of linoleic acid of FFA gradually increased with increase in irradiation dose in both Indica and Japonica rice seeds, the rate was relatively faster in the Japonica rice seeds irradiated with or without intact hull. The increase or decrease in the levels of oleic acid of FFA was identical in both Indica and Japonica cultivars, irradiated with or without intact hull. The increase in the levels of linoleic acid in FFA was higher in all the varieties irradiated without intact hull, indicating that the rice lipids in such seeds had undergone more extensive damage than the corresponding seeds irradiated with hull.

TABLE 1

Fatty Acid Composition (%) of Lipids from Control Rice Seeds Used for γ Irradiation Studies[a]

	Free fatty acids			Neutral lipids			Phospholipids		
VAR	16:0	18:1	18:2	16:0	18:1	18:2	16:0	18:1	18:2
KAT	27.5	29.3	43.2	25.6	41.3	33.1	31.4	34.3	34.3
CP	35.7	27.6	36.6	23.5	44.1	32.4	27.6	35.6	36.7
K-184	36.6	28.9	34.5	22.0	43.3	34.6	27.3	37.4	35.3
IR-8	34.1	25.2	40.6	23.7	40.7	35.6	26.7	36.8	36.5
KOS	48.9	22.6	28.4	18.0	44.9	37.1	27.0	34.3	38.7
KUS	49.5	20.8	29.7	18.1	41.7	40.2	21.4	37.0	41.6
SAC	47.7	25.2	27.1	18.6	42.6	38.8	24.9	35.8	39.4
HIM	42.7	24.7	32.5	16.6	43.5	39.9	21.3	36.5	42.2

[a] Results are averages of duplicate experiments.
16:0, palmitic acid; 18:1, oleic acid; 18:2, linoleic acid.
VAR, cultivar; KAT, Katakutara; CP, Century Patna; KOS, Koshihikari; KUS, Kusabue; SAC, Sachiwatari; HIM, Himenomochi.

TABLE 2

Effect of 5 kGy γ Irradiation on the Fatty Acid Composition (%) of Lipids from Rice Seeds Irradiated With and Without Intact Hull[a]

	Free fatty acids			Neutral lipids			Phospholipids		
VAR	16:0	18:1	18:2	16:0	18:1	18:2	16:0	18:1	18:2
KAT	27.0 (24.3)	29.0 (29.1)	44.0 (46.2)	25.0 (25.2)	41.0 (41.2)	34.0 (33.6)	32.3 (33.7)	33.8 (33.9)	33.9 (32.4)
CP	35.4 (30.9)	26.4 (28.6)	38.2 (40.5)	23.7 (23.6)	43.5 (42.6)	32.8 (33.7)	28.6 (29.4)	35.9 (36.6)	35.5 (34.0)
K-184	35.3 (33.1)	29.2 (28.5)	35.5 (38.4)	20.9 (23.1)	43.8 (43.8)	35.3 (33.1)	28.0 (29.6)	38.1 (38.3)	33.9 (32.1)
IR-8	32.2 (28.7)	26.8 (27.4)	41.1 (43.9)	25.6 (25.6)	39.4 (41.2)	35.0 (33.2)	26.2 (29.8)	37.9 (35.7)	35.9 (34.5)
KOS	49.0 (43.2)	21.0 (23.1)	30.0 (33.7)	17.3 (21.2)	45.2 (42.6)	37.5 (36.2)	28.0 (29.4)	33.1 (33.7)	38.9 (36.9)
KUS	48.9 (42.9)	21.1 (23.0)	30.1 (34.2)	17.3 (19.6)	42.8 (41.8)	39.9 (38.6)	24.5 (26.3)	36.1 (36.4)	39.4 (37.3)
SAC	46.5 (39.4)	25.0 (26.7)	28.5 (33.9)	19.3 (17.2)	42.7 (43.3)	38.0 (39.5)	27.9 (30.0)	35.6 (34.2)	36.5 (35.8)
HIM	38.0 (36.1)	25.8 (26.5)	36.2 (37.4)	16.8 (16.1)	42.9 (43.8)	40.3 (40.1)	22.5 (26.6)	35.9 (35.5)	41.6 (37.9)

[a] Figures in parentheses refer to percentage composition of fatty acids, when irradiated without hull.
Abbreviations as in Table 1.

Neutral lipid was found to be resistant to oxidative damage induced by gamma irradiation as indicated by the constant levels of fatty acids in the seeds irradiated with or without intact hull at all irradiation doses.

