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Food Chemistry 97 (2006) 80–86
Food Chemistry
www.elsevier.com/locate/foodchem

Effect of radiation processing of lamb meat on its lipids

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Received 26 April 2004; received in revised form 16 March 2005; accepted 16 March 2005

Abstract

The changes in lipid content of radiation processed lamb meat were investigated. Meat from the rib region of lamb had almost double the lipid content found in the leg region. No differences were observed in the lipid profile of the radiation processed and non-irradiated meat. TLC detected all the five major classes of lipids in both irradiated and non-irradiated meat. However, there was a radiation dose dependent decrease in phospholipid (PL) and cholesterol content, while an increase was observed in the free fatty acid content. The predominant fatty acids present were oleic acid, palmitic acid and stearic acid. There was a significant decrease in the ratio of PUFA/SFA of phospholipids on irradiation. Lipid peroxidation measured in terms of thiobarbituric acid-reactive substances (TBARS) increased on irradiation and chilled storage.

Keywords: Lipid; Irradiation; Lamb; Fatty acid composition; Phospholipids

1. Introduction

Radiation processing of meat is recognised as a safe and effective method among the existing technologies for meat preservation. It is one of the best emerging technologies to ensure the microbiological safety of meat. In developed as well as developing countries, an increase in the incidence of food-borne diseases, especially of animal origin, has been noticed. Radiation processing of fresh meat extends the shelf life and protects the host against pathogenic bacteria. In the USA, the US FDA approved radiation processing of meat in 1997 and the USDA in 1999 (USDA, 1999). Radiation processed ground beef and poultry have appeared since on the market shelves of several states in the US. Meat and meat products pasteurised by radiation have been successfully marketed in Belgium, France, China, Indonesia, the Netherlands, South Africa and Thailand for a number of years (Diehl, 1995).

In India, the Ministry of Health and Family Welfare approved in 1998 meat and meat products for radiation preservation under Prevention of Food Adulteration Rules. However, one of the major concerns with any processing of meat, including radiation processing, is that these processes enhance lipid peroxidation and thereby affect sensory attributes. The susceptibility of irradiated meat to oxidative rancidity is related to the nature, proportion, degree of saturation in fatty acids and the composition of phospholipids in cell membranes (Ahn, Jo, Du, Olson, & Nam, 2000).

Several factors affect consumer decisions to purchase meat, but an important one is the perception of quality. The perceived ''healthiness'' of a food is becoming the key issue for consumers and in the case of meat; this is largely related to its fat content and its fatty acid composition. Fat content of meat is a primary factor that determines product shelf-life/storage stability of meat and meat products. The class of lipid and fatty acid composition of meat is also important for quality traits of meat such as nutritional value, flavour, and textural properties. It varies widely depending on species, degree of cutting and trimming, nature of cooking, processing

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doi:10.1016/j.foodchem.2005.03.024

and on the meat preservation techniques employed (Rhee, 1992). There are some studies on lipid peroxidation of irradiated beef, pork and poultry (Ahn et al., 2000; Lescano, Narvaiz, Kairiyama, & Kaupert, 1991; Luchsinger et al., 1997); however, the information on changes in lipids of radiation processed lamb meat is lacking. Therefore, studies were undertaken to examine the changes in lipids of lamb meat due to radiation processing.

2. Materials and methods

2.1. Chemicals

Phosphatidyl choline (PC), phosphatidyl ethanolamine (PE), phosphatidyl serine (PS), phosphatidyl inositol (PI), sphingomylein, cholesterol, oleic acid, tripalmitin and 1,2 dipalmitin were purchased from Sigma Chemicals Co. (St. Louis, MO). All other reagents were of analytical grade.

2.2. Preparation of meat samples

The leg and rib region of lamb meat was procured 4 h after slaughter. The meat was trimmed of all external fat and ligaments. It was then minced and packaged in polythene bags (700 gauge; WVTR 0.4 g/m^2/day; OTR 1800 ml/m^2/day).

2.3. Irradiation

The packed samples were irradiated under chilled condition (0–3 °C) in a Food Package Irradiator (Nordion Intl. Inc., Canada) with a ^{60}Co source at a dose rate of 3 kGy/h. The samples received minimal doses of 2.5 or 5 kGy with an overdose ratio of 1.3. Dosimetry was performed by cerric–cerrous dosimeter calibrated against Fricke's dosimeter. Dosimetry intercomparison was carried out with National Standards established by Radiological Physics and Advisory Division (RP&AD), Bhabha Atomic Research Centre (BARC), Mumbai, India. Non-irradiated lots served as controls.

