Changes of Endogenous Antioxidants and Fatty Acid Composition in Irradiated Rice Bran during Storage†

Tai-Sun Shin and J. Samuel Godber*

Department of Food Science, Louisiana Agricultural Experimental Station, Louisiana State University Agricultural Center, Baton Rouge, Louisiana 70803

The effects of γ-irradiation (5, 10, and 15 kGy) on antioxidants and lipid composition of rice bran were studied. The free fatty acid (FFA) level in rice bran irradiated at 5 kGy was not significantly different from that of raw rice bran. Increases in irradiation doses to 10 and 15 kGy resulted in an increase in FFA levels. γ-Irradiation at 15 kGy resulted in greater ($p \leq 0.05$) loss of phospholipids in rice bran during storage. Also, γ-irradiation had deleterious effects on lipid stability, E vitamers, and oryzanol in rice bran during irradiation and subsequent storage. The decomposition of individual E vitamers increased with an increase in irradiation level. The loss of total E vitamers and oryzanol occurred in two stages: 50−82% and 12−33% immediately following irradiation and a further 10−35% and 39−42% during storage.

Keywords: *Rice bran; γ-irradiation; vitamin E vitamers; fatty acids*

INTRODUCTION

Radiation processing of food may be used to achieve a wide variety of technological objectives and to serve many valuable purposes. γ-Radiation has been used to inhibit sprouting in potatoes (Kodenchery and Nair, 1972) and garlic bulbs (Kwon et al., 1985), to reduce cooking time of legumes (Rao and Vakil, 1985), to reduce toxic constituents such as antithiamin in tuna (Hilker et al., 1972), to reduce flatulence-causing oligosaccharides in green gram (Rao and Valkil, 1983), to inactivate chymotrypsin inhibitors (Iyer et al., 1980), to reduce gossypol in cottonseed (Jaddou et al., 1983), and to reduce the number and/or the activity of viable microorganisms in peanuts (Chiou et al., 1990), barley malt (Kempe et al., 1964), and chickens (Lamuka et al., 1992; Katta et al., 1991). However, the high irradiation doses involved may produce unacceptable off-flavors and odors, undesirable color changes, and textural and nutritional losses (Delincée, 1983a).

Rice bran contains valuable components such as oil, proteins, vitamins, and essential minerals as well as enzymes, microorganisms, insects, natural toxicant constituents, harmful contaminants, and adulterants (Barber and Barber, 1980). Enzymes, microorganisms, and insects are major causes of deterioration of rice bran during storage (Barber and Barber, 1980; Loeb and Mayne, 1952). Lipases, both endogenous to the bran and of microbial origin, hydrolyze bran oil to produce long-chain free fatty acids that are responsible for acidic and soapy flavors (Saunders and Heltved, 1985).

The purpose of this investigation was to study the effect of three irradiation doses, 5, 10, and 15 kGy, on antioxidants and stability of oil in rice bran during post-γ-irradiation storage.

MATERIALS AND METHODS

γ-Irradiation. Freshly milled raw rice bran from mixed long-grain varieties (Tebonnet and Lemont) was transported from the Rivianna Mill in Abbeville, LA, in 37 kg containers on dry ice to Louisiana State University, where they were placed immediately into a freezer (−20 °C). One hundred gram samples of 10.5% moisture were irradiated at doses of 5, 10, and 15 kGy using a cobalt-60 source. The radiation dose was computed by taking into account the strength of the source and time of exposure. All irradiation treatments were carried out at 20 °C with a dose rate of 0.98 kGy/h. Samples to be irradiated were packed in Whirl plastic bags (Koch Supplies Inc., Kansas City, MO) and placed in an ultralow-temperature freezer (−85 °C) until the irradiation procedure was carried out. The irradiation procedure with triplication was randomized and required 4 days. Samples were taken immediately after irradiation for determination of baseline levels of free fatty acids and oxidative degradation products. Samples were stored at ambient temperature (22−26 °C) for 0, 1, 3, 7, 24, and 52 weeks. At the end of each storage period, samples were vacuum packed and placed into an ultralow-temperature freezer (−85 °C) until analyzed.

Antioxidant Quantification. *Standards.* Tocopherols and tocotrienols were prepared from natural sources (Shin and Godber, 1994). Oryzanol was isolated from crude rice bran oil (Kim and Kim, 1991).

Sample Preparation for HPLC. The analytical method was based on the method reported by Shin and Godber (1993). Five hundred milligrams of rice bran was placed in a 15 mL test tube with 5 mL of ethanol and 0.1 g of ascorbic acid. The test tube was placed in an 80 °C water bath for 10 min, after which time 0.15 mL of 80% KOH was added. The sample was saponified for 15 min at 80 °C, following which the flask was placed in an ice bath, and 3 mL of water and 5 mL of hexane were added. The mixture was transferred to centrifuge bottles and centrifuged at 120*g* for 1 min. The upper layer was transferred to a 125 mL separatory funnel. Extraction of the sample with 5 mL of hexane was repeated twice. The pooled hexane layer was washed three times with 5 mL of water, filtered through Na_2SO_4, and then evaporated under a stream of nitrogen. The crude oil sample was diluted with 1 mL of isooctane.

High-Performance Liquid Chromatography. The HPLC system consisted of Waters (Milford, MA) M-45 pump, a 715 Ultra WISP injector, and a 470 scanning fluorescence detector with excitation at 290 nm and emission at 330 nm; a Sulpecosil (Supelco, Bellefonte, PA) LC-Si, 5 μm, 5 cm × 4.6 mm i.d., column was used. The mobile phase was isooctane/ethyl acetate/acetic acid/2,2-dimethoxypropane (98.15:0.9:0.85:0.1) with a flow rate of 1.6 mL/min. A Baseline 810 chromatog-

* Author to whom correspondence should be addressed.

† Approved for publication by the Director of the Louisiana Agricultural Experiment Station as Manuscript 95-21-9194.

