Lipid and Fatty Acid Composition of Rabbit Meat as Affected by Gamma Irradiation

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Abstract: The present study was conducted to investigate the changes in lipid and fatty acid composition of gamma irradiated rabbit meat. Samples of rabbit meat were aerobically packaged and exposed to gamma irradiation at doses of 0 and 3 kGy at room temperature and 0 and 7 kGy at the frozen state. Lipids were extracted and isolated as neutral lipids and phospholipids classes, then the individual fractions of these classes were separated by thin layer chromatography. Furthermore, the observed neutral lipids and total phospholipids as well as the major phospholipids fractions, phosphatidylcholine and phosphatidylethanolamine, were converted to fatty acid methyl esters and analyzed by gas chromatography. The obtained results revealed that irradiation of rabbit meat samples had no significant effect on their contents of total lipids and the observed fractions for neutral and phospholipid classes were similar in lipids separated from irradiated and non-irradiated rabbit meat. Moreover, irradiation treatments showed no significant effects on the contents of triglycerides, however, significant decreases in the contents of cholesterol and phospholipids were observed. The fatty acids of neutral lipid class showed no significant changes due to irradiation of rabbit meat, while significant decreases, but of minor interest, were observed in the polyunsaturated fatty acids of total phospholipids and their major fractions. The observed results demonstrate that irradiation of rabbit meat samples at doses recommended for irradiation of poultry had no adverse effects on their lipid and fatty acid composition and the observed decreases in the contents of cholesterol or phospholipids and their polyunsaturated fatty acids were of minor interest.

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1. Introduction

The consumption of rabbit meat showed a considerable increase in the world. In the last 50 years, world rabbit meat production has increased by 2.5 fold reaching 1.6 million tons in 2009. Rabbit meat is routinely consumed in many European and north African countries, including Egypt, and its production plays an important role in the national economy of most of these countries (FAOSTAT,2010 and Dalle Zotte and Szendrő, 2011).In comparison to meat of other animal species, rabbit meat is appreciated for its nutritional and dietetic properties as it is lean, rich in protein of high biological value, besides being low in cholesterol and sodium and rich in phosphorus and magnesium (Hermida *et al.* , 2006 and Lukefahr *et al.*, 1989). It also has a high digestibility and can be fortified with bioactive components to obtain functional meat (Dalle Zotte and Szendrő, 2011). However, as with all other meats, rabbit meat was found to be contaminated with different microorganisms including foodborne pathogens (Badr, 2004 and Rodríguez-Calleja *et al*., 2004, 2005).

Irradiation processing is a safe and effective method for preservation of meats, being one of the best technologies to ensure their microbiological safety. Due to its effectiveness in treating packaged food, thereby minimizing the possibility of cross-contamination prior to consumer use, most food safety officials and scientists view irradiation as an effective Critical Control Point in a Hazard Analysis and Critical Control Points (HACCP) System for meat and poultry processing (Ahn *et al.*, 2006 and Satin, 2002). The application of irradiation treatment was found to be effective to control bacterial pathogens in rabbit meat with extending their refrigerated market life (Badr, 2004).

There is increasing interest in the fat content as well as lipid classes and fatty acid composition of edible meat and fat of domestic animals owing to its importance for quality traits of meat such as nutritional value and flavor (Kanatt *et al.*, 2006). In contrast to other meats, there is a lack of comparable studies on the lipid composition and individual fatty acid patterns from the neutral and polar lipids of rabbit meat as affected by irradiation treatment. The maximum dose approved for irradiation of poultry by the USDA-FDA, 2003 is 3 kGy, however, a dose of 7 kGy was approved for irradiation of frozen poultry in other countries (IAEA, 1998). Therefore, the present study was undertaken to provide a detailed analysis of lipid and fatty acid composition of rabbit meat irradiated at dose of 3 kGy at room temperature and 7 kGy in the frozen state as compared with the non-irradiated rabbit meat samples.

2. Materials and methods

Preparation of samples:

Eight New Zealand white rabbits (four males and

four females) at live weights of 2.1 - 2.3 Kg were slaughtered, then the head, viscera, and skin were immediately removed and the meat was separated from the bone. The separated meat was minced and well mixed to form a composed of homogenate of the meat. Then the minced rabbit meat was divided into 20 samples of 500 g which were aerobically packaged in polyethylene pouches. The obtained pouches were sealed by heat and subdivided into two portions. Samples of the first portion were used for irradiation at room temperature, while samples of the other portion were frozen stored at -18 ˚C for 2 days to be irradiated at the frozen state.