2.4. Extraction of lipid

Lipids were extracted from the minced tissue by the dry column method of Marmer and Maxwell (1981). Lipids were eluted from the column with a mixture of dichloromethane/methanol (90:10 v/v). For separation of the total lipid into neutral and polar fractions, sequential elution was carried out. Neutral lipids free of polar lipids were eluted first with dichloromethane, followed by elution of polar lipids with a mixture of dichloromethane/methanol (90:10, v/v). Lipids were also extracted by the method of Folch, Lees, and Sloane-Stanley (1957) using chloroform:methanol (2:1, v/v) for validation of dry extraction procedure. Phospholipid, cholesterol, and free fatty acid content were determined colorimetrically (Duncombe, 1963; John & Stewart, 1980; Zlatkis, Zak, & Boyle, 1953).

2.5. Thin layer chromatography (TLC) of lipids

Total lipids were separated into the various classes by silica gel TLC. The solvent system used was petroleum ether-diethyl ether-acetic acid (83:17:1 v/v) (Kanatt, Paul, D'Souza, & Thomas, 1997). For separation of phospholipids into various classes, the solvent system used was ethyl acetate-propane-2-ol-chloroform-methanol-aqueous potassium chloride (25:25:25:10:9 v/v).

2.6. Preparation of fatty acid methyl esters (FAME)

To determine the fatty acid composition, the lipids were hydrolysed and derivatised with diazomethane. Briefly, lipid was refluxed with methanolic KOH (2 N) for 2 h. It was then extracted with diethylether to obtain the hydrolysed fatty acid. Diazomethane was added to the samples and kept overnight for transesterification to obtain fatty acid methyl esters.

2.7. GC–MS analysis

GC quantification of fatty acids was carried out on a Shimadzu QP-5050A gas chromatography/mass spectrometry instrument. The instrument was equipped with a GC-17A gas chromatograph and a DB-1 (Dimethyl polysiloxane, J&W Scientific) capillary column (length 30 m, i.d. 0.25 mm, and film 0.25 μm) The operating conditions were: column temperature programmed from 60 to 200 °C at the rate of 10 °C/min held at final temperature for 20 min. Injector and interface temperatures were maintained at 210 and 230 °C, respectively. Helium was used as carrier gas. Ionisation voltage was 70 eV. Electron multiplier voltage was 1 kV. Compounds of interest were identified by comparing their mass fragmentation pattern with that of standard spectra available in the spectral library (Flavour and Fragrance and Wiley/NIST libraries) of the instrument.

2.8. Measurement of lipid peroxidation

Thiobarbituric acid-reactive substances (TBARS), a measure of lipid peroxidation, were determined using the method of Alasnier, Meynier, Viau, and Gandmer (2000). A 4-g portion of each sample was blended with 16 ml of 5% Trichloroacetic acid (TCA) and BHT (10 μg BHT/g of lipids). It was then filtered through a Whatman filter (No. 4). Equal amount of filtrate and

0.02 M TBA was heated in a boiling water bath for 30 min. The samples were cooled and the pink coloured complex with absorption maxima at 532 nm was measured. TBA was expressed as mg malonaldehyde/kg of meat.

2.9. Statistical analysis

All data are expressed as means ± SD. Differences between variables were tested for significance by one-way ANOVA with Tukey's post test using GraphPad InStat version 3.05 for Windows 95 (GraphPad Software, San Diego California USA, www.graphpad.com). Differences at $p < 0.05$ were considered to be significant and $n = 3$.

3. Results and discussion

3.1. Total lipid content

Leg region of lamb meat had a total lipid content of 2.3 ± 0.2%, while in rib region it was 4.1 ± 0.5%. Extraction of lipid was performed by the dry extraction method and hence to validate this method lipid was also extracted using a conventional method (Folch et al., 1957), and it was seen that the yield of lipid in both the methods was similar. In the dry column method, lesser volumes of solvents are used and dichloromethane is used instead of chloroform, which is a suspected carcinogen. Also, no emulsion problems are encountered, the separation of lipids into its subclasses can be carried out, and therefore, this method was preferred. Meat taken from different regions of the same animal may vary in their total lipid content and its composition. Threshold dose for radiation processing of meat depends upon both these factors. Lipid content from the leg and rib region, the two most popularly consumed parts of lamb meat, was determined. Watson, Mann, Sinclair, and O'Dea (1992) found that the fat content of midloin chops was 56.8% greater than the leg of lamb. Ono et al. (1984) and Hoke et al. (1999) have also reported that the total lipid content in rib roast (8.9–8.2 g/100 g) was greater than that in leg sirloin (4.9–5.1 g/100 g).

