Chlorophyll Breakdown by Gamma Irradiation in a Model System Containing Linoleic Acid

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ABSTRACT: The possible breakdown of chlorophyll by irradiation was investigated. Chlorophyll *b* standard (3 ppm) was added to a methanol solution containing 1% linoleic acid. Irradiation up to 20 kGy was performed with or without N_2-bubbling, and nonirradiated control was also prepared. Residual chlorophyll *b* content was analyzed by HPLC and UV-visible spectra after irradiation, and color was measured during 6 h of light exposure (3,300 lux, light intensity) to induce photooxidation. The added chlorophyll *b* was destroyed by irradiation at 20 kGy with or without N_2-bubbling. With N_2-bubbling the oil sample did not develop lipid oxidation during irradiation, and irradiated samples did not develop photooxidation during storage under light. Without N_2-bubbling, irradiated oil samples had higher PV than nonirradiated samples. The Hunter color a-value of oil increased and the b-value decreased with irradiation at 20 kGy. UV-visible spectra also supported the breakdown of chlorophyll *b* in solution by irradiation. Irradiation at 2.5 kGy or above destroyed all added chlorophyll *b*. The results indicate that irradiation technology could be applied to reduce or eliminate the residual chlorophyll in oil processing without developing lipid oxidation during the irradiation process, which would prolong the shelf life of oil products by protecting them from photooxidation during display.

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KEY WORDS: Breakdown, chlorophyll *b*, irradiation, lipid oxidation, photooxidation.

The presence of green pigments of chlorophyll in soybean oil is of interest not only for its impact on the color of the finished product but also for its potential role in oxidative stability (1). Except for virgin olive oil, where a greenish color is tolerated, an excessive amount of chlorophyll (>20 μg/g) is considered undesirable and is difficult to remove by conventional bleaching processes (2). Chlorophyll pigments not only impart an undesirable color to vegetable oils and promote oxidation in the presence of light (3–6) but also impair hydrogenation (7).

The major chlorophyll pigments (about 90%) in oils are *a*-type (pheophytin *a* and pyropheophytin *a*). Although the ratio of chlorophyll *a*/*b* is about 3:1 in canola seeds, the *b*-type pigments are not as susceptible to extraction from seeds with a nonpolar solvent such as *n*-hexane (2). Chlorophyll decomposition involves the release of chlorophyll from its protein complex followed by dephytolization and possibly pheophytinization (8). Simpson *et al.* (8) reported that oxidation of the ring structure by chlorine occurs, and ultimately colorless end products form. However, the exact mechanism of chlorophyll breakdown is still poorly understood. Wagenknecht and Lee (9) also reported that a loss of chlorophyll in frozen peas was associated with lipoxygenase activity and fat peroxidation.

Food irradiation is a useful technique for controlling pathogenic microorganisms such as *Escherichia coli* O157:H7 and *Salmonella* (10). Besides the sanitation of food, irradiation can be applied to reduce allergens (11) or nitrosamines (12) in food, to enhance the color of meat products without nitrite (13), and to reduce the salt content of traditional fermented foods (14). However, the use of irradiation is limited by foods containing high fat because irradiation may increase lipid oxidation by producing reactive free radicals or ions from absorbed energy (15). This may reduce the application trial of irradiation to oil, making approaches for chlorophyll removal by irradiation very limited. The objective of the present study was to investigate the possibility of chlorophyll removal by gamma irradiation to inhibit photooxidation during storage without accelerating lipid oxidation during the irradiation process.

EXPERIMENTAL PROCEDURES

Chemicals. Chlorophyll *b* and linoleic acid were purchased from Sigma Chemical Co. (St. Louis, MO). HPLC-grade methanol was purchased from Acros Organics (Morris Plains, NJ). Linoleic acid (1%) was dissolved in methanol. Chlorophyll *b* was dissolved in methanol (6 ppm) under dark conditions, then diluted to 3 ppm.