Irradiation of samples:

Packaged rabbit meat samples of the first portion were exposed to gamma irradiation at room temperature at doses of 0 and 3 kGy, while the frozen samples were irradiated at the frozen state at doses of 0 and 7 kGy. Irradiation of samples was carried out using an experimental Co-60 source (providing a dose rate of 2.958 kGy /h) at the National Center for Radiation Research and Technology, Nasr City, Cairo, Egypt.

Extraction and fractionation of lipids:

Total lipids were extracted from the rabbit meat samples with chloroform/ methanol (2:1 v/v) according to the method of Bligh and Dyer (1959) and the extracts were dried under vacuum by a rotary evaporator. The obtained lipids were weighed and expressed in g/100 g of meat (on dry weigh basis after the determination of moisture content of the meat samples according to AOAC 2000 Official Methods). The contents of total cholesterol were determined in the extracted lipids by direct saponification and gas chromatography as described by Hwang *et al*. (2003) and expressed as % of total lipids. Fractionation of the total lipids was carried out using a silicic acid – celite column and successive elution with chloroform, acetone, and methanol according to Christie (1982). Through the applied chromatographic system, three separate fractions including neutral lipids, glycolipids, and phospholipids were obtained and the amounts of the required neutral and phospholipids fractions were calculated and expressed as percentages of the total lipids. Triglycerides were separated from the neutral lipid fraction by thin layer chromatography as described by Cambero *et al.* (1991a) and their amounts were expressed as percentages of the total neutral lipids. Fractions of phospholipids were separated by thin layer chromatography according to Christie (1982) and their amounts were determined based on their phosphorus content which was determined as described by Alexander and Griffiths (1993). The separated fractions were expressed as percentages of total phospholipids.

Fatty acid composition:

Fatty acid methyl esters were prepared for neutral lipids following the method of Morrison and Smith (1964), while prepared for phospholipids according to Berry *et al.* (1965). The analysis of fatty acid methyl esters was then accomplished using a 6890 Hewlett Packard gas chromatograph equipped with flam ionization detector. The derivatives were fractionated by a capillary column (30m x 0.32mm internal diameter), HP-INNOWAX - cross linked polyethylene glycol. The injector and detector temperatures were 260 ˚C and 275 ˚C, respectively. The temperature program was 150 ˚C for 1 min, 150-235˚C at 17˚C /min, and 235˚C – 245˚C at 1˚C/min at which the oven was held for a maximum of 5 min. The flow rate of carrier gas (nitrogen) was set at 1.5 ml/min. The peak areas and retention times were measured using a Hewlett Packard 3392A integrator. Identification of fatty acid methyl esters was based on comparing their relative retention times with those of reference fatty acid methyl esters.

Statistical analysis:

Determinations were carried out using three separate pouches of samples per treatment, while analyses were replicated twice per each pouch replicate. Data were statistically analyzed by using the generalized linear model procedure of the SAS software (SAS Institute, 1998), while the differences among means (p<0.05) were compared by using Duncan's multiple range test.

3. Results and discussion

Total lipids and lipid classes of rabbit meat as affected by gamma irradiation:

As shown in Table 1, non-irradiated rabbit meat had a total lipid contents of 31.05% (on dry weigh basis), in which their classes of neutral lipids and phospholipids reached 85.32% and 10.92%, respectively, while total cholesterol content reached 0.27 % of the total lipids. Analysis of the neutral lipid extracts by thin layer chromatography revealed the presence of ten spots, however, triglycerides were only considered in this study and their contents reached 90.96% of the total neutral lipids. Meanwhile, fractionation of phospholipids by thin layer chromatography revealed the presence of seven phospholipids in lipids of the non-irradiated meat samples and they were characterized as phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, lysophosphatidylcholine, Sphingomyelin, lysophosphatidylethanolamine, and cardiolipin. Phosphatidylcholine was the major phospholipid constituting 57.70% of the total

phospholipids followed by phosphatidylethanolamine (21.85%) and cardiolipin (6.61%). Phosphatidylserine and lysophosphatidylcholine represented 6.98% of the total phospholipids as a whole because it was not possible to achieve a definitive separation. Similarly, sphingomyelin and lysophosphatidylethanolamine could not be achieved separately and they constituted 6.82% of the total phospholipids as a whole. Similar results were observed for rabbit lipids (Cambero *et al*., 1991a, b and Alasnier *et al.*, 1996).

