Alterations in Polyunsaturated Fatty Acid Composition of *Voandzeia subterranea* Seeds upon γ Irradiation

Rivo H. Andrianarison, Zafisolo Rakotoarisoa, Marie Tixier, and Jean L. Beneytout*
Laboratoire de Biochimie, Faculté de Pharmacie, 2 Rue du Docteur Marcland, 87025 Limoges Cedex, France

Exposure of *V. subterranea* seeds, a herbaceous plant from Madagascar belonging to the family of legumes, to γ irradiation resulted in a polyunsaturated fatty acids decrease associated with the formation of UV-absorbing substances. The finding that products containing conjugated diene structure are formed during lipid extract irradiation indicates that hydroperoxy fatty acids may arise not only by enzymatic reactions but also by nonenzymatic oxygenation of polyunsaturated fatty acids promoted by ionizing radiation. Dehulled green seeds, flour made from dehulled green seeds, and lipid extract were studied for irradiation dose dependent changes in fatty acids compositions and hydroperoxydiene synthesis. The irradiation dose is more efficient in lipid extract than in dehulled green seeds or in flour made from these seeds, suggesting that the formation of UV-absorbing products is not a reliable clue for enzyme activity owing to the absence of protein in lipid extract. A homolytic pathway for the biogenesis of hydroperoxy fatty acids from polyunsaturated fatty acids is proposed. This involves an initiating radical which promotes a chain mechanism in which the O_2 adsorbed is converted to hydroperoxide. Conclusively, preservation of fatty acid oxygenation should be a primary goal in the ionizing radiation processes of *V. subterranea* seeds and generally in the preservation of food of plant origin by ionizing radiation.

INTRODUCTION

Recently, several investigations have reported the preservation of food of animal or plant origin by ionizing radiation (Dodd et al., 1985; Raffi et al., 1988). In animal cells, the metabolites of polyunsaturated fatty acids including hydroperoxy fatty acids represent an important class of biologic mediators which are released after ionizing radiation (Steel et al., 1988). It would be of great interest, therefore, to determine whether ionizing radiation may be involved in the synthesis of dioxygenated polyunsaturated fatty acids from plant cells and particularly in food of plant origin.

The polyunsaturated fatty acids are susceptible to oxidation by radical processes (Jore and Ferradini, 1988). On the other hand, it is well-known that free radicals are formed in food by ionizing radiation (Ehrenberg et al., 1969; Wills, 1980). However, little information exists about the possible formation of hydroperoxy fatty acids resulting from exposure to ionizing radiation of fatty acids (Chipault and Mizuno, 1964; Hyde and Verdin, 1968). Therefore, efficient strategies for preventive lipid peroxidation in ionizing radiation processes of food of plant origin are also lacking. Chemical characteristics as well as fatty acid composition govern food quality. Studies conducted in our laboratory revealed alterations in fatty acid composition of *Voandzeia subterranea* seeds upon γ-irradiation treatment. The present investigation was undertaken to determine the effect of ionizing radiation on fatty acid composition of seeds, flour, and lipid extract made from these seeds.

MATERIALS AND METHODS

Plant Materials and Irradiation. *V. subterranea* seeds were from Malagasy cultivation harvested in 1990. Samples (dehulled green seeds, flour, or lipid extract made from dehulled green seeds) were placed in plastic containers and irradiated in a bilateral ^{60}Co γ-ray field using a IBL 460 ionizer. Total dose was 2, 4, 6, 8, or 10 kGy, delivered at a dose rate of 331 967 Gy/h. Irradiation was carried out at room temperature.

Lipid Extraction. *V. subterranea* samples were extracted by shaking (3000 rpm, at room temperature) for 15 min in 5 volumes of chloroform–methanol (2:1 v/v) (Folch et al., 1957) using an Ultra Turrax homogenizer. The extraction was repeated for 15 min with the same amount of chloroform–methanol. The extracts were combined and dried with anhydrous $CaCl_2$. After filtration, the organic solvent was removed under vacuum. Lipids were dissolved in 10 mL of hexane divided as 1-mL portions in test tubes and stored at −20 °C before analysis. The samples were used to determine fatty acid composition and hydroperoxide formation.

Preparation and Analysis of Fatty Acid Methyl Esters. Fatty acid methyl esters were prepared by saponification and methylation essentially as described by Suutari et al. (1990). The methyl esters were analyzed by gas–liquid chromatography. The major fatty acids were identified by comparing their retention times with those of standards (Sigma). The extractable fatty acids were determined by adding 30 μg of heptadecanoic acid methyl ester (as internal standard) prior to saponification.