Irradiation. The prepared solution was transferred into three different bottles (1 L). One bottle was introduced to irradiation at 20 kGy. The next bottle was irradiated with N_2 (99.999% ultrapure)-bubbling during the process. Nitrogen tubing was inserted into the bottom of the bottle and nitrogen pressure was regulated outside the irradiation chamber by

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extending the gas tube outside. Irradiation was performed in a Co-60 gamma irradiator (point source, AECL, IR-79; Nordion International Co., Ltd., Ontario, Canada) with a source strength of 100 kCi. The dose rate was 83.3 Gy/min at 12 ± 0.5°C, and the applied absorbed doses were 0 to 20 kGy. Dosimetry was performed using 5-mm-diameter alanine dosimeters (Bruker Instruments, Rheinstetten, Germany), and the free radical signal was measured using a Bruker EMS 104 EPR analyzer. The actual dose was within ±2% of the target dose. The last bottle was a nonirradiated control and was placed outside of the irradiation chamber.

Light storage. The prepared samples (40 mL) in duplicate were transferred into serum bottles. The bottles were sealed with rubber septa and aluminum caps and were stored in a light storage box as described by Jung *et al.* (16). Half of the samples were covered by aluminum foil to avoid light during storage. The light intensity at the sample level was 3,300 lux. The temperature in the light storage box was 25°C. The sample bottles were taken out at 0, 1, 2, 4, and 6 h and analyzed.

PV and UV-visible spectrum. PV was measured by the AOAC method 28.025 (17). Irradiated and photooxidized samples were dissolved in chloroform/acetic acid (2:3, vol/vol) solution, and saturated potassium iodide (0.5 mL) dissolved in deionized distilled water (DDW) was added. The mixture was vortexed for 1 min and stored under dark conditions for 5 min. Then DDW (75 mL) and 1% starch indicator solution (2.5 mL) were added to the mixture. The sample was titrated with $Na_2S_2O_3$ (0.005 N), adding it gradually and with constant agitation. Titration was continued until the yellow iodine color disappeared. The PV was calculated by $[(A - B) \times N \times 1000]/S$, where A = amount of $Na_2S_2O_3$ (mL) for titration of the sample, B = amount of $Na_2S_2O_3$ (mL) for titration of the control, N = concentration of $Na_2S_2O_3$, and S = sample weight (g).

The UV-visible spectrum was obtained using a spectrophotometer (UV-1601PC; Shimadsu, Co., Tokyo, Japan) in the range of 200 to 800 nm.

Color measurement. The sample (10 mL) was transferred to a glass cell (CM A-98, 10 mm in width), placed on a cell holder, and measured using a Color Difference Meter (spectrophotometer CM-3500d; Minolta Co. Ltd., Osaka, Japan) with an illuminant D65/10° observer. A large-size aperture was used and the measurement was triplicated. The Hunter color L-, a-, and b- values were obtained using Spectra Magic software (version 2.11; Minolta Cyberchrom Inc., Osaka, Japan).

Chlorophyll b *and linoleic acid content.* Chlorophyll *b* was determined using HPLC with a mobile phase of ethylacetate/methanol/DDW at a ratio of 60:30:10, by vol (18). Solvents were filtered through a 0.45-µm Nylon 6 filter and degassed by sonicator for 1 h before use. The HPLC system was composed of a Waters model 2690 separation module (Waters Co., Milford, MA) and a Shiseido reversed-phase (C18; Shiseido, Tokyo, Japan) column, and chlorophyll *b* was monitored at 658 and 436 nm. The chromatograms were integrated and recorded on Millenium^{32} software (Waters Co.). Absorbance spectra were obtained using a photodiode array detector (Waters model 996). An authentic chlorophyll *b* standard was used to develop the standard curve for chlorophyll *b* content.

Linoleic acid was separated by the extraction method of Folch *et al.* (19), and the methyl ester was separated on an Agilent gas chromatograph (model 6890, Agilent Technologies, Inc., Palo Alto, CA) equipped with an FID. A split inlet (split ratio, 20:1) was used to inject samples into a capillary column (DB Wax, 60 m × 250 µm × 0.25 µm nominal; J&W Scientific Inc., Folsom, CA), and a ramped oven temperature was used (180°C for 5 min, increased to 220°C at 2.5°C/min, held for 20 min). Injection was achieved by an Agilent model 7683 injector (Agilent Technologies, Inc.). Inlet and detector temperatures were 210°C. Nitrogen was the carrier gas at a constant flow rate of 1.1 mL/min. Detector air, N_2, and makeup gas (N_2) flows were 300, 30, and 28 mL/min, respectively. The chromatogram obtained was integrated using GC Chemstation software (Rev. A.08.03; Agilent Technologies, Inc.).