3.2. Lipid profile

The lipids from control (non-irradiated) and irradiated meat were analyzed by TLC to investigate if there was any change in the lipid profile due to irradiation. The various classes of lipids obtained from the point of application were phospholipids, diglycerides, cholesterol, free fatty acids and triglycerides. No significant difference was observed between the lipid profiles of irradiated and control samples. There was also no difference in the lipid profile of leg and rib meat.

3.3. Phospholipid profile

In the above solvent system, phospholipids did not get separated and thus were seen at the point of application of lipids. Various classes of phospholipids were further resolved using a system of ethyl acetate, propan-2-ol, chloroform, methanol, and 25% aq KCl. In this system, neutral lipids moved to the solvent front. Some changes were observed between the control and irradiated samples. Seven different classes of phospholipids were obtained in control samples. In irradiated samples, there was a dose dependent decrease in the intensity of the first spot, which was identified to be sphingomylein using standards. Muscle phospholipids are mainly composed of phosphatidyl choline (PC) and phosphatidyl ethanolamine (PE), which account for 45–60% and 20–30%, respectively. Sphingomylein and phosphatidyl serine (PS) are present in small amounts (less than 2%).

3.4. Quantitation of phospholipids

PL content of the control and irradiated samples from the leg and rib region of lamb meat was determined colorimetrically and is depicted in Fig. 1. It can be seen that the PL content of leg meat was 27.4%, while in the rib meat it was only 13.7%. On radiation processing, there was a decrease in the PL content in meat taken from both the leg and rib region. The PL content decreased to 22.9% and 20.3% in leg meat irradiated at 2.5 and 5.0 kGy, respectively, as compared to non-irradiated meat (leg region) samples. Similar trends were observed in rib meat irradiated at 2.5 and 5 kGy, which had PL content of 11.4% and 10.8%, respectively. The oxidation of PL in the leg meat on radiation processing was found to be 2-fold greater as compared to that of the rib meat. Gandemer and Kim (1993) have reported that the total

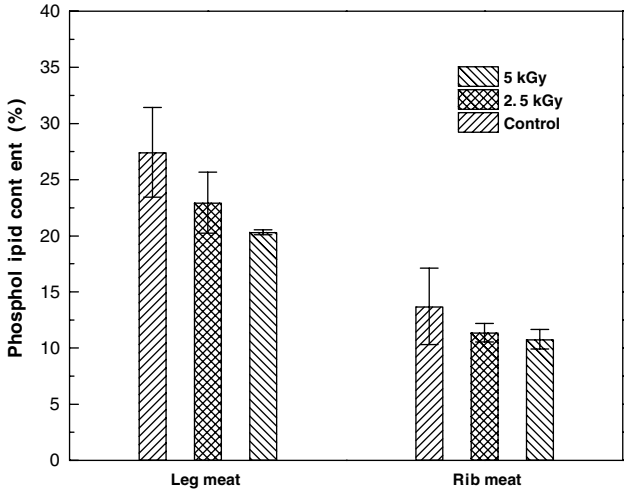

Fig. 1. Phospholipid content of lamb meat. Each value represents mean ± standard deviation.

phospholipid content in chicken was slightly reduced in breast glycolytic muscles (10%), whereas it decreased markedly in drumstick muscles (35%), which are more oxidative muscles.

3.5. Quantitation of FFA

The FFA of the leg and rib region of lamb meat is shown in Fig. 2. In leg meat, the FFA content was 5.1%, while in rib meat it was only 3.0%. This corroborates the finidings of Alasnier et al. (2000) who have reported that in chicken meat the thigh muscles contained three times more FFAs than the breast muscles. The higher FFA content of leg meat could probably be due to the oxidative nature of leg muscle compared to the more glycolytic rib region. The FFA contents of both leg and rib meat showed a dose dependent increase on irradiation (Fig. 2). Lescano et al. (1991) have also reported that chicken half breasts packed in polystyrene trays and wrapped with PVC film that are irradiated at a dose of 4.5 kGy show a higher FFA content compared to non-irradiated control samples. In frozen poultry, Gruiz and Kiss (1987) have reported an increase in FFA content on irradiation at 4 kGy.