Gas Chromatography. A Packard-Becker Model 417 chromatograph equipped with a flame ionization detector, a capillary inlet system, and a OV-1 (25 m × 0.32 mm × 0.2 μm) column was used. The column temperature was programmed from 180 to 240 °C at the rate of 2 °C/min. The injector and detector were maintained at 300 °C. Peak areas were measured by using a Hewlett-Packard Model 3365 A integrator.

Calculations. The absolute amount of the individual fatty acids was calculated per 1 g of sample by comparison of the peak area to that of the methyl ester internal standard. The total amount of fatty acids was a sum of all fatty acids.

UV Spectra Analysis. Diene formation was monitored by taking UV spectra from 330 to 190 nm using a Perking-Elmer Lambda 5 UV–vis spectrophotometer. Twenty-five microliters of lipid extract was used. Hexane was removed under N_2 and replaced by 3 mL of ethanol. Formation of conjugated diene was visualized by the increase of absorbance at 234 nm as previously described (Andrianarison et al., 1990).

RESULTS AND DISCUSSION

V. subterranea seeds, flour, or lipid extract from dehulled green seeds were studied for irradiation dose dependent changes in the fatty acids compositions and hydroperoxydiene synthesis. Details of dose measurements have been described previously (Snyder et al., 1986).

* Author to whom correspondence should be addressed.

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Table I. Fatty Acid Composition (Percent) of *V. subterranea* Seeds

fatty acid	dehulled green seeds[a]	dehulled dry seeds
16:0ω	13.73 ± 0.31	13.20 ± 0.20
18:0ω	8.93 ± 0.22	9.39 ± 0.11
18:1*n*-9	20.73 ± 0.51	24.64 ± 0.23
18:1*n*-7	1.72 ± 0.05	1.78 ± 0.10
18:2*n*-6,9	45.20 ± 0.70	43.29 ± 0.61
18:3*n*-6,9,12	4.96 ± 0.12	2.33 ± 0.15
20:0ω	2.66 ± 0.13	2.95 ± 0.12
22:6*n*-3,6,9,12,15,18	1.09 ± 0.10	0.87 ± 0.01
others[b]	0.98 ± 0.15	1.53 ± 0.05

[a] Mean values are from three replicated determinations. Mean ± SD. [b] Includes 20:1(*n*-9), 20:1(*n*-7), and unifentified acids.

Table II. Effect of Irradiation Dose (0–10 kGy) on Polyunsaturated Fatty Acid Composition in Samples[a] from *V. subterranea* Seeds

		polyunsaturated fatty acids[b]		
sample	kGy	18:2*n*-6,9	18:3*n*-6,9,12	22:6*n*-3,6,9,12,15,18
A	0	45.21 ± 1.32	4.96 ± 0.06	1.09 ± 0.01
	2	45.49 ± 0.67	4.55 ± 0.14	0.90 ± 0.02
	4	44.68 ± 0.36	4.13 ± 0.21	0.68 ± 0.02
	6	43.03 ± 1.21	3.76 ± 0.33	0.49 ± 0.02
	8	42.11 ± 0.29	3.65 ± 0.22	0.44 ± 0.01
	10	40.43 ± 0.56	3.48 ± 0.25	0.23 ± 0.00
B	0	48.01 ● 2.19	5.96 ± 0.22	0.75 ± 0.06
	2	47.53 ± 1.65	5.35 ± 0.25	0.73 ± 0.04
	4	46.99 ± 1.38	5.07 ± 0.28	0.67 ± 0.02
	6	46.55 ± 1.56	4.91 ± 0.25	0.61 ± 0.02
	8	45.50 ± 1.47	4.84 ± 0.23	0.49 ± 0.01
	10	44.87 ± 1.06	4.72 ± 0.24	0.40 ± 0.02
C	0	46.74 ± 1.58	4.81 ± 0.23	0.96 ± 0.05
	2	45.77 ± 0.61	4.76 ± 0.22	0.94 ± 0.04
	4	45.73 ± 1.76	4.50 ± 0.21	0.82 ± 0.02
	6	43.75 ± 1.89	4.46 ± 0.22	0.66 ± 0.01
	8	42.71 ± 1.56	4.17 ± 0.10	0.46 ± 0.02
	10	41.68 ± 0.72	4.01 ± 0.25	0.39 ± 0.06

[a] A, lipid extract. B, flour from green seeds. C, green seeds. [b] Mean values were from three replicated determinations. Mean ± SD.