0021-8561/96/1444-0567$12.00/0 © 1996 American Chemical Society

Table 1. Tocopherols, Tocotrienols, and Oryzanol of Raw and Irradiated Rice Bran[a−d]

irradiation (kGy)	α-T	α-T3	β-T	γ-T	γ-T3	δ-T	δ-T3	Ory	total T	total T3
0	63.16[e]	38.19[e]	8.99[e]	32.26[e]	119.71[e]	1.99[e]	7.27[e]	3101.52[e]	106.40[e]	165.17[e]
5	30.18[f]	12.61[f]	4.65[f]	21.44[f]	61.92[f]	1.14[f]	5.69[f]	2757.62[f]	57.41[f]	80.22[f]
	(52.2)	(67.0)	(48.2)	(33.5)	(48.2)	(42.6)	(21.5)	(11.1)	(46.0)	(51.4)
10	19.35[g]	6.86[g]	4.05[g]	16.50[g]	38.72[g]	0.72[g]	5.63[f]	2542.39[g]	40.62[g]	51.21[g]
	(69.3)	(82.0)	(54.9)	(48.8)	(67.6)	(63.8)	(22.4)	(18.0)	(61.8)	(68.9)
15	13.05[h]	4.12[h]	3.36[h]	10.41[h]	15.83[h]	0.62[h]	4.16[g]	2406.93[g]	27.44[h]	24.11[h]
	(79.3)	(89.2)	(62.6)	(67.7)	(86.7)	(69.0)	(42.6)	(22.4)	(74.2)	(85.4)

[a] mg/kg of rice bran. [b] Means ($n = 4$) with different superscripts are different ($p \leq 0.05$). [c] T, tocopherol; T3, tocotrienol; Ory, oryzanol; total T, total tocopherol; total T3, total tocotrienol. [d] Figures in parentheses refer to percentage loss of E vitamers or oryzanol.

raphy workstation was used to determine peak areas (Waters, Milford, MA).

Lipid Analysis. *Extraction.* Total lipids in rice bran (10 g) were extracted three times with a cold chloroform/methanol mixture (2:1 v/v). The solvent was evaporated from the combined extracts under reduced pressure at 40 °C, and the residue was dissolved in 2:1 chloroform/methanol. Traces of carbohydrates and proteins were removed from the crude extract according to procedures described by Folch et al. (1957).

Separation into Lipid Classes. A 100 mesh Mallinckrodt silicic acid column (25 mm i.d. × 30 cm) was used to separate the neutral lipid (NL), glycolipid (GL), and phospholipid (PL) fractions. A loading ratio of 300 mg of lipid extract/30 g of silica gel was used. Before packing, the silica gel was prepared by first washing with methanol and then with H_2O to remove fines. The silica gel was then activated overnight in an oven at 120 °C, mixed with the first elution solvent ($CHCl_3$), and packed in the column as a gel. NL were eluted with chloroform (400 mL), GL with acetone (600 mL), and PL with methanol (400 mL), successively. These fractions were evaporated to dryness in a rotary evaporator under reduced pressure at 40 °C. All fractions were identified by thin-layer chromatography (Kozukue and Kozukue, 1981) with GL and PL contents quantitated gravimetrically.

Fractionation of Free Fatty Acid (FFA) and Nonpolar Lipid (NPL). A modification of the method of Mattick and Lee (1959) was used in which the FFA was separated as sodium salts using sodium bicarbonate from the NL fraction obtained from column chromatography. Sodium salts were acidified with H_2SO_4 to obtain the free fatty acid form. The NL fraction (ca. 300 mg) with 4 mL of heptadecanoic acid (1 μg/μL in hexane) as an internal standard was transferred to a separatory funnel and treated with a 30 mL mixture of diethyl ether and hexane (1:1 v/v) and 0.5% Na_2CO_3 (10 mL). The contents were shaken vigorously for 30 s and allowed to stand for 20 min. The bottom aqueous layer was drawn into another separatory funnel while the upper, diethyl ether layer was treated with 0.5% Na_2CO_3 (10 mL). The contents were shaken vigorously for 30 s and allowed to stand for 20 min. The bottom aqueous layer was pooled into a separatory funnel, and the procedure was repeated one additional time. The aqueous layer was acidified with 5 mL of 10% H_2SO_4 and was extracted with 10 mL of diethyl ether three times. The diethyl ether extracts were pooled, washed with distilled water until free from acid, and dried to dryness. The FFA samples were transferred into sample bottles, dried, and weighed. The diethyl ether layer (nonpolar lipid) left after separation of the FFA was twice washed with 20 mL of distilled water, dried over anhydrous Na_2SO_4, filtered, and evaporated in a stream of nitrogen at 40 °C. FFA and NPL contents were quantitated gravimetrically.

Preparation of Fatty Acid Methyl Ester (FAME). A modification of the procedure described by Christie (1982) was used. The lipid fraction (up to 50 mg) was dissolved in hexane (1 mL) in a 15 mL test tube, and 5% methanolic hydrogen chloride (2 mL) was added. Heptadecanoic acid methyl ester (1 μg/μL) was added as an internal standard in all but FFA fractions. The mixture was refluxed for 8 h at 80 °C, then water (5 mL) containing 5% sodium chloride was added, and the required esters were extracted twice with 5 mL of hexane using Pasteur pipets to separate the layers. The hexane layer was washed twice with 10 mL of 5% potassium bicarbonate and dried over anhydrous Na_2SO_4. The solution was filtered and the solvent removed in a stream of nitrogen with a 40 °C water bath.

Analysis of Methyl Ester. Analysis of the FAME, in duplicate, was carried out on a Hewlett-Packard (San Fernando, CA) 5890 gas chromatograph equipped with a split/splitless capillary inlet system and a flame ionization detector with a Supelco SP-2380 capillary column (0.20 μm stationary phase thickness, 30 m × 0.25 mm i.d.). Other operation parameters were as follows: injector temperature, 250 °C; detector temperature, 250 °C; helium carrier gas flow rate, 20 cm/s; split ratio, 1/100. A Maxima 820 chromatography workstation was used to determine peak areas (Waters). Fatty acids were identified and quantified using known reference compounds.

Statistical Analysis. A statistical analysis of the results was performed using the general linear model of the Statistical Analysis System (SAS). Tests for least significant differences (lsd) were applied when differences among treatments were significant ($p < 0.05$) as determined by analysis of variance (SAS Institute, 1989).