3.6. Quantitation of cholesterol

The cholesterol content of both the leg and rib region of lamb meat is shown in Fig. 3. The cholesterol content in rib meat was 3.3%, while in leg meat it was 3.8%. On radiation processing, the cholesterol content of meat from both the regions decreased. Radiation processing leads to oxidation of cholesterol. The free radicals generated by the process of lipid peroxidation co-oxidise several molecules, especially cholesterol which is in the lipid fraction and membranes. Production of cholesterol

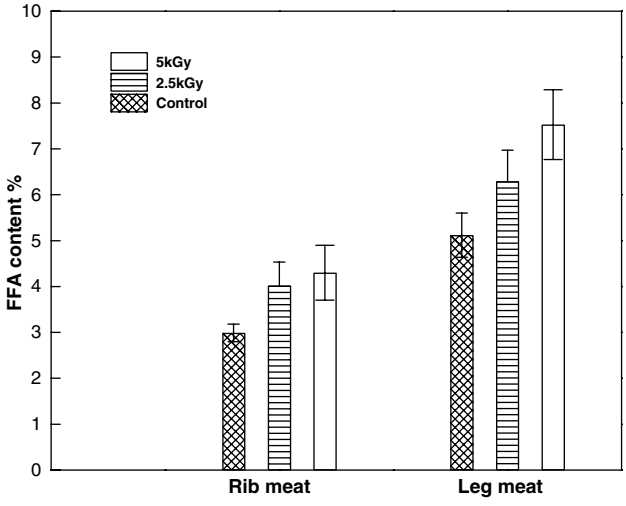

Fig. 2. Free fatty acid content of lamb meat. Values are means ± S.D. of three determinations.

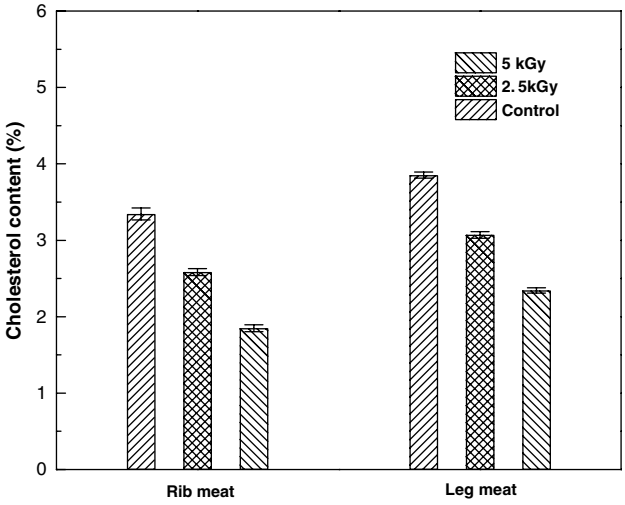

Fig. 3. Cholesterol content of lamb meat. Values are means ± S.D. of three determinations.

oxides has been observed in meat and meat products that have been processed by other methods and also after chilled storage.

3.7. Fatty acid composition of total lipid

The fatty acid compositions of the total lipid extracted from the rib and leg region of lamb meat are shown in Table 1. Similar to other livestock species reared for meat production, major fatty acids in muscle lipids of lamb are oleic ($C_{18:1}$), palmitic ($C_{16:0}$) and stearic ($C_{18:0}$). The most abundant fatty acid was oleic acid ($C_{18:1}$) and this is in good agreement with the findings of Park and Washington (1993) who have reported that $C_{18:1}$ was around 42.2% in *Longissimus dorsi*. Meat flavor is influenced by fatty acid composition. Saturated fatty acids increase hardness of fat and being easily solidified upon cooling influence meat palatability. On the other hand, unsaturated fatty acids increase the potential for oxidation which influences shelf life. Radiation processing of meat did not significantly alter the fatty acid composition of meat from both the regions.