Statistical analysis. All experiments were duplicated and ANOVA was performed using SAS software (20). The Student-Newman-Keuls multiple-range test was used to find differences among mean values, and significance was defined at $P < 0.05$. Mean values and pooled SEM were reported.

RESULTS AND DISCUSSION

Chlorophyll b *content and UV-visible spectrum.* The chlorophyll *b* content of nonirradiated and unfoiled samples was 2.91 ppm immediately after irradiation and decreased during light storage to about half after 6 h of storage (Table 1). All the chlorophyll *b* was destroyed in the samples irradiated at 20 kGy with and without N_2 gas-bubbling. The foil-covered sample, which was treated to avoid photooxidation during storage, showed no difference during 6 h of photooxidation in the nonirradiated control and maintained chlorophyll *b* at the original level in the solution. Endo *et al.* (21) reported that amounts of chlorophyll pigments (including chlorophyll *a* and *b*, pheophytin *a* and *b*, pheophorbide *a*, methylpheophorbide *a*, and pyropheophytin *a*) in canola seed, meal, and crude oil were 19.9, 3.3, and 50.7 ppm, respectively. The *b*-type pigments cannot be extracted from seeds with a nonpolar solvent such as *n*-hexane, although the proportion of the *b*-type pigments found in meals is similar to that in oils. Owing to their higher polarity, *b*-type pigments are also more easily extracted in comparison with *a*-type (21).

The absorbance spectra of the sample irradiated with or without N_2-bubbling differed significantly ($P < 0.05$) from the nonirradiated control (Fig. 1). In the spectra, the nonirradiated sample had two main peaks at 658 and 436 nm, but no peak was detected in irradiated samples. Ramirez-Niño *et al.* (22) reported that differential measurement of opacity [opacity is equal to $(\text{transmittance})^{-1}$ and is dimensionless] using two light sources [blue light-emitting diode (LED) with 450 nm emission and infrared LED with an emission at 910 nm] for optical absorption measurement showed significantly different signals with different irradiation doses and proposed its use as a dosimeter.

TABLE 1
Chlorophyll Content (ppm) of 20 kGy-Irradiated and Photooxidized Linoleic Acid Solution (1% in methanol) Containing Chlorophyll *b* (3 ppm) by HPLC

		Photooxidation time (h)[a]					
	Irradiation	0	1	2	4	6	SEM[b]
Unfoiled	0 kGy	2.91a[c]	2.99a	2.34a	1.81b	1.51b	0.144
	20 kGy	ND	ND	ND	ND	ND	—
	20/N_2[d]	ND	ND	ND	ND	ND	—
Foiled[e]	0 kGy	2.88	3.05	3.02	2.85	2.73	0.108
	20 kGy	ND	ND	ND	ND	ND	—
	20/N_2[d]	ND	ND	ND	ND	ND	—

[a]Light intensity was 3,300 lux at 25°C. ND, not detected.
[b]SEM, pooled standard errors of the mean (n = 10).
[c]Different letters (a, b) within a row differ significantly at $P < 0.05$.
[d]Sample was bubbled with ultrapure N_2 gas during irradiation.
[e]Bottles were covered by aluminum foil to avoid photooxidation.

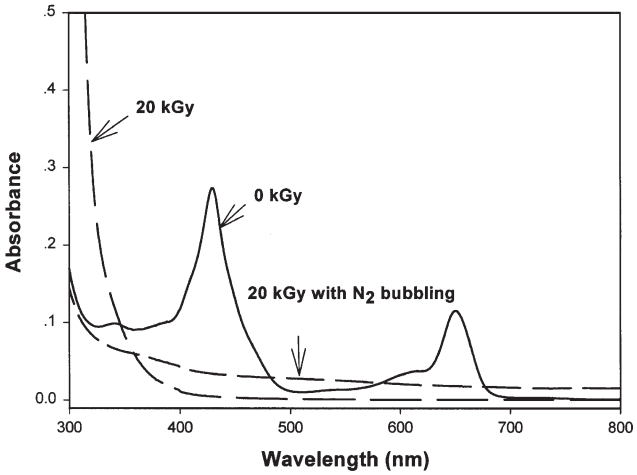

FIG. 1. Typical UV-visible spectra of linoleic acid solution (1% in methanol) containing chlorophyll *b* (3 ppm) treated with 20 kGy-irradiation with and without N_2 or left untreated (0 kGy).