RESULTS AND DISCUSSION

Antioxidants in Rice Bran. Many studies have been reported on the effect of γ-irradiation on α-tocopherol or total vitamin E vitamers during irradiation. However, only a few papers note the effect of irradiation and postirradiation storage on individual E vitamers. The decomposition of tocopherols (T), tocotrienols (T3), and oryzanol in rice bran during irradiation is shown in Table 1. The decomposition of individual E vitamers increased significantly ($p < 0.05$) with an increase in irradiation level. Oryzanol contents in rice bran irradiated at 5 kGy were lower ($p < 0.05$) than that in raw bran. Decomposition of oryzanol increased ($p < 0.05$) with an increase from 5 to 10 kGy. However, the decrease in oryzanol was not significant ($p > 0.05$) with an increase from 10 to 15 kGy. α-Tocotrienol in rice bran was the most sensitive to γ-irradiation. Rice bran irradiated at 5, 10, and 15 kGy lost 67, 82, and 89% of its α-tocotrienol, respectively. The order of loss of vitamin E vitamers in bran irradiated at 5 kGy was α-T3 > α-T > β-T = γ-T3 > δ-T > γ-T > δ-T3, with only slight differences in this order with increased irradiation dose. An increase in decomposition of total tocotrienol was higher than that of total tocopherols with increased irradiation level.

Available papers do not provide information on the order of stability of vitamin E vitamers either in irradiated food samples or in model systems. Double bonds between certain carbon atoms in long-chain fatty acids esterified with glycerol are selectively attacked by free radicals produced by irradiation, particularly the superoxide and hydroxyl radicals (Murray, 1990). The tocotrienols with three double bonds in their side chains were more susceptible to γ-irradiation than tocopherols without double bonds. However, δ-tocotrienol was the most stable to γ-irradiation. Ramarathnam et al. (1989) reported that the α-tocopherol content decreased markedly in rice seeds irradiated with or without hull. At a dose of 15 kGy, only traces of α-tocopherol could be detected in rice seeds irradiated with and without intact hull.

Table 2. Retention of Vitamin E Vitamers in Irradiated Rice Bran during 52 Weeks of Storage[a,b]

		vitamin E vitamers[c]								
irradiation dose (kGy)	storage (weeks)	α-T	α-T3	β-T	γ-T	γ-T3	δ-T	δ-T3	total T	total T3
5	0	30.18^{d}	12.61^{d}	4.65^{d}	21.44^{d}	61.92^{d}	1.14^{d}	5.69^{d}	57.41	80.22
	1	24.18^{e}	9.53^{e}	3.89^{de}	18.76^{e}	48.50^{e}	1.04^{d}	4.72^{e}	47.87	62.75
	3	20.18^{f}	7.96^{e}	3.27^{e}	17.65^{e}	45.46^{ef}	0.82^{ef}	4.49^{e}	41.92	57.91
	7	19.45^{f}	5.08^{f}	2.79^{ef}	15.41^{f}	43.72^{f}	0.73^{fg}	4.39^{ef}	38.38	53.19
	24	12.09^{g}	3.22^{fg}	2.16^{f}	13.26^{g}	32.39^{g}	0.69^{gh}	4.06^{f}	28.20	39.67
	52	9.65^{g}	2.46^{g}	1.92^{f}	11.86^{g}	29.86^{g}	0.64^{h}	3.31^{g}	24.07	35.63
10	0	19.36^{d}	6.85^{d}	4.05^{d}	16.50^{d}	38.73^{d}	0.72^{d}	5.63^{d}	40.61	51.21
	1	12.08^{e}	3.22^{e}	3.59^{e}	13.26^{e}	32.39^{e}	0.63^{d}	4.72^{e}	29.56	40.33
	3	9.66^{ef}	2.47^{ef}	1.92^{fg}	11.85^{ef}	29.85^{ef}	0.55^{ef}	4.36^{e}	23.98	36.68
	7	7.16^{fg}	1.90^{fg}	1.50^{gh}	10.59^{f}	28.22^{f}	0.53^{f}	3.93^{ef}	19.78	34.05
	24	6.26^{gh}	1.20^{gh}	1.43^{hi}	8.23^{gh}	16.81^{g}	0.49^{f}	3.86^{f}	16.41	21.87
	52	4.45^{h}	1.05^{h}	1.27^{i}	7.72^{h}	11.85^{h}	0.39^{g}	2.76^{g}	13.83	15.66
15	0	13.05^{d}	4.12^{d}	3.36^{d}	10.41^{d}	15.83^{d}	0.62^{d}	4.16^{d}	27.44	24.11
	1	8.08^{e}	1.80^{ef}	2.27^{e}	8.43^{ef}	13.84^{ef}	0.58^{d}	3.64^{e}	19.36	19.28
	3	6.26^{ef}	1.20^{fg}	1.43^{ef}	8.23^{fg}	12.81^{f}	0.49^{e}	3.26^{e}	16.41	17.27
	7	4.45^{fg}	1.15^{fgh}	1.27^{f}	7.73^{g}	10.85^{g}	0.41^{f}	2.76^{f}	13.86	14.76
	24	3.06^{g}	1.01^{h}	1.03^{fg}	5.73^{h}	6.23^{h}	0.33^{g}	2.10^{g}	10.15	9.34
	52	1.23^{h}	0.83^{h}	0.84^{g}	3.34^{i}	4.22^{i}	0.22^{i}	1.21^{i}	5.63	6.26

[a] mg/kg of rice bran. [b] Means ($n = 4$) with different superscripts are different ($p \leq 0.05$). [c] T, tocopherol; T3, tocotrienol.

Rice bran irradiated at 5, 10, and 15 kGy lost 11, 18, and 22% of its oryzanol, respectively. Oryzanol was found to be relatively stable to γ-irradiation. Ramarathnam et al. (1989) found that the oryzanol content ranged from 96 to 246 μg/g of lipid in rice seed, while the decrease in oryzanol in rice seed irradiated at 5−15 kGy ranged from 7 to 42% of its oryzanol content. In the present study, reduction of oryzanol in rice bran was less than that reported for rice seeds. This could be due to the higher amount of total E vitamers in rice bran. The most striking difference between lipids extracted from nonirradiated and irradiated wheat was an increase in the amount of free sterol (Tipples and Norris, 1965). It was noted that the irradiation could have severed or weakened the linkage by which sterol was bound, thus making the sterol more completely available for solvent extraction. The results suggest that ester linkage in oryzanol could be affected by γ-irradiation and, thus, could be lost.

The destruction of total tocopherols in wheat varied from variety to variety (Tipples and Norris, 1965). Manitoba wheat irradiated at 1, 10, and 100 kGy lost 19, 39, and 79% of its total tocopherols, while Minister wheat lost 8, 13, and 39%, respectively. Knapp and Tappel (1961) studied comparative radiosensitivities of fat-soluble vitamins under controlled conditions. Vitamin E was by far the most sensitive, followed in order of decreasing senstivity by carotene and vitamins A, D, and K.