3.8. Fatty acid composition of phospholipids

The fatty acid composition of phospholipids is shown in Table 2. The major fatty acids identified in the PL fraction of lamb meat were oleic ($C_{18:1}$), palmitic ($C_{16:0}$), stearic ($C_{18:0}$), linoleic ($C_{18:2}$) and arachidonic acid ($C_{20:4}$). The degree of unsaturation of a fatty acid affects oxidation rate significantly. A higher proportion of PUFA characterises the fatty acid composition of phospholipids. Comparison of fatty acid contents between control and irradiated lamb meat showed that they were significant for oleic ($C_{18:1}$), linoleic ($C_{18:2}$) and arachidonic ($C_{20:4}$) acids whose contents decreased

Table 1
Major fatty acids (mean percentages) of total lipid extracted from lamb meat

Fat constituents	Rib meat						Leg meat					
	Control		2.5 kGy		5 kGy		Control		2.5 kGy		5 kGy	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
$C_{15:0}$	4.75	0.30	5.8	0.12	5.19	0.20	–		–		–	
$C_{16:0}$	29.4	1.81	32.6	0.64	31.1	1.18	28.0	1.42	31.1	1.31	31.2	1.82
$C_{18:0}$	14.5	0.93	15.1	0.32	15.7	0.61	17.8	0.94	15.6	0.50	15.9	0.96
$9C_{18:1}$	43.0	1.19	44.5	0.96	43.8	1.44	47.8	2.62	44.3	1.76	43.5	2.77
Total SFA	48.6		53.5		52.02		45.8		46.7		47.0	
MUFA/TFA	0.46		0.45		0.45		0.51		0.49		0.48	
MUFA/SFA	0.88		0.83		0.84		1.07		0.94		0.95	

SFA, saturated fatty acid; MUFA, monounsaturated fatty acid; TFA, total fatty acid; SD, standard deviation.

Table 2
Major Fatty acids (mean percentages) of phospholipids extracted from rib and leg region of lamb meat

Fat constituents	Rib meat						Leg meat					
	Control		2.5 kGy		5 kGy		Control		2.5 kGy		5 kGy	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
$C_{16:0}$	26.3	3.80	29.4	0.29	31.1	5.9	24.1	0.26	26.8	5.15	27.3	4.78
$C_{18:0}$	20.3	3.05	20.4	5.40	25.8	2.89	21.5	2.45	21.4	1.55	24.1	2.50
$9C_{18:1}$	32.1	5.44	28.9	7.98	21.9	3.79	31.1	0.26	30.4	2.32	23.8	2.10
$C_{18:2}$	10.2	3.16	7.48	0.49	5.76	0.58	11.0	2.19	6.94	3.64	5.99	2.70
$5,8,11,14C_{20:4}$	6.27	2.52	4.24	0.35	2.38	0.28	7.82	1.56	6.19	0.23	2.22	0.19
Total SFA	46.5		49.8		56.9		45.5		48.2		51.4	
Total PUFA	16.5		11.7		8.14		18.8		13.1		8.21	
PUFA/SFA	0.35		0.23		0.14		0.41		0.27		0.16	
MUFA/TFA	0.33		0.31		0.25		0.32		0.33		0.28	
PUFA/TFA	0.17		0.13		0.09		0.19		0.14		0.10	
UFA/TFA	0.51		0.44		0.34		0.52		0.47		0.38	

SFA, saturated fatty acid; PUFA, polyunsaturated fatty acid; MUFA, monounsaturated fatty acid; TFA, total fatty acid, SD, standard deviation.

on radiation processing. From Table 2, it can be seen that the ratio of PUFA/SFA also decreased significantly on radiation processing. In studies carried out by Maxmell and Rady (1989), no new fatty acid residues or other artifacts due to γ irradiation of air and vacuum packed chicken tissues were found in detectable amounts by GC in any of the lipid fractions isolated. Minor changes of interest, however, were observed for the PUFA residues in the polar fraction of muscle tissue, especially at higher irradiation doses (6 and 10 kGy). The changes in PUFA are minimised when irradiation was carried out under frozen (−20 °C) conditions. Raddy, Maxwell, Wierbicki, and Phillips (1988) found that fewer alterations in PUFA composition occurred in chicken when irradiation was carried out in frozen state rather than at 2–5 °C, indicating that it would be preferable to irradiate meat at lower temperatures.