Fatty acid composition of PL was seen to change markedly, especially the levels of oleic acid and linoleic acid, in rice lipids of seeds irradiated without intact hull. The changes in

TABLE 3

Effect of 10 kGy γ Irradiation on the Fatty Acid Composition (%) of Lipids from Rice Seeds Irradiated With and Without Intact Hull[a]

	Free fatty acids			Neurtral lipids			Phospholipids		
VAR	16:0	18:1	18:2	16:0	18:1	18:2	16:0	18:1	18:2
KAT	27.2	27.5	45.3	25.6	42.0	32.4	32.3	35.0	32.7
	(23.7)	(28.5)	(47.8)	(27.8)	(40.1)	(32.1)	(34.1)	(35.1)	(30.8)
CP	35.1	25.2	39.7	23.9	45.0	31.1	28.4	37.4	34.2
	(30.1)	(28.0)	(41.9)	(26.7)	(41.4)	(32.1)	(30.5)	(38.5)	(31.0)
K-184	33.0	29.5	37.5	21.6	44.3	34.1	28.1	39.4	32.5
	(32.9)	(28.1)	(39.0)	(25.1)	(42.7)	(32.2)	(29.4)	(39.1)	(31.4)
IR-8	29.5	27.2	43.3	23.1	40.4	36.6	29.4	36.0	34.6
	(26.7)	(28.0)	(45.3)	(24.0)	(41.8)	(34.2)	(32.7)	(35.9)	(31.4)
KOS	48.7	19.7	31.6	19.0	43.2	37.8	28.0	34.6	37.4
	(41.2)	(23.7)	(35.1)	(20.1)	(43.2)	(36.8)	(30.4)	(35.5)	(34.0)
KUS	41.4	22.2	36.4	18.6	41.2	40.1	25.6	36.9	37.5
	(39.7)	(23.8)	(36.5)	(19.3)	(40.9)	(39.8)	(26.9)	(36.9)	(36.2)
SAC	41.5	26.1	32.4	19.6	41.9	38.5	30.1	34.5	35.4
	(38.3)	(27.2)	(34.6)	(20.1)	(42.0)	(37.9)	(32.1)	(33.9)	(34.0)
HIM	32.1	27.4	40.5	14.3	44.5	41.2	26.5	35.2	38.2
	(37.0)	(24.1)	(38.9)	(20.1)	(42.8)	(37.2)	(30.2)	(35.1)	(34.7)

[a] Figures in parentheses refer to percentage composition of fatty acids, when irradiated without hull.
Abbreviations as in Table 1.

TABLE 4

Effect of 15 kGy γ Irradiation on the Fatty Acid Composition (%) of Lipids from Rice Seeds Irradiated With and Without Intact Hull[a]

	Free fatty acids			Neutral lipids			Phospholipids		
VAR	16:0	18:1	18:2	16:0	18:1	18:2	16:0	18:1	18:2
KAT	26.7	24.6	48.7	25.7	40.1	34.2	32.7	35.8	31.5
	(21.8)	(27.6)	(50.6)	(27.6)	(40.8)	(31.6)	(41.1)	(32.1)	(26.8)
CP	34.5	24.6	40.9	24.0	42.1	33.9	29.2	38.1	32.7
	(26.5)	(28.8)	(44.7)	(23.6)	(42.7)	(33.7)	(34.1)	(37.4)	(28.5)
K-184	30.7	30.0	39.4	18.6	45.6	35.8	29.4	39.5	31.1
	(29.1)	(29.6)	(41.3)	(22.1)	(44.5)	(33.4)	(33.7)	(38.6)	(27.7)
IR-8	25.6	27.9	46.5	24.7	41.1	34.3	33.4	35.7	30.9
	(23.9)	(28.2)	(47.9)	(20.9)	(42.6)	(36.5)	(33.1)	(38.7)	(28.1)
KOS	48.1	18.5	33.4	18.4	45.3	36.3	29.0	35.1	35.9
	(39.3)	(24.4)	(36.3)	(21.4)	(43.6)	(35.0)	(33.7)	(36.5)	(29.7)
KUS	37.4	24.4	38.2	16.1	42.4	41.5	27.4	36.5	36.1
	(37.2)	(24.5)	(38.3)	(18.4)	(39.1)	(42.6)	(33.3)	(35.4)	(31.3)
SAC	34.4	27.9	37.7	19.6	43.5	36.9	32.4	34.1	33.5
	(36.2)	(27.4)	(36.5)	(21.8)	(41.7)	(36.5)	(30.9)	(37.3)	(31.9)
HIM	28.1	29.1	42.8	16.2	43.3	40.5	30.5	34.6	34.9
	(33.9)	(25.4)	(40.7)	(21.7)	(41.6)	(36.7)	(29.5)	(38.1)	(32.4)

[a] Figures in parentheses refer to percentage composition of fatty acids, when irradiated without hull.
Abbreviations as in Table 1.

the levels of oleic and linoleic acids were less severe in Indica than in Japonica rice seeds.

The above observations indicated that γ irradiation of rice seeds with intact hull minimized the increase in the amount of unsaturated fatty acids in the FFA, which are prone to autoxidation in the presence of oxygen radicals generated by γ irradiation (8,9). Thus, it can be concluded that rice hull played a vital role in offering protection to the rice lipids from the drastic effects of oxidative damage induced by γ irradiation.

Effect of γ irradiation on the endogenous antioxidant contents in rice seeds irradiated with and without intact hull. Rice lipid is known to have unusual storage stability, which is attributed mainly to its tocopherol and oryzanol contents (16). It was assumed that these endogenous antioxidants could offer a chemical defense against damage induced by oxygen radicals generated during γ irradiation. The protective effect of these agents could be due to their radical-scavenging ability. In providing this protection, the natural antioxidants are liable to undergo self-destruction depending on the extent of their participation in the antioxidative defense mechanism (8).