Table 1 further shows that the applied irradiation treatments had no significant effect on the contents of total lipids in the rabbit meat samples. Furthermore, fractionation of the observed classes by thin layer chromatography showed that the observed fractions for both neutral and phospholipids classes were similar in lipids separated from all samples and there were no fractions in the patterns of lipids of the irradiated meat that do not observed in those of the control meat samples. Irradiation of rabbit meat at the different applied doses had no significant effect on the contents of triglycerides in the neutral lipid class, but significantly decreased its cholesterol contents. However, the content of cholesterol is low and the observed decrease, although statistically significant, may be of no real importance quantitatively (Table 1). The oxidation of cholesterol due to radiation processing of meats was reported (Kanatt *et al*., 2006 and Nam *et al.*, 2001). On the other hand, irradiation treatments induced slight, but significant, decreases in the total phospholipids as well as the contents of Phosphatidylserine + lysophosphatidylcholine and sphingomyelin + lysophosphatidylethanolamine fractions of the phospholipids, but the decrease was lower when meat samples were irradiated at the frozen state. However, these phospholipids fractions present in small amounts as compared with phosphatidylcholine and phosphatidylethanolamie fractions which account for 79.55% of the total phospholipids and constitute the major fractions of muscle phospholipids. Similar results were observed by Bakalivanova *et al.* (2009) and the decrease in sphingomyelin due to irradiation treatment was reported by Kanatt *et al*. (2006).

Fatty acids profile of neutral lipids separated from non-irradiated and irradiated rabbit meat:

Gas chromatographic analysis of fatty acid methyl esters from neutral lipids of non-irradiated rabbit meat revealed that palmitic acid was the major saturated fatty acid reaching 36.95% followed by stearic (12.94%) and myristic (4.41%), while oleic acid was the major unsaturated fatty acid (23.97%) followed by linoleic (13.99%) and palmitoleic (3.98%) as shown in Table 2. These results are in good agreement with the findings of Cambero *et al*. (1991a). Neutral lipids of the irradiated rabbit meat samples showed a fatty acid patterns similar to those of the non-irradiated control samples indicating that irradiation of rabbit meat samples at the doses applied in this study had no significant effects on the fatty acid composition of their neutral lipid class. No new fatty acids were observed in the fatty acids profile of the neutral lipid class due to irradiation of rabbit meat including the unidentified fatty acids (which appeared to be identical from comparisons in both irradiated and non-irradiated samples). Similar results were observed by Rady *et al.* (1988) and Maxwell and Rady (1989).

Fatty acids profile of phospholipids separated from non-irradiated and irradiated rabbit meat:

Table 3 represents the fatty acids composition of total phospholipids separated from non-irradiated and irradiated rabbit meat. The major saturated fatty acids in the total phospholipids of the control non-irradiated rabbit meat were palmitic (29.98%) and stearic (17.52%), while the major unsaturated fatty acids were found to be linoleic (19.58%), oleic (16.48%), arachidonic (9.88), and linolenic (1.78%). As shown, a higher content of polyunsaturated fatty acids characterizes the fatty acids profile of the total phospholipids and these results agree with those observed by Cambero *et al*. (1991b). Subjecting rabbit meat to gamma irradiation at doses used in this study induced slight, but statistically significant, decreases in the contents of arachidonic, linolenic, and linoleic acids accompanied by slight, but significant, increase in the contents of oleic and palmitic acids. The observed decreases were minimized in the frozen irradiated samples. Similar findings were reported by Kanatt *et al*. (2006). It was illustrated that one possible pathway to account for the decomposition of the polar polyenoic fatty acids in the presence of ionizing radiation would be the loss of some double bond character in these compounds and the subsequent formation of monoenoic fatty acids (Maxwell and Rady, 1989). The fatty acid composition of total phospholipids depends on the relative proportion of the individual phospholipids fractions in the total phospholipids and on the fatty acid composition of the individual fraction (Alasnier and Gandemer, 1998).Chromatographic analysis was also performed for the fatty acid methyl esters of the major phospholipids fractions, phosphatidylcholine and phosphatidylethanolamine (Tables 4 &5). Generally, phosphatidylcholine contained higher saturated fatty acids than phosphatidylethanolamine, which showed a lower percentage of palmitic acid accompanied by a higher percentage of arachidonic acid. The changes in fatty acids profiles of these major phospholipids fractions due to irradiation of rabbit meat were similar