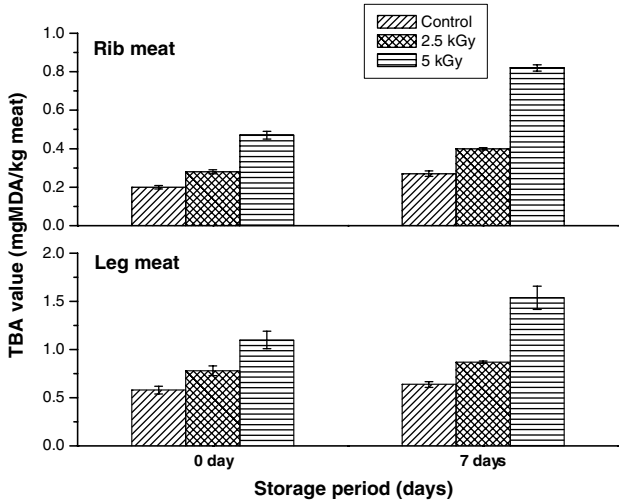

Fig. 4. Effect of radiation processing on the formation of thiobarbituric acid-reactive substances in lamb meat stored at 0–3 °C. Data represent means ± standard deviation.

3.9. Lipid oxidation

3.9.1. Effect of irradiation on oxidative rancidity of lamb meat

Lipid oxidation in lamb meat was measured in terms of TBA values (Fig. 4). There was dose dependent increase in TBA number on radiation processing (2.5 and 5 kGy) of lamb meat. As compared to the non-irradiated samples, irradiation at 2.5 and 5 kGy

resulted in 34% and 89% increase in TBA values, respectively. Several workers have reported the role of ionising radiation in the generation of free radicals that induce lipid peroxidation. Our results are in agreement with the findings of other investigators who have also reported an increase in lipid peroxidation of radiation processed meat and meat products (Jo & Ahn, 2000; Luchsinger et al., 1996). Presence of oxygen affects the rate of oxidation. The higher TBA values of irradiated meat are due to the fact that autooxidation of fat is accelerated by free radicals produced during irradiation to form hydroperoxides which break down into various decomposition products including aldehydes, of which malonaldehyde is the major TBA-reactive substance (Hoyland & Taylor, 1991). The TBA test, despite its limitations, remains the most commonly used method to measure lipid oxidation in meat, especially on a comparative basis. If all the TBA-reactive substances are determined by a single method, the change in TBA numbers show the relative amount of lipid oxidation occurring during storage and/or processing (Gray & Monahan, 1992).

3.9.2. Effect of section of meat taken on oxidative rancidity

The region from which meat is taken is another factor that influences the degree of rancidity. Lipid peroxidation measured in terms of TBA number was less in meat taken from the rib region as compared to that taken from the leg region (Fig. 4). The initial TBA value of non-irradiated leg meat was almost 2-fold greater than that of rib meat. The TBA number of irradiated (5 kGy) leg meat was also 2-fold greater than the corresponding rib meat. The metabolic type of the fibres is a major factor involved in the heterogeneity of muscle quality within a carcass. The oxidative muscles are more sensitive to oxidation than the glycolytic ones. This is partly related to their higher phospholipid content and to their higher long chain PUFA content. Phospholipids are the primary substrates of lipid oxidation in muscle foods (Gandemer, 1999).

3.9.3. Effect of chilled storage on oxidative rancidity

TBA values were affected by storage time. On storage (0–3 °C), there was a 40% increase in the TBA values in the case of leg meat irradiated at 5 kGy, while in rib meat the TBA value increased by 74% (Fig. 4). Any process causing disruption of the muscle membrane system, such as grinding, cooking and deboning, accelerates development of oxidative rancidity (Renerre, 1990). The accelerated development of oxidative rancidity observed in this study could be attributed to the grinding of raw meat, which disrupts the integrity of the membrane. Strange, Benedict, Gugger, Metzger, and Swift (1974) found that the TBA values of ground meat samples were 2–3 times the values of ungrounded meat under identical conditions, reflecting an increased rate of lipid peroxidation.

4. Conclusion

The present studies attempted to establish whether radiation processing of lamb meat at chilled temperature resulted in changes to the composition of lipids. In the range of radiation doses employed (0–5 kGy), there was no change in the lipid profile qualitatively. However, on quantification, some changes, such as decreases in the phospholipid and cholesterol levels, were observed. Fatty acid profiles of total lipids did not show significant changes, but in the case of phospholipids there was a decrease in the PUFA content. Oxidative rancidity also increased on irradiation. Hence, the beneficial use of radiation processing in improving the safety and extending the shelf life of meat could be utilised to the fullest if these changes in lipids could be minimised. This can be achieved by altering the packaging conditions or by addition of antioxidants, which is being investigated further.

Acknowledgements

The authors thank Dr. Variyar P.S for his help in GC/MS analysis.

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