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Lipid and Cholesterol Oxidation, Color Changes, and Volatile Compounds Production in Irradiated Raw Pork Batters with Different Fat Content

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Abstract

An emulsion-type product was prepared to determine the effect of irradiation on lipid and cholesterol oxidation, color change, and volatile production in raw pork with different fat content. Lipid oxidation increased with an increase in fat content or irradiation dose. Irradiated batters had higher cholesterol oxides than did non-irradiated batters, and the major cholesterol oxides formed in irradiated pork batters were 7α- and 7β-hydroxycholesterol. Hunter color a- and b-values of raw pork batters were decreased by irradiation regardless of fat content. Irradiation significantly increased the amount of volatile compounds. Although lipid oxidation of high fat products (10 and 15% fat) was higher than that of low fat products (4%), high fat products did not always produce greater amount of volatile compounds in raw pork batters. In summary, irradiation increased lipid and cholesterol oxidation, and volatile compounds production, and had detrimental effects on the color of raw pork batter under aerobic conditions.

I. Introduction

The effect of irradiation in controlling microorganisms is well known. However, one of the major concerns with irradiating meat is its effect on meat quality. Ionizing radiation generates hydroxyl radicals that can accelerate lipid oxidation and produce color change and off-odor that significantly impact consumer acceptance (Ladikos and Lougovois, 1990). A less stable display of color was also noted for frozen boneless pork chops irradiated in aerobic packaging (Luchsinger et al. 1996). Patterson and Stevenson (1995) showed that dimethyltrisulfide is the most potent off-odor compound in irradiated raw chicken meat.

Several cholesterol oxidation products (COPs) are known to be cytotoxic, atherogenic, mutagenic and carcinogenic, and cholesterol and lipid oxidation were positively correlated in cooked pork. Lebovics et al. (1992) reported that the chemical changes induced

in cholesterol by ionizing radiation were similar in nature to those known to occur during autoxidation, and non-irradiated egg powder stored for 1 month contained approximately the same quantity of 7-hydroxycholesterols as a freshly produced egg powder irradiated at 1 kGy. Maerker and Jones (1991) reported that irradiation produced a large amount of 7-hydroxycholesterol by oxidizing cholesterol in liposomes. They showed that the ratio of 7-ketocholesterol/cholesterol 5,6-epoxides generated by irradiation was less than 1, much lower than that by autoxidation, and suggested the use of these unique product ratios as indicators of irradiation.

The objective of this study was to determine the influence of fat content on lipid oxidation, cholesterol oxides formation, color change, and the production of volatile compounds in irradiated raw pork batters.

II. Materials and methods

Sample Preparation

Lean pork and pork backfat were purchased from a local packing plant and ground twice through a 9-mm plate. An emulsion-type pork product was prepared using ground meat, NaCl (2% of meat weight), ice water (10% of meat weight), and pork backfat. Three levels of backfat (0, 10, or 20% of meat weight) were used to adjust fat content in meat batters. Raw ground pork without NaCl, water, or fat was also prepared to use as a no additive-added control. Batters (approximately 100 g each) were packaged in oxygen-impermeable nylon/polyethylene bags (9.3 ml $O_2/m^2/24$ h at 0C, Koch, Kansas City, MO) and stored overnight at 4°C to minimize changes before irradiation. The next morning, packaging bags were cut open and meat batters were irradiated at 0 or 4.5 kGy (average absorbed dose; dose rate was 107 kGy/min) using a linear accelerator (Circe IIIR, Thomson CSF Linac, France). Lipid oxidation, color, and volatile compounds of samples were analyzed 3 h after irradiation. Total fat content of patties was also determined.

Lipid Oxidation and Color Measurement

Lipid oxidation was determined using a spectrophotometric 2-thiobarbituric acid reactive substances (TBARS) method described by Ahn et al. (1998b). A CIE Lab color scale (Hunter Lab colorimeter, Hunter Associates Lab., Inc. Reston, VA) was used to measure the degree of lightness (L-value), redness (a-value) and yellowness (b-value) of samples. The surface color was measured at three different positions on each patty, and the mean values of the three measurements were used as the color values of each patty.