to those observed in the fatty acids of the total phospholipids showing the same observed phenomenon. As with neutral lipid class, no new fatty acids were observed in the fatty acid profiles of the total phospholipids and their major fractions due to irradiation of the rabbit meat. It could be concluded that irradiation of rabbit meat samples at doses recommended for irradiation of poultry had no adverse effects on their lipid and fatty acid composition. Although significant decreases were observed in the contents of cholesterol and phospholipids as well as the polyunsaturated fatty acids of phospholipids, the observed decreases were of minor interest.

Table 1: Lipid classes of rabbit meat as affected by gamma irradiation

Components	% / Irradiation dose			
	At room temperature		At frozen state	
	0.0 kGy	3.0 kGy	0.0 kGy	7.0 kGy
Total lipids (% on dry weigh)	31.05±1.07	31.03±1.23	31.01±1.13	31.07±1.20
Neutral lipids (% of total lipids)	85.32±0.16	85.17±0.22	85.32±0.13	85.21±0.19
Triglycerides (% of total neutral lipids)	90.96±0.20	90.98±0.12	90.97±0.16	90.95±0.26
Total cholesterol (% of total lipids)	0.27±0.01a	0.20±0.02c	0.27±0.02a	0.23±0.01b
Phospholipids (% of total lipids)	10.92±0.18a	8.89±0.24c	10.92±0.20a	9.91±0.29b
Phosphatidylcholine (% of total phospholipids)	57.70±0.29	57.74±0.17	57.71±0.24	57.68±0.32
Phosphatidylethanolamine (% of total phospholipids)	21.85±0.20	21.77±0.17	21.85±0.22	21.82±0.31
Cardiolipin (% of total phospholipids)	6.61±0.14	6.55±0.17	6.61±0.09	6.60±0.15
PS+LPC (% of total phospholipids)	6.98±0.09a	6.87±0.06c	6.98±0.07a	6.91±0.09b
S+LPE (% of total phospholipids)	6.82±0.08a	5.03±0.11c	6.82±0.09a	5.49±0.10b

Means with a different letter within each component are different significantly ($P< 0.05$).
PS: Phosphatidylserine, LPC: Lisophosphatidylcholine, S: Sphingomylin, LPE: Lisophophatidylethanolamine.

Table 2: Fatty acid composition of neutral lipids separated from irradiated and non-irradiated rabbit meat

Fatty acids	% / Irradiation dose			
	At room temperature		At frozen state	
	0.0 kGy	3.0 kGy	0.0 kGy	7.0 kGy
10:0	0.55±0.03	0.55±0.01	0.54±0.01	0.54±0.02
12:0	0.89±0.02	0.88±0.02	0.89±0.04	0.89±0.03
14:0	4.41±0.05	4.43±0.03	4.42±0.04	4.42±0.06
15:0	0.58±0.01	0.58±0.02	0.57±0.02	0.57±0.03
Unidentified	0.11±0.01	0.11±0.02	0.11±0.01	0.11±0.01
16:0	36.95±0.03	36.96±0.07	36.96±0.05	36.95±0.06
16:1	3.98±0.02	3.95±0.04	3.97±0.03	3.98±0.03
17:0	0.64±0.06	0.64±0.04	0.64±0.05	0.64±0.07
17:1	0.29±0.01	0.28±0.03	0.29±0.02	0.29±0.02
Unidentified	0.06±0.01	0.06±0.01	0.05±0.01	0.06±0.02
18:0	12.94 ±0.06	12.97±0.03	12.95±0.06	12.96±0.07
18:1	23.97 ±0.04	23.98±0.02	23.97±0.03	23.98±0.04
18:2	13.99±0.03	13.97±0.03	14.00±0.02	13.98±0.04
18:3	0.09±0.01	0.09±0.02	0.09±0.01	0.08±0.01
20:0	0.09±0.02a	0.08±0.01c	0.09±0.01a	0.09±0.01b
20:1	0.46±0.02	0.47±0.03	0.46±0.02	0.46±0.01
Total SFA	57.05±0.24	57.09±0.31	57.06±0.27	57.06±0.23
Total UFA	42.78±0.19	42.74±0.26	42.78±0.21	42.77±0.28
Total MUFA	28.70±0.20	28.68±0.23	28.69±0.19	28.71±0.25
Total PUFA	14.08±0.10	14.06±0.09	14.09±0.11	14.06±0.07
TotalUnidentified	0.17±0.01	0.17±0.02	0.16±0.01	0.17±0.02