Effect of Post-γ-irradiation Storage on Vitamin E Vitamers. Our results for individual vitamin E vitamers are shown in Table 2. There was no apparent pattern in the order of decomposition of E vitamers in irradiated rice bran during storage. γ-Tocopherol and δ-tocopherol in irradiated rice bran had relatively higher retention during storage, and α-tocopherol and α-tocotrienol had relatively less retention. Increased irradiation dose did not significantly ($p > 0.05$) increase the decomposition rate of total E vitamers during storage (Figure 1A). The retention of vitamin E vitamers during storage was dependent on the amount retained during irradiation. During 7 weeks of storage, the relative decomposition rate of total E vitamers in raw rice bran was higher than that of irradiated rice bran. This was considered to be due to the lower amount of vitamin E vitamers in irradiated brans. Diehl (1981) found that irradiation of hazelnuts to 1 kGy produced an 18% loss

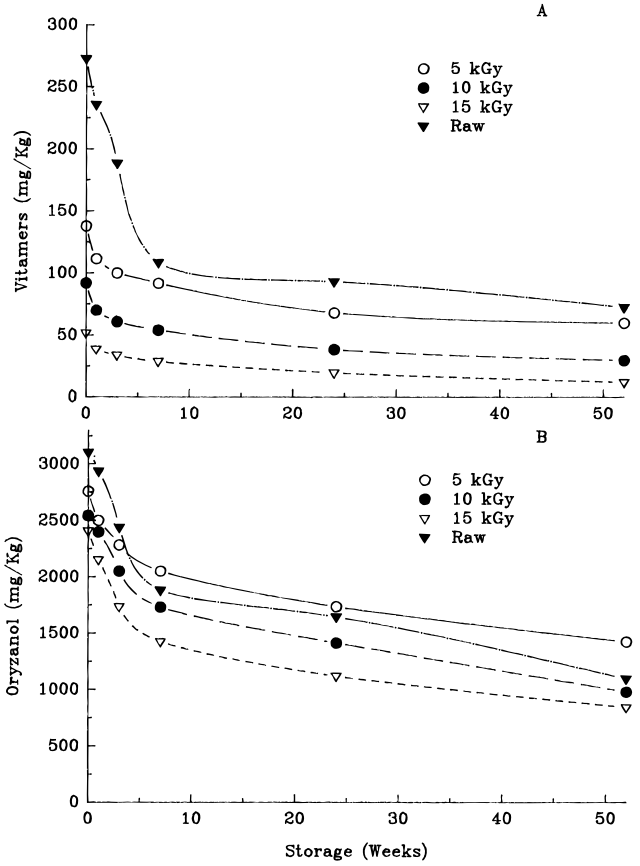

Figure 1. Total E vitamers (A) and oryzanol (B) in raw and irradiated rice bran during 52 weeks of storage.

of α-tocopherol, while baking produced a 13% loss. When the nuts were irradiated and then cooked, the total loss of tocopherol was 67%. The researcher proposed that apparent synergistic loss was due to free radicals and secondary reaction products that may attack vitamin E during processing or subsequent storage. Oryzanol content in rice bran irradiated at 5 kGy was lower than that of raw rice bran during 3 weeks of storage (Figure 1B). Thereafter, it was higher than that of raw rice bran. During 7 weeks of storage, the loss of oryzanol in irradiated rice bran was about 60% of its loss during 52 weeks of storage.

Free Fatty Acids in Irradiated Rice Bran. Table 3 shows the contents of major lipid classes in crude rice

Table 3. Major Lipid Classes of Crude Bran Oil Extracted from Raw Rice Bran and Their Fatty Acid Composition[a]

		fatty acid composition (%)								
lipid class[b]	wt %	14:0	16:0	18:0	18:1	18:2	18:3	20:0	saturated	unsaturated
TL[c]	20.1	0.40	22.21	2.21	38.85	34.58	1.14	0.61	25.43	74.57
NL[d]	89.2	0.43	23.41	1.88	37.24	35.29	1.07	0.68	26.40	73.60
GL[d]	6.8	0.09	27.34	0.18	36.45	35.76	0.18		27.61	72.39
PL[d]	4.0	0.11	22.13	0.16	38.11	39.32	0.17		22.40	77.60

[a] Values are means of three replicate analyses. [b] TL, total lipids in raw rice bran; NL, neutral lipids (nonpolar lipid and free fatty acid); GL, glycolipids; PL, phospholipids. [c] Crude rice bran oil extracted as described in the text. [d] Fractionated by column chromatography.

Table 4. Changes of Free Fatty Acids (FFA) and Phospholipids in Rice Bran Immediately following γ-Irradiation[a]

irradiation (kGy)	total FFA of (g/100 g of rice bran oil)	phospholipids (g/100 g of rice bran oil)
0	3.60[d]	4.02[d]
5	4.20[d]	4.01[d]
10	5.34[e]	3.92[d]
15	6.05[f]	3.66[e]

[a] Means ($n = 4$) with different superscripts are different ($p \leq 0.05$).

bran oil extracted from raw rice bran and fatty acid composition. The effects of γ-irradiation on quantitative changes in FFA levels in rice bran is shown in Table 4. The FFA level in rice bran irradiated at 5 kGy was not significantly different from that of raw rice bran. An increase in irradiation to doses of 10 and 15 kGy resulted in a significant ($p < 0.05$) increase in FFA levels. The increase in FFA levels at both doses resulted from radiolysis of glycerides. Also, enzymatic hydrolysis of glycerides might contribute an increase in FFA level during γ-irradiation since rice bran was irradiated at 20 °C for about 10 and 15 h for 10 and 15 kGy, respectively.

Free fatty acids were more susceptible to irradiation change than esterified acids (Tipples and Norris, 1965). Generally, the major radiolytic products of fatty acids are carbon dioxide, hydrogen, carbon monoxide, a series of hydrocarbons, and the C_n aldehydes (Delincée, 1983a). Quantitative analyses indicated a greater yield of various radiolytic compounds from FFA than from the corresponding triglycerides in model systems (Vajdi et al., 1978). Tipples and Norris (1965) reported that silicic acid fractionation results showed a reduction in triglyceride and a corresponding increase in mono- and diglycerides in wheat irradiated at 100 kGy. However, there was no increase in the amount of FFA recovered. This suggests that the splitting of the ester linkage produced degraded molecules or free radicals rather than FFA (Tipples and Norris, 1965). In rice bran, the rate of formation of FFA was higher even though doses of irradiation were relatively low. This might be due to lipolytic enzymes in rice bran active during the irradiation process.