Linoleic acid content in the sample was also investigated by GC, but no difference was found among the treatments (data not shown). When medium- to low-range irradiation doses were used, the degree of unsaturation generally remained unchanged during and after irradiation (15).

PV. PV of the unfoiled sample without irradiation increased slowly during storage because of photooxidation (Table 2). The PV of 20 kGy-irradiated samples were dramatically increased by irradiation and stayed high during photooxidation storage. This finding indicates that although the irradiation process increased lipid oxidation, chlorophyll breakdown in the 20 kGy-irradiated sample did not catalyze photooxidation when the sample was exposed to light. The irradiated sample treated with N_2-bubbling showed no increase in PV even when stored in light, suggesting that N_2-bubbling could completely stop or inhibit lipid oxidation during the irradiation process. During storage under light, photooxidation was also inhibited by removing the photosensitizer, chlorophyll *b*.

The PV of the foil-covered sample did not change at all in the nonirradiated control (Table 2), indicating that photooxi-

TABLE 2
PV of Photooxidized Linoleic Acid Solution (1% in methanol) Containing Chlorophyll *b* (3 ppm) by Gamma Irradiation

		Photooxidation time (h)[a]					
	Irradiation	0	1	2	4	6	SEM[b]
Unfoiled	0 kGy	0c,y[c,d]	24.0 c,y	27.5c,y	49.7b,y	79.0a,y	4.86
	20 kGy	1243.7x	1251.6x	1286.3x	1253.4x	1268.8x	11.44
	20/N_2[e]	0y	0y	0z	0z	0z	—
	SEM[f]	7.81	12.90	3.82	3.38	3.03	
Foiled[g]	0 kGy	0y	0y	0y	0y	0y	—
	20 kGy	1251.7x	1275.4x	1262.1x	1251.8x	1266.5x	17.40
	20/N_2[e]	0y	0y	0y	0y	0y	—
	SEM[f]	10.41	6.19	3.88	11.10	14.82	

[a]Light intensity was 3,300 lux at 25°C.
[b]SEM, pooled standard errors of the mean (n = 20).
[c]Different letters (a–c) within a row differ significantly at $P < 0.05$.
[d]Different letters (x–z) within foiled and unfoiled columns differ significantly at $P < 0.05$.
[e]Sample was bubbled with ultrapure N_2 gas during 20 kGy-irradiation.
[f]SEM: n = 12.
[g]Bottles were covered by aluminum foil to avoid photooxidation.

TABLE 3
Hunter Color L-Value[a] of Photooxidized Linoleic Acid Solution (1% in methanol) Containing Chlorophyll b (3 ppm) by Gamma Irradiation

		Photooxidation time (h)[b]					
	Irradiation	0	1	2	4	6	SEM[c]
Unfoiled	0 kGy	101.94b,c,y[d,e]	101.92c,y	101.95a,b,y	101.98a,y	101.97a,b,y	0.010
	20 kGy	102.35a,x	102.32b,x	102.32b,x	102.32b,x	102.32b,x	1.007
	20/N_2[f]	102.33x	102.30x	102.31x	102.31x	102.29x	0.013
	SEM[g]	0.090	0.010	0.008	0.012	0.011	
Foiled[h]	0 kGy	101.90y	101.89y	101.92y	101.91y	101.88y	0.015
	20 kGy	102.32x	102.32x	102.32y	102.32y	102.32x	0.012
	20/N_2[f]	102.26x	102.30x	103.98x	103.93x	102.30x	0.464
	SEM[g]	0.021	0.009	0.421	0.426	0.013	

[a]Higher L-value indicates increased lightness.
[b]Light intensity was 3,300 lux at 25°C.
[c]SEM, pooled standard errors of the mean (n = 10).
[d]Different letters (a–c) within a row differ significantly at $P < 0.05$.
[e]Different letters (x–z) within foiled and unfoiled columns differ significantly at $P < 0.05$.
[f]Sample was bubbled with ultrapure N_2 gas during 20 kGy-irradiation.
[g]SEM: n = 6.
[h]Bottles were covered by aluminum foil to avoid photooxidation.