Means with a different letter within each fatty acid are different significantly ($P< 0.05$).
SFA: Saturated fatty acids. UFA: Unsaturated fatty acids. MUFA: Monounsaturated fatty acids. PUFA: Polyunsaturated fatty acids.

Table 3: Fatty acid composition of total phospholipids separated from irradiated and non-irradiated rabbit meat

Fatty acids	% / Irradiation dose			
	At room temperature		At frozen state	
	0.0 kGy	3.0 kGy	0.0 kGy	7.0 kGy
14:0	0.58±0.01	0.59±0.01	0.57±0.02	0.58±0.01
15:0	0.25±0.01	0.24±0.02	0.24±0.02	0.24±0.01
Unidentified	0.12±0.01	0.12±0.01	0.13±0.01	0.13±0.01
16:0	29.98±0.11c	30.40±0.14a	30.01±0.09c	30.27±0.13b
16:1	1.65±0.03	1.71±0.01	1.66±0.02	1.67±0.03
17:0	0.72±0.01	0.72±0.01	0.71±0.02	0.71±0.01
17:1	0.45±0.01	0.45±0.01	0.45±0.01	0.45±0.01
18:0	17.52±0.04	17.48±0.07	17.53±0.06	17.50±0.08
18:1	16.48±0.03b	16.65±0.04a	16.50±0.02b	16.60±0.04a
18:2	19.58±0.03a	19.50±0.03b	19.57±0.03a	19.51±0.02b
18:3	1.78±0.01a	1.68±0.02b	1.77±0.01a	1.71±0.01b
Unidentified	0.16±0.01	0.16±0.01	0.15±0.01	0.16±0.01
20:0	0.38±0.03	0.40±0.03	0.37±0.02	0.38±0.03
20:1	0.27±0.01	0.28±0.03	0.27±0.01	0.26±0.01
Unidentified	0.20±0.01	0.19±0.01	0.20±0.01	0.20±0.01
20:4	9.88±0.02a	9.43±0.06c	9.87±0.03a	9.63±0.05b
Total SFA	49.43±0.19	49.83±0.27	49.43±0.23	49.68 ±0.18
Total UFA	50.09±0.22	49.70±0.28	50.09±0.15	49.83±0.26
Total MUFA	18.85±0.16	19.09±0.21	18.88±0.13	18.98±0.18
PUFA Total	31.24±0.13	30.61±0.23	31.21±0.17	30.85±0.15
Total Unidentified	0.48±0.01	0.47±0.01	0.48±0.01	0.49±0.01

Means with a different letter within each fatty acid are different significantly ($P< 0.05$).
FA: Saturated fatty acids. UFA: Unsaturated fatty acids. MUFA: Monounsaturated fatty acids.
PUFA: Polyunsaturated fatty acids.