Fatty Acid Analysis. Small differences were observed between fatty acid compositions of dehulled green or dry seeds of *V. subterranea* (Table I). In the two cases, the major fatty acids were linoleic, oleic, and palmitic acids. Arachidic and linolenic acids occurred in lesser amounts.

Effect of γ Irradiation on Percentage Distribution of Polyunsaturated Fatty Acids. The amount of polyunsaturated fatty acids decreased concomitantly with increase of irradiation dose (Table II), suggesting that the irradiation induced a selective loss of polyunsaturated fatty acids. Loss of fats was thought to be the result of radiolysis (Nawar, 1978), involving primary ionization followed by cleavage at preferential position near the carbonyl groups.

Effect of Irradiation Dose on UV-Absorbing Product Formation. When *V. subterranea* samples (lipid extract, dehulled green seeds, and flour made from those seeds) were subjected to ionizing radiation as described under Materials and Methods, a diene peak (234 nm) appeared concomitantly with increase of irradiation dose (Figure 1), while the peak of nonoxidized lipid acids (206 nm) decreased. The peak at 234 nm corresponds to those obtained by oxidation of polyunsaturated fatty acids by plant lipoxygenase (Andrianarison et al., 1991). Absorption near 234 nm was characteristic of lipid peroxidation, in vitro or in vivo studies (Kappus, 1984).

These results suggest that ionizing radiation may be of major contribution in the peroxidation of polyunsaturated fatty acids. This oxidation process is more effective in lipid extract than in dehulled green seeds or in flour made from these seeds (Figure 1).

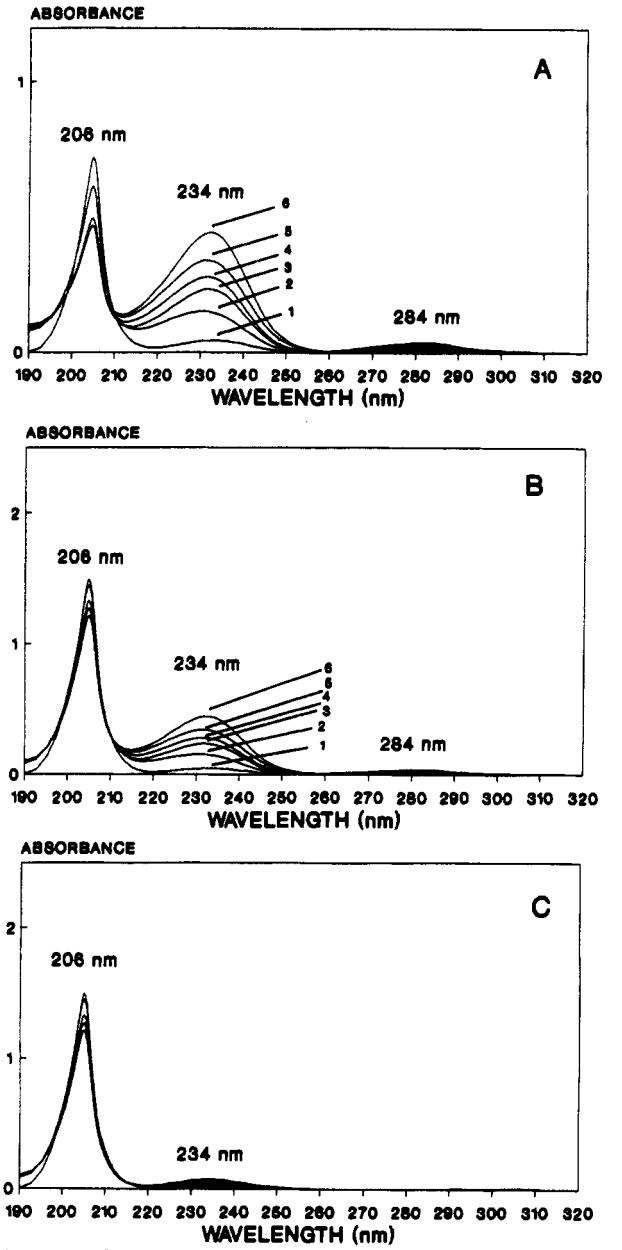

Figure 1. UV spectra analysis. (A) UV spectra of irradiated lipids extract. (B) UV spectra of lipid extract from irradiated flour made from dehulled green seeds. (C) UV spectra of lipid extract from irradiated dehulled green seeds. The doses of irradiation were, from line 1 to line 6, 0, 2, 4, 6, 8, and 10 kGy.