dation is the main reason for the increase in PV in the nonirradiated, unfoiled sample. The PV value of the 20 kGy-irradiated sample with N_2-bubbling stayed at 0 during the full storage period regardless of the foil effect. This indicates a breakdown of all chlorophyll by irradiation, resulting in complete protection of oil from photooxidation. The sample with 20 kGy irradiation without N_2-bubbling had significantly ($P < 0.05$) higher PV from the beginning. The results confirm that oxygen exposure during irradiation can increase lipid oxidation dramatically. Ahn *et al.* (23) reported that exposure to oxygen was a more important factor than irradiation in the development of lipid oxidation in raw meat, a high-fat food, during storage.

Color change. The Hunter color L-value of the photooxidized linoleic acid solution with chlorophyll *b* (3 ppm) was increased by irradiation regardless of foil treatment (Table 3). The L-value of the foil-covered sample increased slightly after 4 h of photooxidation in the nonirradiated samples but decreased in the 20 kGy-irradiated sample. The L-value of the unfoiled sample also increased significantly ($P < 0.05$) owing to the irradiation treatment.

The Hunter color a-value in the nonirradiated, unfoiled sample increased during storage for 6 h (Table 4). The increase in the a-value and decrease in chlorophyll content of the foiled and nonirradiated sample during storage were highly correlated ($r^2 = 0.94$). This indicated that the chlorophyll content of the solution affected greenness, resulting in an increase in the a-value. Irradiation at 20 kGy or 20 kGy with N_2-bubbling increased the a-value significantly ($P < 0.05$). However, the a-value of the foil-covered sample with nonirradiated control did not change during storage (Table 4), indicating that photooxidation in the foiled sample did not de-

TABLE 4
Hunter Color a-Value[a] of Photooxidized Linoleic Acid Solution (1% in methanol) Containing Chlorophyll b (3 ppm) by Gamma Irradiation

		Photooxidation time (h)[b]					
	Irradiation	0	1	2	4	6	SEM[c]
Unfoiled	0 kGy	–2.11e,y[d,e]	–1.91d,z	–1.71c,y	–1.36b,y	–1.01a,y	0.009
	20 kGy	0.01a,b,x	–0.02b,y	0.02a,x	0.01a,b,x	0.00a,b,x	0.007
	20/N_2[f]	0.02x	0.02x	0.03x	0.03x	0.02x	0.007
	SEM[g]	0.005	0.007	0.006	0.011	0.008	
Foiled[h]	0 kGy	–2.10b,z	–2.10b,y	–2.08a,b,z	–2.09a,b,z	–2.06a,y	0.006
	20 kGy	0.00y	0.00x	0.01y	0.00y	0.00x	0.006
	20/N_2[f]	0.02x	0.02x	0.09x	0.09x	0.01x	0.023
	SEM[g]	0.007	0.007	0.027	0.019	0.005	

[a]a-Value indicates redness: +a* is the red direction and –a* is the green direction.
[b]Light intensity was 3,300 lux at 25°C.
[c]SEM, pooled standard errors of the mean (n = 10).
[d]Different letters (a–e) within a row differ significantly at $P < 0.05$.
[e]Different letters (x–z) within foiled and unfoiled columns differ significantly at $P < 0.05$.
[f]Sample was bubbled with ultrapure N_2 during 20 kGy-irradiation.
[g]SEM: n = 6.
[h]Bottles were covered by aluminum foil to avoid photooxidation.

TABLE 5
Hunter Color b-Value[a] of Photooxidized Linoleic Acid Solution (1% in methanol) Containing Chlorophyll *b* (3 ppm) by Gamma Irradiation

		Photooxidation time (h)[b]					
	Irradiation	0	1	2	4	6	SEM[c]
Unfoiled	0 kGy	2.89a,x[d,e]	2.67b,x	2.51c,x	2.10d,x	1.68e,x	0.024
	20 kGy	–0.07y	–0.02y	–0.03y	–0.07y	–0.07y	0.029
	20/N_2[f]	–0.05y	–0.07y	–0.04y	–0.07y	–0.05y	0.029
	SEM[g]	0.024	0.022	0.035	0.027	0.026	
Foiled[h]	0 kGy	2.88x	2.90x	2.91x	2.91x	2.94x	0.028
	20 kGy	–0.06y	–0.07y	–0.06y	–0.06y	–0.06y	0.028
	20/N_2[f]	–0.07y	–0.06y	–0.23y	–0.03z	–0.06y	0.066
	SEM[g]	0.031	0.027	0.068	0.052	0.029	