TABLE 5

Effect of γ Irradiation on α-Tocopherol and Oryzanol Contents of Lipids from Rice Seeds Irradiated With and Without Hull[a]

	α-tocopherol (μg/g lipid)				Oryzanol (μg/g lipid)			
VAR	0kGy	5kGy	10kGy	15kGy	0kGy	5kGy	10kGy	15kGy
KAT	407	257	114	tr	151	134	108	105
		(110)	(tr)	(tr)		(128)	(104)	(93)
CP	385	227	93	tr	246	227	182	170
		(100)	(tr)	(tr)		(207)	(172)	(153)
K-184	392	216	98	tr	178	161	125	116
		(94)	(tr)	(tr)		(146)	(120)	(105)
IR-8	385	219	85	tr	96	82	75	59
		(89)	(tr)	(tr)		(77)	(62)	(52)
KOS	443	274	115	tr	100	89	74	62
		(128)	(tr)	(tr)		(85)	(69)	(56)
KUS	350	204	88	tr	113	89	78	65
		(95)	(tr)	(tr)		(86)	(69)	(55)
SAC	427	231	94	tr	145	119	103	92
		(107)	(tr)	(tr)		(116)	(97)	(73)
HIM	319	195	76	tr	115	96	81	70
		(84)	(tr)	(tr)		(86)	(71)	(63)

[a] Results are averages of three replicates.
Figures in parentheses refer to values, when rice seeds were irradiated without intact hull.

The effect of γ irradiation on the levels of α-tocopherol and oryzanol of rice seeds was examined. Table 5 shows the changes in α-tocopherol content during the course of irradiation. The level of α-tocopherol in control Indica rice seeds, which ranged from 385 to 407 μg/g lipid, decreased drastically to a range of 85 to 114 μg/g lipid in seeds irradiated with intact hull at 10 kGy. This accounted for a 72 to 78% decrease in α-tocopherol with respect to the control. The corresponding loss in the Japonica rice seeds was 74 to 80%. At 15 kGy, both Indica and Japonica rice seeds irradiated with or without hull were found to have undergone complete loss of α-tocopherol. Seeds irradiated without intact hull were found to have undergone even faster losses in α-tocopherol contents. The level of α-tocopherol in Indica rice seeds irradiated without hull ranged from 89 to 110 μg/g at 5 kGy, a loss of 73 to 77%. The levels for Japonica rice seeds at the same irradiation dose ranged from 84 to 128 μg/g lipid, the loss being 71 to 78%. Thus, the extent of destruction undergone by α-tocopherol during irradiation of rice seeds without intact hull was almost identical in Indica and Japonica rice cultivars.

Irradiation of rice seeds at higher doses, before and after dehulling, has clearly indicated that α-tocopherol participated actively in the defense mechanism during oxidative damage induced by γ irradiation. The fall in the levels of α-tocopherol, especially when the rice seeds were irradiated without intact hull, was seen to have a direct relationship with the changes in fatty acid composition, TBA values and germination potentials of rice seeds (11). It could be possible that as a natural antioxidant α-tocopherol participated actively in the radical scavenging mechanism, thereby retaining the ability of the seeds to germinate relative to the extent of undamaged α-tocopherol content of the rice seed. This effect of α-tocopherol was further supplemented by the chemical defense system in rice hull, when the seeds were irradiated with intact hull. These data indicated that the rice seeds were offered additional protection by the rice hull, in preventing oxidative damage caused by oxygen radicals, during γ irradiation.

Oryzanol, a relatively weaker antioxidant than α-tocopherol (12), was found to be fairly stable to γ irradiation (Table 5). The oryzanol contents of Indica and Japonica rice seeds ranged from 96 to 246 and 100 to 145 μg/g lipid, respectively. On irradiation of rice seeds at 15 kGy with intact hull, the decrease in oryzanol contents ranged from 30 to 38% and 37 to 42% for Japonica and Indica rice seeds, respectively. The corresponding loss for rice seeds irradiated without intact hull was 38 to 46% and 44 to 51%. These results indicate that oryzanol, being a weaker antioxidant, perhaps did not play any major protective role in preventing the oxidative damage induced by γ irradiation. However, the changes in the ESR signal intensities, TBA values of rice lipids (11), fatty acid compositions (Tables 1-4), contents of endogenous antioxidants (Table 5) and the final effect on germination potentials of rice seeds during γ irradiation (11) have strongly indicated that protection from oxidative damage was offered by the rice hull.

Thus, it is evident that an effective antioxidative defense system exists in rice hull that has the ability to offer chemical protection to the rice grain and hence the germ, which is mainly responsible for germination. This model investigation with high-energy radiation proved that rice hull provided not only physical protection to the rice germ from direct attack by such radiation, but also chemical protection due to the reasons stated. This protective effect could be supplementary to the endogenous rice antioxidants α-tocopherol and oryzanol. As an overall conclusion, the protective system seems to be relatively more efficient in Indica rice seeds than in Japonica cultivars.

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