During the exposure to ionizing radiation of lipid extract or flour, we found a second peak at 284 nm (Figure 1). Absorption near 280 nm was characteristic of a conjugated dienone chromophore (Andrianarison et al., 1989).

This is evidence that the formation of conjugated diene and dienone chromophores is not a reliable clue to lipoxygenase activity owing to the absence of enzyme in lipid extract. It is well-known that radiation damage resulting from exposure to ionizing radiation is primarily an indirect effect initiated by free radicals produced during the radiation process (Ehrenberg et al., 1969).

The mechanism in the formation of the conjugated diene or dienone products was not investigated. However, we proposed a mechanism which can explain these syntheses (Figure 2). It seems likely that the conjugated diene products were formed from polyunsaturated fatty acids containing a pentadiene system, as, for example, linoleic or linolenic acid (the most abundant polyunsaturated fatty acids in *V. subterranea* seeds) (LH). Loss of hydrogen radical (H•), which is transferred into the

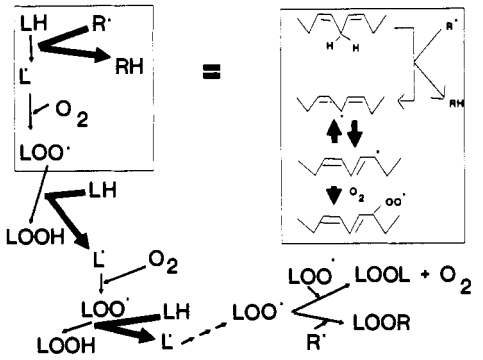

Figure 2. Proposed schematic mechanism for the formation of lipid hydroperoxide in samples from *V. subterranea* green seeds upon γ irradiation. LH, polyunsaturated fatty acid; R·, initiating radical; L·, pentadienyl radical; LOO·, peroxyl radical.

initiating radical (R·), produced during the radiation process, results in the formation of a new free pentadienyl radical (L·) which would be oxidized rapidly by molecular oxygen. A peroxyl radical would be expected (LOO·). Such an equilibrium between peroxyl radical and pentadienyl radical plus O_2 is already known from research of the free-radical chemistry of autoxidizing polyunsaturated lipids. The peroxyl radical may promote a loss of hydrogen radical from another polyunsaturated fatty acid molecule followed by the formation of a hydroperoxy fatty acid (LOOH) and a free pentadienyl radical (L·). This radical process may be ended by the reaction between two peroxyl radicals ($2LOO· \rightarrow LOOL + O_2$) or between the initiating radical and a peroxyl radical ($LOO· + R· \rightarrow LOOR$). Patterson and Redpath (1977), on the one hand, and Metwally and Moore (1987), on the other hand, have shown that the γ ray induced oxidation of unsaturated fatty acids in aqueous solution and the predominant chain-initiating radical was OH. The dienone chromophore, showing absorbance at 284 nm, may be the result of the reactions of water radiolysis species with hydroperoxy fatty acids (Greenstock and Wiebe, 1981).

V. subterranea is a plant whose dry seeds are used as food in some countries of Africa and in Madagascar. The seeds of this plant are receiving nutritional interest because of their high-quality protein and good fatty acid composition. However, the development of food applications of *V. subterranea* is often hampered by conservation problem due to infection by seed parasite. One issue may be the use of ionizing radiation. Unfortunately, this process induces peroxidation of fatty acids.

The high concentration of hydroperoxy fatty acids in the diet may cause several abnormalities in the human body. In addition, linolenic acid, one of the unsaturated essential fatty acids (Achaya, 1987), was reduced upon ionizing radiation treatment of *V. subterranea*, suggesting that the quality of seeds is rendered poorer upon ionizing radiation. Then, preservation of fatty acid oxygenation should be a primary goal in the ionizing radiation processes of *V. subterranea* seeds and generally in the preservation of food of plant origin by ionizing radiation. Chipault and Mizuno (1966) showed that several antioxidants were also destroyed by high-energy radiations in the presence of oxygen and did not prevent the peroxidation of fats.

It is hoped that further studies utilizing free polyunsaturated fatty acids or polyunsaturated fatty acids esterified in phospholipids will aid in the investigation and ultimate understanding of the relevant effect of ionizing radiation.

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