Table 4: Fatty acid composition of the phosphatidylcholine separated from lipids of irradiated and non-irradiated rabbit meat

Fatty acids	% / Irradiation dose			
	At room temperature		At frozen state	
	0.0 kGy	3.0 kGy	0.0 kGy	7.0 kGy
14:0	0.51±0.02	0.52±0.01	0.51±0.01	0.51±0.02
15:0	0.55±0.01	0.55±0.01	0.54±0.02	0.56±0.01
Unidentified	0.16±0.02	0.16±0.02	0.16±0.02	0.16±0.02
16:0	38.96±0.09	39.10±0.10	38.97±0.11	38.99±0.09
16:1	2.58±0.07	2.59±0.06	2.60±0.06	2.63±0.07
17:0	0.93±0.02	0.92±0.01	0.91±0.02	0.91±0.02
17:1	0.94±0.02	0.94±0.03	0.94±0.03	0.94±0.02
18:0	12.46±0.03	12.40±0.06	12.45±0.04	12.44±0.04
18:1	22.84±0.02b	23.07±0.03a	22.85±0.03b	22.97±0.04a
18:2	18.77±0.04a	18.60±0.02b	18.78±0.03a	18.69±0.04b
18:3	0.74±0.02a	0.61±0.04b	0.74±0.03a	0.65±0.02b
Unidentified	0.19±0.01	0.19±0.01	0.18±0.01	0.18±0.02
20:0	0.09±0.02	0.08±0.02	0.09±0.01	0.09±0.02
20:1	0.28±0.04	0.27±0.03	0.28±0.03	0.28±0.02
Total SFA	53.50±0.11	53.57±0.23	53.47±0.18	53.50±0.13
Total UFA	46.15±0.16	46.08±0.11	46.19±0.21	46.16±0.15
Total MUFA	26.64±0.08	26.87±0.13	26.67±0.21	26.82±0.17
Total PUFA	19.51±0.10	19.21±0.19	19.52±0.09	19.34±0.13
Total Unidentified	0.35±0.03	0.35±0.03	0.34±0.02	0.34±0.03

Means with a different letter within each fatty acid are different significantly ($P< 0.05$).
SFA: Saturated fatty acids. UFA: Unsaturated fatty acids. MUFA: Monounsaturated fatty acids.
PUFA: Polyunsaturated fatty acids.

Table 5: Fatty acid composition of the phosphatidylethanolamine separated from lipids of irradiated and non-irradiated rabbit meat

Fatty acids	% / Irradiation dose			
	At room temperature		At frozen state	
	0.0 kGy	3.0 kGy	0.0 kGy	7.0 kGy
14:0	0.77±0.01	0.79±0.03	0.77±0.03	0.78±0.03
15:0	0.26±0.01	0.26±0.01	0.25±0.01	0.25±0.02
Unidentified	0.16±0.02	0.16±0.02	0.16±0.02	0.16±0.02
16:0	29.50±0.06	29.60±0.04	29.52±0.04	29.61±0.05
16:1	2.36±0.04	2.39±0.02	2.34±0.02	2.36±0.03
17:0	0.79±0.01	0.79±0.01	0.79±0.01	0.79±0.01
17:1	0.64±0.01	0.64±0.03	0.64±0.03	0.64±0.02
18:0	15.67±0.03	15.69±0.04	15.66±0.01	15.66±0.04
18:1	22.43±0.03b	22.76±0.05a	22.45±0.02b	22.70±0.04a
18:2	20.46±0.04a	20.28±0.06b	20.46±0.03a	20.35±0.05b
18:3	1.31±0.01a	1.18±0.03b	1.32±0.01a	1.24±0.02b
Unidentified	0.11±0.02	0.11±0.02	0.11±0.01	0.11±0.01
20:0	0.29±0.03	0.30±0.03	0.28±0.02	0.28±0.02
20:1	0.26±0.04	0.27±0.04	0.25±0.03	0.25±0.04
20:4	4.99±0.03a	4.78±0.05b	5.00±0.02a	4.82±0.03b
Total SFA	47.28±0.18	47.43±0.26	47.27±0.14	47.37±0.22
Total UFA	52.45±0.23	52.30±0.19	52.46±0.13	52.36±0.25
Total MUFA	25.69±0.09b	26.06±0.09a	25.68±0.12b	25.95±0.10a
Total PUFA	26.76±0.07a	26.24±0.10c	26.78±0.10a	26.41±0.13b
Total Unidentified	0.27±0.02	0.27±0.03	0.27±0.01	0.27±0.01

Means with a different letter within each fatty acid are different significantly ($P< 0.05$).
SFA: Saturated fatty acids. UFA: Unsaturated fatty acids. MUFA: Monounsaturated fatty acids. PUFA: Polyunsaturated fatty acids.

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