The effect of storage on total FFA in rice bran oil extracted from raw and irradiated rice bran is shown in Figure 2A. Irradiated and raw rice bran showed similar increases in FFA content. FFA levels in rice bran irradiated at 5 kGy were not different ($p > 0.05$) from that of raw rice bran during storage. FFA contents in irradiated rice bran increased ($p < 0.05$) during 24 weeks of storage when the doses were increased. FFA content in rice bran irradiated at 15 kGy decreased ($p < 0.05$) after 24 weeks of storage. FFA contents in raw rice bran and rice bran irradiated at 5 and 10 kGy increased slightly. Unlike rice bran, FFA content in irradiated cereal grain and flour usually increased during irradiation (10−100 kGy) and was similar to that of control during storage. Ismail et al. (1977) reported

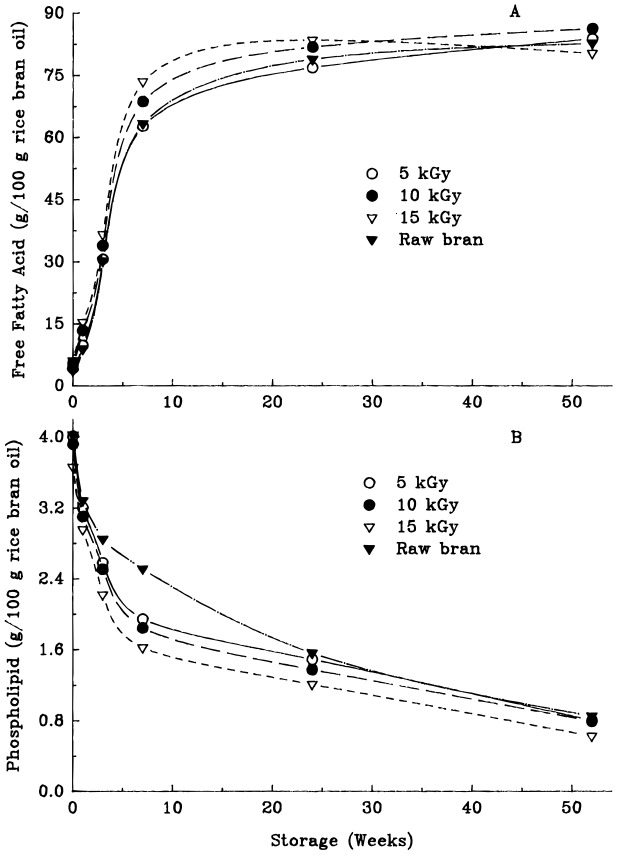

Figure 2. Changes in total free fatty acid (A) and phospholipid (B) in rice bran oil extracted from raw and irradiated rice bran during 52 weeks of storage.

that fat acidity in brown rice irradiated at 0.1, 0.2, 0.3, and 0.5 kGy decreased slightly compared to the control brown rice during 5 months of storage. The development of FFA in the lipid of wheat flour irradiated at 50 and 100 kGy did not appear to be impaired by irradiation (Tipples and Norris, 1965). After 680 days of storage of whole-meal flour, the FFA content had risen to about 40%.

Similar FFA levels in irradiated and raw rice bran during storage (Figure 2B) presumably indicated that γ-irradiation in rice bran does not decrease lipolytic enzyme activities in the range used in this study. Delincée (1983b) reported that enzymes causing autolysis during storage of high-protein foods generally cannot be inactivated at radiation dose levels for radurization (2−5 kGy) or radappertization (50 kGy), thus long-term stability of radiation-sterilized meats is achieved by enzyme inactivation through heating. An increase in enzyme activity was observed in irradiated fruits and vegetables, while a decrease was observed for others (Delincée, 1983b). In banana subjected to γ-irradiation (0.15−2 kGy), skin browning was observed to be due to an activation of polyphenol oxidase (Thomas and Nair, 1971). Ogawa and Uritani (1970) demonstrated that γ-irradiation induced tissue browning of potato tubers

Table 5. Free Fatty Acid Composition of Raw and Irradiated Rice Bran during 52 Weeks of Storage[a]

		fatty acid composition (%)								
irradiation dose (kGy)	storage (weeks)	14:0	16:0	18:0	18:1	18:2	18:3	20:0	saturated	unsaturated
raw	0	0.37	20.11	1.30	40.86	35.90	0.62	0.85	22.62	77.38
	1	0.58	22.41	1.36	40.34	33.82	0.61	0.88	25.23	74.77
	3	0.91	23.07	1.44	40.70	32.26	0.55	1.07	26.49	73.51
	7	0.95	23.37	1.72	41.85	30.39	0.55	1.17	27.21	72.79
	24	1.35	23.86	1.69	41.86	29.70	0.42	1.12	28.02	71.98
	52	1.67	24.75	1.93	42.03	27.90	0.37	1.35	29.70	70.30
5	0	0.38	20.84	1.32	40.77	35.23	0.60	0.86	23.40	76.60
	1	0.59	21.65	1.38	41.59	33.34	0.57	0.88	24.50	75.50
	3	0.81	22.16	1.46	41.90	32.06	0.52	1.09	25.52	74.48
	7	0.98	22.71	1.76	42.38	30.49	0.48	1.20	26.65	73.35
	24	1.38	23.37	1.73	42.32	29.63	0.43	1.14	27.62	72.38
	52	1.71	23.80	1.98	42.88	27.86	0.39	1.38	28.87	71.13
10	0	0.38	20.90	1.33	41.01	34.95	0.56	0.87	23.48	76.52
	1	0.59	21.78	1.38	41.67	33.15	0.54	0.89	24.64	75.36
	3	0.86	22.20	1.47	42.09	31.76	0.53	1.09	25.62	74.38
	7	1.07	22.78	1.77	42.48	30.18	0.51	1.21	26.83	73.17
	24	1.39	23.44	1.74	42.43	29.38	0.47	1.15	27.72	72.28
	52	1.75	23.76	2.04	43.12	27.49	0.43	1.41	28.96	71.04
15	0	0.38	21.69	1.35	41.22	33.98	0.51	0.87	24.29	75.71
	1	0.61	22.15	1.40	42.15	32.33	0.46	0.90	25.06	74.94
	3	0.94	22.65	1.50	42.32	30.99	0.49	1.11	26.20	73.80
	7	1.24	23.08	1.63	42.94	29.40	0.49	1.22	27.17	72.83
	24	1.42	23.28	1.88	42.89	28.88	0.37	1.28	27.86	72.14
	52	1.77	23.90	2.06	43.14	27.35	0.36	1.42	29.15	70.85