Cholesterol Oxidation Products (COPs)

A column chromatography method of Li et al. (1996) was used with some

modifications. Extracted fat (0.2 g) was loaded onto a glass column (12 mm x 30 cm) packed with silicic acid, Celite 545 (Sigma, St. Louis, MO) and $CaHPO_4$ (10:9:1, w/w). After washing with 50 ml of solvent I (hexane:ethylacetate = 9:1) and 60 ml of solvent II (hexane:ethylacetate = 4:1, v/v), the cholesterol oxidation products (COPs) were eluted with 40 ml of solvent III (acetone:ethylacetate:methanol = 10:10:1, v/v/v). The eluent was added with 50 μl as an external standard (5α-cholestane, 100 μg/ml), was dried under nitrogen, and was derivatized with 200 μl pyridine and 100 μl Sylon BFT [Bis(trimethylsilyl)trifluoroacetamide:Trimethylchlorosilane = 99:1, Supelco Park, Bellefonte, PA] in 80C waterbath for 1 h. Analysis of cholesterol oxides was performed with a gas chromatography (GC) (Hewlett Packard 6890) equipped with an on-column capillary injector and a flame ionization detector (FID).

Volatile Compounds Analysis

Precept II and Purge-and-Trap concentrator 3000 (Tekmar-Dohrmann, Cincinnati, OH) were used to purge and trap volatile compounds, and a GC (Hewlett Packard, Model 6890, Wilmington, DE) equipped with a flame ionization detector (FID) was used to analyze volatile compounds from meat batters. Meat (3 g) was sampled and analyzed using the conditions described in detail by Ahn et al. (1998b).

Statistical Analysis

Data were analyzed using the SAS program (SAS Institute 1989). Two-way analysis of variance (ANOVA) was used for TBARS values, and one-way ANOVA for COPs within irradiation treatment at 10% fat content and for volatile compounds within a fat content. Four replications were conducted and significance level used was $P<0.05$. The Student-Newman-Keul's multiple range test was used to compare differences among mean values ($P<0.05$). Mean values and standard errors of the mean (SEM) were reported.

III. Results and discussion

Lipid Oxidation

Fat contents of meat batters prepared with 0, 10%, and 20% added backfat were 4, 10, and 15%, respectively. Increase of fat content resulted in the increase of TBARS values in both non- or irradiated raw pork batters (Table 1). Added NaCl and ice water did not influence lipid oxidation of non-irradiated and irradiated meat. Wettasinghe and Shahidi (1996) studied the oxidative stability of comminuted lean pork with different salts extensively and found that NaCl at 0.60 to 1.2% had no prooxidative effect. However, irradiation increased the TBARS values of raw pork batters by about 1.5 times of the control (Table 1).

Table 1. Effect of fat content and irradiation on lipid oxidation of raw pork batters[1]

Treatment	Fat content				
	Raw pork[2]	4 %	10 %	15 %	SEM[3]
	---------- TBARS values (mg MDA/kg meat) ----------				
Non-irradiated	0.24^{by}	0.25^{by}	0.27^{by}	0.35^{ay}	0.014
Irradiated	0.36^{bx}	0.38^{bx}	0.48^{ax}	0.53^{ax}	0.024
SEM	0.019	0.013	0.029	0.012	

[1]Pork batters with NaCl (2%) and ice water (10%).

[2]Ground raw pork with no NaCl, water, or backfat.

[3]Standard errors of the mean: Among the means of different fat content within a same irradiation treatment. n=16.

[4]Standard errors of the mean: Among the means of different irradiation treatment within a same fat content. n=8.

[a,b]Different letters within a row are significantly different ($P<0.05$). n=16.

[x,y]Different letters within a column are significantly different ($P<0.05$). n=8.