[a]b-Value indicates yellowness: +b is the yellow direction and – b is the blue direction.
[b]Light intensity was 3,300 lux at 25°C.
[c]SEM, pooled standard errors of the mean (n = 10).
[d]Different letters (a–e) within a row differ significantly.
[e]Different letters (x–z) within foiled and unfoiled columns differ significantly.
[f]Sample was bubbled with ultrapure N_2 during 20 kGy-irradiation.
[g]SEM: n = 6.
[h]Bottles were covered by aluminum foil to avoid photooxidation.

velop because it was not exposed to light. The sample with 20 kGy irradiation had a higher a-value than the nonirradiated control.

The Hunter color b-value of the unfoiled, nonirradiated sample decreased with storage under photooxidation conditions (Table 5). Irradiation at 20 kGy, regardless of N_2-bubbling, showed significantly ($P < 0.05$) lower b-values in the unfoiled sample. The b-value of the foil-covered sample remained constant (Table 5).

The dose required for complete breakdown of added chlorophyll *b* (3 ppm) was 2.5 kGy or above in the methanol model system (Fig. 2). However, in real oil processing the irradiation dose requirement for complete chlorophyll removal will be higher. At a given irradiation dose, a substance dissolved in water or other polar liquids such as alcohols is damaged more than the pure, dry substances because of the high reactivity of intermediate radical species (24). When the irradiation dose is increased to achieve successful chlorophyll removal during oil processing, the development of lipid oxidation is a concern. However, the results in Table 2 indicate that irradiation even at 20 kGy does not result in lipid oxidation when the irradiation is carried out in the presence of N_2 gas.

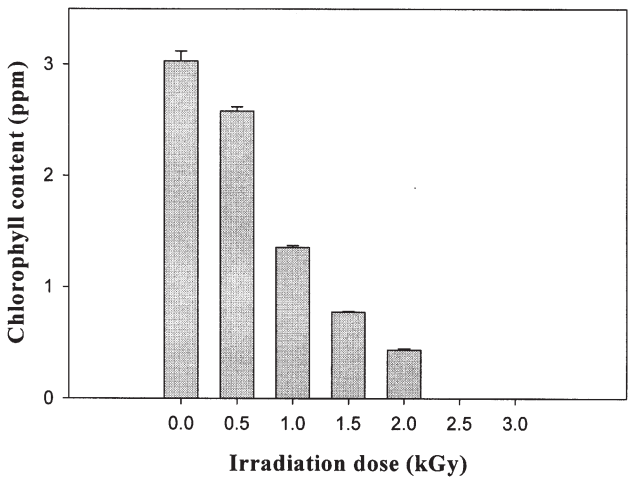

FIG. 2. Chlorophyll *b* content (ppm) in linoleic acid solution (1% in methanol) irradiated at various doses measured immediately after irradiation. Error bars refer to SD.