[a] Values are means of four analyses.

and was accompanied by a marked increase in peroxidase activity and a transient increase in *o*-diphenol oxidase activity. The radiation dose was computed by taking into account the strength of the source and time of exposure. All irradiation treatments were carried out at 20 °C with a dose rate of 0.98 kGy/h. At low radiation doses of 0.1−3 kGy used for fruits and vegetables, the effect on enzymes seems to be due to metabolic changes induced by irradiation (Delincée, 1983b). Patel et al. (1965) studied lipase activity of γ-irradiated groundnut (0.05−1.2 kGy) and indicated that the lipase activity of dormant seed was affected by radiation. However, lipolytic enzymes in rice bran do not seem to be affected by γ-irradiation at 5, 10, and 15 kGy.

FFA Composition of Rice Bran Oil. The effect of γ-irradiation on FFA composition of rice bran oil extracted from irradiated rice bran during storage is shown in Table 5. Upon irradiation, the linoleic and linolenic acids in rice bran irradiated at 15 kGy were significantly ($p < 0.05$) different from those in raw rice bran. With longer storage, the FFA of nonirradiated and irradiated samples showed a decrease in linoleic and linolenic acid, with a corresponding apparent increase in oleic and palmitic acid. Differences in FFA composition between raw and irradiated rice bran samples were slight during storage. Also, the changes of FFA composition among irradiated rice brans were not significantly ($p > 0.05$) different during storage.

Tipples and Norris (1965) reported that the peroxide values of wheat flour lipids increased with increasing irradiation. Upon storage (185 days), the increase in peroxide value became less with increasing irradiation and the value was higher in the control than in irradiated samples. Also, a decrease in linoleic and linolenic acid was observed to be less in irradiated samples than in the control (Tipples and Norris, 1965).

Phospholipids in Rice Bran. Phospholipids are an important part of biological and food systems, even though they are not a major constituent of these systems. No information has been reported concerning the effect of γ-irradiation and postirradiation storage on phospholipids in rice bran.

Increasing γ-irradiation doses from 5 to 10 kGy did not ($p > 0.05$) lower the phospholipid content in rice bran. At 15 kGy, phospholipids did decrease ($p < 0.05$) (Table 4). There were slight changes of fatty acid composition of phospholipids in rice bran irradiated at 5 and 10 kGy (Table 6). Hafez et al. (1989) reported that γ-irradiation at high doses (20, 40, 60, 80, and 100 kGy) significantly ($p < 0.05$) decreased the phospholipid content of soybean. Furthermore, an increase in the moisture content of soybean seeds and irradiation at doses of 56 and 65 kGy resulted in a significant ($p < 0.05$) reduction in phospholipids. They noted that an increase in the amount of reactive species formed by radiolysis of water was sufficiently high to cause severe damage to phospholipids. Phosphatidic acid and the lysophospholipids were the major radiolytic products observed for the phospholipids in model systems (Tinsley and Maerker, 1993). Bancher et al. (1972) observed decreases in phosphatidylcholine (lecithin) and simultaneous increases in phosphatidic acid following irradiation of walnuts and groundnuts. Phospholipids of walnuts were more sensitive to irradiation than phospholipids of ground nuts. This was explained on the basis of a greater degree of unsaturation of walnut lipids compared to groundnuts. In contrast, the most likely explanation for the decrease in phospholipids extracted from irradiated wheat was that the phospholipids were "denatured" and thus were less available for solvent extraction (Tipples and Norris, 1965) rather than the effect of radiolysis on actual phospholipids.

Effects of Post-γ-irradiation Storage on Phospholipids. Very little information is available regarding changes in phospholipids during post-γ-irradiation storage. Total phospholipids in raw and irradiated rice bran decreased significantly ($p < 0.05$) during storage (Figure 2B). The retention of phospholipids in raw rice bran and that of rice bran irradiated at 5 and 10 kGy were not significantly ($p > 0.05$) different during 52 weeks of storage except between 3 and 7 weeks in raw rice bran. γ-Irradiation at 15 kGy resulted in significantly ($p < 0.05$) different retention of phospholipids in rice bran during strage. The fatty acids in rice bran oil

Table 6. Composition of Phospholipids in Raw and Irradiated Rice Bran during 52 Weeks of Storage[a]

		fatty acid composition (%)								
irradiation dose (kGy)	storage (weeks)	14:0	16:0	18:0	18:1	18:2	18:3	20:0	saturated	unsaturated
raw	0	0.11	22.13	0.16	38.11	39.32	0.17		22.40	77.60
	1	0.13	22.52	0.16	38.89	38.15	0.15		22.81	77.19
	3	0.12	23.44	0.18	38.97	37.12	0.17		23.74	76.26
	7	0.13	23.99	0.18	38.80	36.77	0.13		24.30	75.70
	24	0.14	24.81	0.21	39.43	35.27	0.14		25.16	74.84
	52	0.15	25.20	0.22	39.67	34.63	0.13		25.57	74.43
5	0	0.11	22.20	0.16	38.23	39.14	0.16		22.47	77.53
	1	0.12	22.51	0.17	38.89	38.16	0.15		22.80	77.20
	3	0.12	23.71	0.18	38.83	36.99	0.17		24.01	75.99
	7	0.13	23.99	0.18	38.80	36.77	0.13		24.30	75.70
	24	0.13	24.83	0.20	39.43	35.28	0.13		25.16	74.84
	52	0.14	25.22	0.21	39.05	35.26	0.12		25.57	74.43
10	0	0.11	22.59	0.16	38.13	38.86	0.15		23.86	76.14
	1	0.12	22.89	0.16	38.82	37.85	0.16		23.17	76.83
	3	0.14	23.39	0.18	38.99	37.15	0.15		23.71	76.29
	7	0.13	23.46	0.19	39.15	36.93	0.14		23.78	76.22
	24	0.12	24.78	0.18	39.52	35.28	0.12		25.08	74.92
	52	0.14	24.94	0.19	39.23	35.40	0.10		25.27	74.73
15	0	0.12	23.20	0.16	38.42	37.98	0.12		23.48	76.52
	1	0.13	23.35	0.16	38.38	37.86	0.12		23.64	76.36
	3	0.14	24.07	0.17	38.40	37.10	0.12		24.38	75.62
	7	0.14	24.52	0.17	38.69	36.37	0.11		24.83	75.17
	24	0.14	25.44	0.18	39.12	35.03	0.09		25.76	74.24
	52	0.14	25.75	0.21	39.44	34.38	0.08		26.10	73.90

[a] Values are means of four analyses.