Cholesterol Oxidation Products (COPs)

Table 2 indicates that the production of COPs in irradiated meat was about 4-fold that of the non-irradiated. Osada et al. (1993) studied the interaction of lipid oxidation and cholesterol oxidation in samples with various fats and found that lipid oxidation precedes cholesterol oxidation. Increased COPs production in irradiated raw pork batters with high TBARS suggested the presence of interaction between lipid and cholesterol oxidation. The amounts of α-epoxide, β-epoxide, and cholestantriol were not changed, but those of 7α-hydroxycholesterol, 7β-hydroxycholesterol, and total COPs were increased dramatically by irradiation. The COPs are not increased only by irradiation but by other processing such as heating.

Color

Irradiation had no effect on the color L-values of raw pork batters except for the samples with no fat added. Irradiation reduced color a- and b-values of raw pork products regardless of fat content (Figure 1). Irradiation adversely affected oxidative rancidity and color of pork chops in aerobic packaging, but the color of irradiated meat in vacuum packaging was redder and more stable than non-irradiated when stored at refrigerated and frozen temperature conditions (Luchsinger et al. 1996). Richards and Morrison (1971) observed the conversion of reduced myoglobin to metmyoglobin by irradiation. Our results also indicated that irradiation accelerated lipid oxidation and changed meat color to brown. Higher fat content decreased a-value but increased color b-value on raw pork batters (Figure

1).

Table 2. Cholesterol oxidation products (COPs) in non- or irradiated raw pork batter with 10% added backfat[1]

COPs	Non-irradiated	Irradiated	SEM[2]
	---------- COPs (μg/g) meat ----------		
7α-Hydroxycholesterol	17.6[b]	72.6[a]	12.66
7β-Hydroxycholestero	5.3[b]	70.5[a]	10.68
20-Hydroxycholesterol	nd[b]	4.7[a]	0.16
α-Epoxide	11.7	8.5	2.21
β-Epoxide	7.2	5.4	3.05
Cholestantriol	5.0	5.4	3.04
22-Ketocholesterol	nd	nd	-
25-Hydroxycholesterol	nd	nd	-
6-Ketocholesterol	nd	nd	-
7-Ketocholesterol	nd[b]	14.5[a]	1.24
Total cholesterol oxides	46.8[b]	181.6[a]	12.06

[1]Pork batters with NaCl (2%) and ice water (10%).

[2]Standard errors of the mean. n=8.

[a,b]Different letters within a row are significantly different ($P<0.05$). nd: not detected.

Volatile compounds

Several aldehydes, such as 2-methylpropanal, 2-methylbutanal and hexanal, and alcohols had high correlation coefficients (Table 3) with TBARS values of raw pork batters with 4% fat. Hexanal, a well-known indicator of oxidative deterioration in meat, was not found in non-irradiated meat with 4% fat (Table 4). However, the increase of fat content or irradiation dose rapidly increased hexanal content in raw pork batters.

Many unknown compounds with short GC retention time (less than 2 min) were produced from irradiated raw pork batters. Irradiation increased most of the volatile components in low fat batter (0% fat added). However, irradiation increased only 2-methyl butanal and hexanal in high fat batters (10 and 20% fat added). Although 1-heptene was considered a marker of irradiated cooked sausages (Ahn et al. 1998a), only a trace amount of 1-heptene was found in all irradiated raw meat batters. Morehouse and Ku (1990) reported that the detection of odd-number hydrocarbons (C15:0, C17:0, C17:1, and C17:2) using a gas chromatographic method enabled them to identify irradiated frog legs and confirmed by the

electron spin resonance method. It should be pointed out that only identified volatile compounds were used to calculate total volatile compounds in this study, and many unknown compounds from irradiated pork products were not included in total volatile calculations. If unknown volatile compounds are included, irradiated meat had higher ($P<0.05$) total volatile compounds than nonirradiated.

Unlike lipid oxidation, the increase of volatile compounds was not linear with fat content in raw pork batters. The amounts of volatile compounds produced in pork batter with 15% fat were smaller than those with 10% fat. Jo and Ahn (1998) also showed that the increase of fat content in an oil emulsion system decreased volatile compounds production in oil emulsion when determined using the purge-and-trap dynamic headspace method.