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REFERENCES

1. Lee, K.H., Photooxidation Inhibition Effect of Ascorbic Acid and Ascorbyl Palmitate, Ph.D. Thesis, Chungnam National University, Korea, 1998.
2. Daun, J.K., The Relationship Between Rapeseed Chlorophyll, Rapeseed Oil Chlorophyll, and Percentage Green Seeds, *J. Am. Oil Chem. Soc. 59*:15–18 (1982).
3. Tan, Y.A., C.L. Chong, and K.S. Low, Crude Palm Oil Characteristics and Chlorophyll Content, *J. Sci. Food Agric. 75*:281–288 (1997).
4. Usuki, R., T. Susuki, Y. Endo, and T. Kaneda, Residual Amounts of Chlorophylls and Pheophytins in Refined Edible Oils, *J. Am. Oil Chem. Soc. 61*:785–788 (1984).
5. Fakourelis, N., E.C. Lee, and D.B. Min, Effects of Chlorophyll and β-Carotene on the Oxidation Stability of Olive Oil, *J. Food Sci. 52*:234–235 (1987).
6. Gutiérrez-Rosales, F., J. Garrido-Fernández, L. Gallardo-Guerrero, B. Gandul-Rojas, and M.I. Mínguez-Mosquera, Action of Chlorophylls on the Stability of Virgin Olive Oil, *J. Am. Oil Chem. Soc. 69*:866–871.
7. Abraham, V., and J.M. deMan, Hydrogenation of Canola Oil as Affected by Chlorophyll, *Ibid. 63*:1185–1188 (1986).
8. Simpson, K.L., T.C. Lee, D.B. Rodriguez, and C.O. Chichester, Metabolism in Senescent and Stored Tissues, in *Chemistry and Biochemistry of Plant Pigments*, 2nd edn., edited by T.W. Goodwin, Academic Press, New York, 1976, Vol. 1, p. 780.
9. Wagenknecht, A.C., and F.A. Lee, Enzyme Action and Off-Flavor in Frozen Peas, *Food Res. 23*:25–28 (1958).

10. Olson, D.G., Irradiated Food, *Food Technol. 52*:56–62 (1998).
11. Lee, J.W., J.H. Kim, H.S. Yook, K.O. Kang, S.Y. Lee, H.J. Hwang, and M.W. Byun, Effects of Gamma Radiation on the Allergenic and Antigenic Properties of Milk Proteins, *J. Food Prot. 64*:272–276 (2001).
12. Ahn, H.J., H.S. Yook, M.S. Rhee, C.H. Lee, Y.J. Cho, and M.W. Byun, Application of Gamma Irradiation on Breakdown of Hazardous Volatile *N*-Nitrosamines, *J. Food Sci.,* In press.
13. Byun, M.W., J.W. Lee, H.S. Yook, K.H. Lee, and K.P. Kim, The Improvement of Color and Shelf Life of Ham by Gamma Irradiation, *J. Food Prot. 62*:1162–1166 (1999).
14. Byun M.W., K.H. Lee, D.H. Kim, J.H. Kim, H.S. Yook, and H.J. Ahn, Effects of Gamma Radiation on Sensory Qualities, Microbiological, and Chemical Properties of Salted and Fermented Squid, *Ibid. 63*:934–939 (2000).
15. Murano, P.S., Quality of Irradiated Foods, in *Food Irradiation: A Sourcebook*, edited by E.A. Murano, Iowa State University Press, Ames, 1995, pp. 63–87.
16. Jung, M.Y., K.H. Lee, and Y.S. Kim, Retinyl Palmitate Isomers in Skim Milk During Light Storage as Affected by Ascorbic Acid, *J. Food Sci. 63*:597–600 (1995).
17. *Official Methods of Analysis of AOAC International*, 14th edn., Association of Official Analytical Chemists International, Arlington, VA, 1984.
18. Schwartz, S.J., and T.V. Lorenzo, Chlorophyll Stability During Continuous Aseptic Processing and Storage, *J. Food Sci. 56*:1059–1062 (1991).
19. Folch, J., M. Lees, and G.M. Sloane-Stanley, A Simple Method for the Isolation and Purification of Total Lipides [*sic*] from Animal Tissues, *J. Biol. Chem. 226*:497–509 (1957).
20. *SAS User's Guide*, SAS Institute, Inc., Cary, NC, (1989).
21. Endo, Y., C.T. Thorsteinson, and J.K. Daun, Characterization of Chlorophyll Pigments Present in Canola Seed, Meal, and Oil, *J. Am. Oil Chem. Soc. 69*:564–568 (1992).
22. Ramirez-Niño, J., D. Mendoza, and V.M. Castano, A Comparative Study on the Effect of Gamma and UV Irradiation on the Optical Properties of Chlorophyll and Carotene, *Radiat. Meas. 29*:195–202 (1998).
23. Ahn, D.U., C. Jo, and D.G. Olson, Analysis of Volatile Components and the Sensory Characteristics of Irradiated Raw Pork, *Meat Sci. 54*:209–215 (2000).
24. World Health Organization, Food Irradiation, in *Safety and Nutritional Adequacy of Irradiated Food*, WHO, Geneva, 1994, pp. 5–13.

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