Table 7. Composition of Nonpolar Lipid from Raw and Irradiated Rice Bran during 52 Weeks of Storage[a]

		fatty acid composition (%)								
irradiation dose (kGy)	storage (weeks)	14:0	16:0	18:0	18:1	18:2	18:3	20:0	saturated	unsaturated
raw	0	0.50	23.78	1.71	37.71	34.67	0.98	0.65	26.64	73.36
	1	0.48	24.48	1.68	37.63	34.08	0.89	0.76	27.40	72.60
	3	0.34	23.03	1.92	38.44	34.56	0.90	0.81	26.10	73.90
	7	0.32	23.76	1.96	38.11	34.20	0.99	0.66	26.70	73.30
	24	0.32	23.82	2.05	38.29	33.83	0.97	0.72	26.91	73.09
	52	0.29	25.27	2.11	37.88	32.87	0.88	0.70	28.37	71.63
5	0	0.26	24.07	1.85	37.66	34.55	0.96	0.65	26.83	73.17
	1	0.27	24.28	1.76	37.99	34.05	0.90	0.75	27.06	72.94
	3	0.32	24.19	1.78	38.18	33.89	0.86	0.78	27.07	72.93
	7	0.36	25.42	2.12	37.52	32.96	0.96	0.66	28.56	71.44
	24	0.36	25.50	2.52	37.08	32.90	0.94	0.70	29.08	70.92
	52	0.39	26.53	2.46	37.09	31.97	0.87	0.69	30.07	69.93
10	0	0.25	24.01	1.77	37.75	34.65	0.93	0.64	26.67	73.33
	1	0.27	24.22	1.67	38.09	34.15	0.86	0.74	26.90	73.10
	3	0.31	24.13	1.69	38.29	33.97	0.83	0.78	26.91	73.09
	7	0.35	25.39	2.04	37.61	33.03	0.93	0.65	28.43	71.57
	24	0.35	25.49	2.45	37.11	32.99	0.92	0.69	28.98	71.02
	52	0.38	26.51	2.39	37.18	32.02	0.84	0.68	29.96	70.04
15	0	0.24	23.55	1.86	38.29	34.51	0.87	0.68	26.33	73.67
	1	0.31	23.45	1.78	38.27	34.54	0.93	0.72	26.26	73.74
	3	0.25	24.85	1.88	37.69	33.62	0.94	0.77	27.75	72.25
	7	0.35	24.86	2.00	37.43	33.79	0.89	0.68	27.89	72.11
	24	0.40	25.49	2.08	37.39	32.95	0.92	0.77	28.74	71.26
	52	0.40	26.35	2.26	37.39	31.82	0.99	0.79	29.80	70.20

[a] Values are means of four analyses.

extracted from irradiated rice bran contained a reduced proportion of linoleic and linolenic acids with increased palmitic and oleic acid composition during 52 weeks of storage. The fatty acid profile in phospholipids was similar to the fatty acid composition of the nonpolar lipids from irradiated rice bran (Table 7). The effect of γ-irradiation on fatty acid composition was slightly greater with phospholipids than with nonpolar lipids. Quantitative analysis showed that the amounts of volatile products produced from phospholipids during irradiation were significantly less than those formed in glycerides (Delincée, 1983a). Also, the contribution of free fatty acids to the total amount of radiolytic products was relatively small compared to the yield from triglycerides. This accounts for the majority of lipids present in food, since fat-containing foodstuffs usually contain only small amounts of FFA.

Nonpolar Lipids and Glycolipids in Rice Bran. The amount of nonpolar lipids and glycolipids (data not shown) decreased with longer storage of rice bran, with a corresponding increase in the amounts of free fatty acids and a decrease in the amounts of phospholipids. The trends of changes in fatty acid composition of glycolipids (data not shown) were similar to that of nonpolar lipid (Table 7). The changes in contents of lipid classes in raw and irradiated rice bran during postirradiation storage were due to the lipolytic en-

zymes. γ-Irradiation did not provide any beneficial effects during postirradiation storage.

LITERATURE CITED

Bancher, E.; Washvettl, J.; Goller, H. J. Effect of gamma irradiation on lipids or groundnuts and walnuts. II. Qualitative and quantitative examination of polar lipids. *Sefen-eole-fette-waschse* **1972**, *98*, 505−508.

Barber, S.; Benedito de Barber, C. Rice Bran: Chemistry and Technology. In *Rice: Production and Utilization*; Luh, B. S., Ed.; AVI Publishing: Westport, CT, 1980; pp 790−862.

Chiou, R. Y.-Y.; Lin, C. M.; Shyu, S.-L. Property characterization of peanut kernels subjected to gramma irradiation and its effect on the outgrowth and aflatoxin production by *Aspergillus parasiticus*. *J. Food Sci.* **1990**, *55*, 210−217.

Christie, W. W. The preparation of derivatives of lipid. In *Lipid Analysis*; Pergamon Press: New York, 1982; p 53.

Delincèe, H. Recent advances in radiation chemistry of lipids. In *Recent Advances in Food Irradiation*; Elias, P. S., Cohen, A. J., Eds.; Elsevier Biomedical Press: Amsterdam, The Netherlands, 1983a; pp 89−114.

Delincèe, H. Recent advances in radiation chemistry of proteins. In *Recent Advances in Food Irradiation*; Elias, P. S., Cohen, A. J., Eds.; Elsevier Biomedical Press: Amsterdam, The Netherlands, 1983b; pp 129−147.

Diehl, J. F. Effects of combination processes on the nutritive value of food. In *Combination Processes in Food Irradiation*; Proceedings of a Symposium, Colombo, Nov 24−28 IAEA-SN-250; International Atomic Energy Agency: Vienna, 1981; pp 349−366.

Folch, J.; Lees, M.; Sloane Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497−509.