Table 3. Correlation coefficients between volatile compounds and TBARS of raw pork batter with different fat content

Volatile compounds	Ground pork	0% fat	10% fat	20% fat
1-Heptene	-	0.79	0.35	0.18
2-Methylpropanal	0.001	0.96	0.21	0.42
2-Methylbutanal	0.69	0.93	0.27	0.38
Pentanal	0.001	0.53	0.70	0.15
Hexanal	0.71	0.95	0.74	0.86
1-Butanol	0.008	0.92	0.31	0.34
Total volatile compounds	0.19	0.82	0.44	0.13

n = 8.

IV. Conclusion

Under aerobic conditions, irradiation increased lipid oxidation, cholesterol oxides formation, and volatile compounds production, and had detrimental effects on the color of raw pork batters. Because the amounts of volatile compounds in meat with the same degree of lipid oxidation vary significantly, fat content of compared samples should be taken into consideration before using volatile compounds content as a criterion for meat quality.

Table 4. Major volatile component of irradiated raw pork batter with different fat content

Volatile compounds	Non-irradiated					Irradiated				
	Raw[1]	0%[2]	10%	20%	SEM[3]	Raw[1]	0%[2]	10%	20%	SEM[3]
	area (pA x sec)									
1-Heptene	nd	nd	nd	nd	-	nd	0.8	0.8	0.5	0.23
2-Methylpropanal	11.0[a]	7.8[b]	8.3[b]	7.1[b]	0.68	11.4[a]	11.9[a]	9.4[b]	9.0[b]	0.52
2-Methylbutanal	nd[b]	nd[b]	16.8[a]	16.2[a]	1.18	16.8	23.0	22.9	24.8	2.81
Pentanal	nd[b]	nd[b]	14.4[a]	12.3[a]	1.22	0.8	3.5	6.2	6.3	2.50
Hexanal	nd	nd	5.4	6.3	1.49	0.8[b]	17.2[a]	20.1[a]	18.2[a]	0.83
1-Butanol	5.3[a]	2.0[b]	6.7[a]	0.8[b]	0.85	5.5	7.2	2.5	3.5	1.32
Total	49.3[c]	33.8[c]	150.1[a]	115.0[b]	7.74	72.5[b]	127.5[a]	124.0[a]	128.3[a]	9.52

[1]Ground raw pork. [2]Raw pork batter. [3]Standard errors of the mean. n=32.

[a,b,c]Different letters within a row of same irradiation dose are significantly different ($P<0.05$).

V. References

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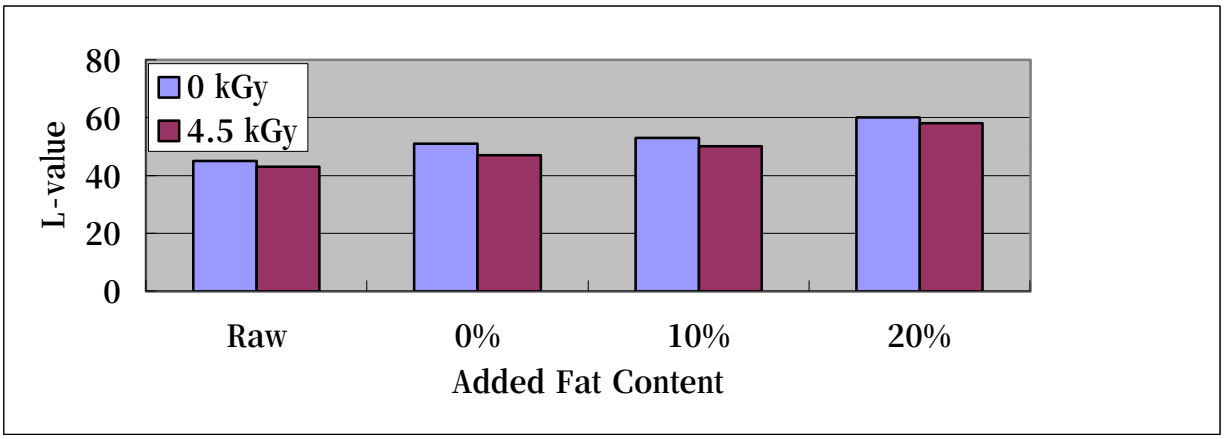
Figure 1. Effect of fat content and irradiation on color changes of raw pork batters. [a,b.c,d]Different letters among added fat content within a same irradiation dose are significantly different ($P<0.05$). [x,y]Different letters between irradiation doses within a same added fat content are significantly different ($P<0.05$).