Hafez, Y. S.; Mohammed, A. I.; Perera, P. A.; Singh, G.; Hussein, A. S. Effects of microwave heating and gamma irradiation on phytate and phospholipid contents of soybean (*Glycine max. L.*). *J. Food Sci.* **1989**, *54*, 958−962.

Hilker, D. M.; Tang, N. Y.-A.; Chan, K.-C. The effect of gamma irradiation on the antithiamine activity of skipjack tuna. *J. Food Sci.* **1972**, *37*, 596−598.

Ismail, F. A.; El-Wakeil, F. A.; El-Dash, S. M. Tolerance, quality and storability of gamma-irradiated Egyptian rice. In *Food Preservation by Irradiation*; International Atomic Energy Agency: Vienna, 1977; pp 501−515.

Iyer, V.; Salunkhe, D. K.; Sathe, S. K.; Rockland, L. B. Quick-cooking beans (*Phaseolus vulgaris L.*): 1. Investigations on quality. *Qual. Plant Plant Foods Hum. Nutr.* **1980**, *30*, 27−31.

Jaddou, H.; Al-Hakim, M.; Al-Adamy, L. Z.; Mhaisen, M. T. Effect of gamma-radiation on gossypol in cottonseed meal. *J. Food Sci.* **1983**, *48*, 988−989.

Katta, S. R.; Rao, D. R.; Sunki, G. R.; Chawan, C. B. Effect of gamma irradiation of whole chicken carcasses on bacterial loads and fatty acids. *J. Food Sci.* **1991**, *56*, 371−372.

Kempe, L. L; Graikoski, J. T.; Stratton, J. R.; Day, W. H. Sterilization of barley malt with gamma radiation. *J. Agric. Food Chem.* **1964**, *12*, 98−100.

Kim, I.; Kim, C. Separation of oryzanol from the refining by-product of rice bran oil. *Korean J. Food Sci. Technol.* **1991**, *23*, 76−80.

Knapp, F. W.; Tappel, A. L. Comparison of radiosensitivities of the fat-soluble vitamins by gamma irradiation. *J. Agric. Food Chem.* **1961**, *9*, 430−433.

Kodenchery, U. K.; Nair, M. P. Metabolic changes induced by sprout-inhibition dose of γ-irradiation in potatoes. *J. Agric. Food Chem.* **1972**, *20*, 282−285.

Kozukue, E.; Kozukue, N. Lipid content and fatty acid composition in bamboo shoots. *J. Food Sci.* **1981**, *46*, 751−755.

Kwon, J. H.; Byun, M. W.; Cho, H. O. Effects of gamma irradiation dose and timing of treatment after harvest on the storability of garlic bulbs. *J. Food Sci.* **1985**, *50*, 379−381.

Lamuka, P. O.; Sunki, G. R.; Chawan, C. B.; Rao, D. R.; Shackelford, L. A. Bacteriological quality of freshly processed broiler chickens as affected by carcass pretreatment and gamma irradiation. *J. Food Sci.* **1992**, *75*, 330−332.

Loeb, J. R.; Mayne, R. Y. Effect of moisture on the microflora and formation of free fatty acids in rice bran. *Cereal Chem.* **1952**, *29*, 163−175.

Mattick, L. R.; Lee, F. A. A note on a method for the extraction of free fatty acids from lipid material. *J. Food Sci.* **1959**, *24*, 451−452.

Murray, D. R. Radiolytic products and selective destruction of nutrients. In *Biology of Food Irradiation*; Wiley: New York, 1990; pp 71−135.

Ogawa, M.; Uritani, I. Tissue browning of potato tubers induced by gamma irradiation. *Agric. Biol. Chem.* **1970**, *34*, 870−874.

Patel, G. M.; Vyas, D. N.; Patel, K. C. Behavior of lipase activity of the gamma-irradiated groundnut during germination. *J. Am. Oil Chem. Soc.* **1965**, *42*, 617−619.

Ramarathnam, N.; Osawa, T.; Namiki, M.; Kawakishi, S. Studies on changes in fatty acid composition and content of endogenous antioxidants during gamma irradiation of rice seeds. *J. Am. Oil Chem. Soc.* **1989**, *66*, 105−108.

Rao, V. S.; Vakil, U. K. Effect of gamma-radiation on cooking quality and sensory attributes of four legumes. *J. Food Sci.* **1985**, *50*, 372−375.

Rao, V. S.; Vakil, U. K. Effects of gamma-radiation on flatulence-causing oligosaccharides in green gram (*Phaseolus areus*). *J. Food Sci.* **1983**, *48*, 1971−1795.

SAS Institute. *SAS User's Guide: Statistics*, version 6.04 ed.; SAS Institute: Cary, NC, 1987; pp 499−640.

Saunders, R. M.; Heltved, F. Fluorimetric assay of lipase in rice bran and its application to determination of conditions for rice bran stabilization. *J. Cereal Sci.* **1985**, *3*, 79−86.

Shin, T.-S.; Godber, J. S. Improved high-performance liquid chromatography of vitamin E vitamers on normal-phase columns. *J. Am. Oil Chem. Soc.* **1993**, *70*, 1289−1291.

Shin, T.-S.; Godber, J. S. Isolation of four tocopherols and four tocotrienols from a variety of natural sources by semi-preparative high performance liquid chromatography. *J. Chromatogr.* **1994**, *678*, 49−58.

Thomas, P.; Nair, P. M. Effect of gamma irradiation on polyphenol oxidase activity and its relation to skin browning in bananas. *Phytochemistry* **1971**, *10*, 771−774.

Tinsley, P. W.; Maerker, G. Effect of low-dose gamma-radiation on individual phospholipids in aqueous suspension. *J. Am. Oil Chem. Soc.* **1993**, *70*, 187−191.

Tipples, K. H.; Norris, F. W. Some effects of high levels of gamma irradiation on the lipids of wheat. *Cereal Chem.* **1965**, *42*, 437−451.

Vajdi, M.; Nawar, W. W.; Merritt, J. C., Comparison of radiolytic compounds from saturated and unstaturated triglycerides and fatty acids. *J. Am. Oil Chem. Soc.* **1978**, *55*, 849−850.

Received for review June 26, 1995. Accepted November 13, 1995.[⊗] We acknowledge the financial support of the Louisiana Board of Regents under Grant LEQSF(93-95)RD-B-01.

JF950386A

[⊗] Abstract published in *Advance ACS Abstracts*, January 15, 1996.