Figure 1.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Raw
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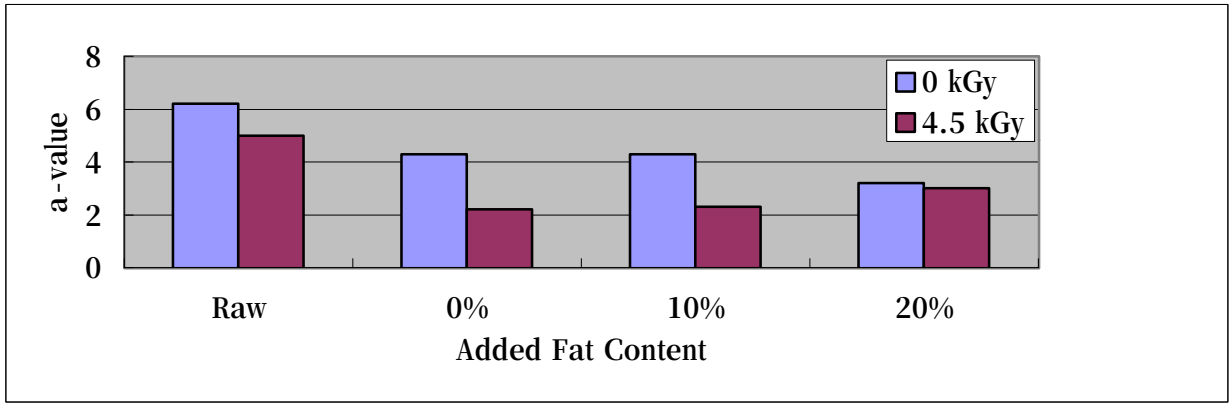
Added Fat Content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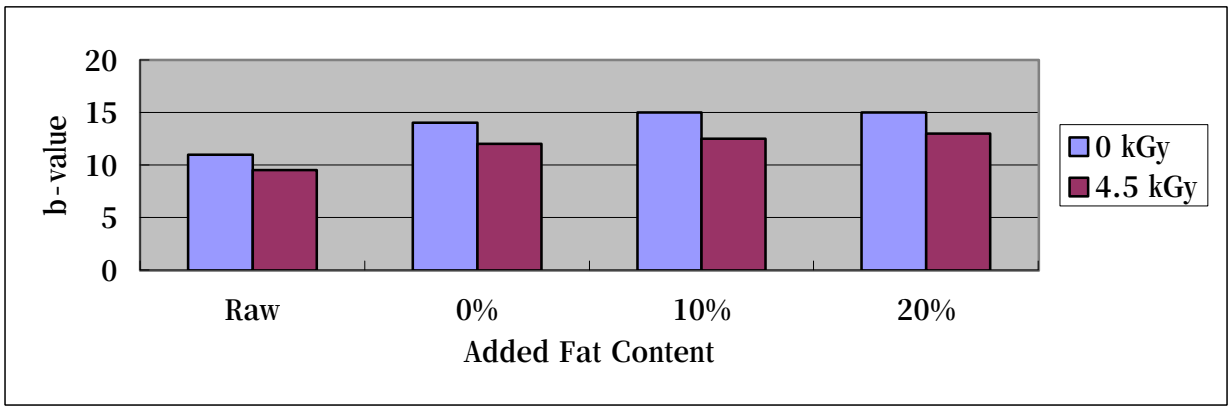
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