EFFECT OF GAMMA IRRADIATION ON MICROBIAL LOAD OF SHEEP MEAT

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Abstract

Sheep (*Ovis aries)* is well-thought-out to be a significant source of nutrition around the world. Pathogenic microorganisms have caused numerous outbreaks of food-borne illnesses in many countries in the past, in spite of the mighty efforts their government employed to avoid contamination. Appropriate hygienic practices can lower the level of contamination. This study was conducted to monitor reduction in microbial contents especially the pathogens of sheep meat after Gamma irradiation. The sheep meat control sample used in this study contained coliform ($2.4\times10^6\pm1.26$ cfu/g), Staphylococcus ($8.04\times10^6\pm0.489$ cfu/g) and hemolytic bacteria ($0.7\times10^5\pm0.89$ cfu/g). A Gamma radiation dose of 1kGy was adequate to eliminate coliform while a dose of 1.5 kGy was required to get rid of *Staphylococcus* and Hemolytic bacteria. Further control sample harbored acid tolerant bacteria that could survive in pH 2 ($0.1\times10^4\pm0.089$ cfu/g) and pH3 ($0.6\times10^4\pm0.7$ cfu/g) and were eliminated at 0.5 and 1.5kGy dose respectively. The sample contained ampicillin resistant bacteria ($6.7\times10^5\pm0.9$ cfu/g) that were eliminated at a dose of 1.5kGy. In conclusion a radiation dose of 1.5 kGy was found optimal to eliminate harmful bacteria from sheep meat.

Introduction

Sheep meat is an excellent source for sustenance and provides for a better nourishment of individual who consumes it. Sheep population in Pakistan during the year 2005-06 was 25.5 million, with an annual growth rate of 0.90 for the period of 2002-2006. Sheep breeds found in Pakistan add up to 28 in total. Mutton production in Pakistan during the year 2005-06 was 782.1 thousand tons, with sheep meat contributing up to 30% (Pasha and Afzal, 2006)

The sheep breed of most significance in Pakistan is Lohi breed. It is also the biggest breed in Pakistan making some 40% of the Punjab and 15% of the national sheep production. Watered areas of the central Punjab are home to Lohi breed but it is widely found in other expanses of the province also. There exists a wide variety in traits of production for this breed, which implies that there is still room for more improvement (Babar *et al.*, 2004).

Meat, a rich protein source is highly susceptible to contamination by microbes, which can lead to its breakdown, following food borne ailments in human, causing a great deal of losses at economic and health level. Even though muscle tissues of healthy animals are free of any microorganisms, contamination can occur during numerous phases of slaughtering and transportation(Komba *et al.*, 2012).

There exists a wide variety of microorganisms that can house fresh meat, but what type will override depends upon pH, composition, textures, storage temperature, and transportation means of raw meat (Ahmad *et al.*, 2013).

Sheep may harbor pathogens even without displaying any clinical signs of it. These pathogens may take refuge in the gastrointestinal tract and exterior surfaces of sheep. Foodborne pathogens which have been reported in sheep include *Salmonella* species, Pathogenic *E. coli* (EHEC). Other possible foodborne pathogens associated with sheep meat include *Campylobacter jejuni, Yersinia enterocolitica, Yersinia pseudotuberculosis, salmonella* spp, *staphylococcus* spp, *Cryptosporidium parvum, Toxoplasma gondii.* Sheep may carry pathogens that usually come as part and parcel of handling, and they can potentially be transmitted through consumption of meat. These include: *Burkholderia pseudomallei (Melioidosis), Coxiella burnetii* (Q fever), *Bacillus anthracis* (Anthrax). As far as parasites are concerned, *Toxoplasma* species are present in lamb meat (Dubey *et al.*, 2011).

Materials and Methods

Sample Collection: Fresh samples of sheep meat were purchased from the Zenith store, Lahore. Samples were collected after three to four hours of animal slaughter. Samples were taken to the laboratory in icebox and stored at -18°C.

Sample Preparation Irradiation: Twelve samples, each weighing 40 g, were prepared which were then wrapped in aluminum foil and packed in ziploc bags. Three samples were separated and analyzed as control. Other nine samples were divided into three groups and labeled with their specified doses of 0.5, 1.0 and 1.5 kGy, respectively.

Radiation Treatment: Samples were then packed in ice box for transportation and were sent for the radiation process to the Pakistan Radiation Services (PARAS). Samples were radiated using gamma radiation from Co^{60} source. Samples understudy were irradiated at 0.5kGy, 1.0 kGy and 1.5kGy.

Storage: After irradiation, samples were stored in Freezer at -18°C for 2 weeks.

Sample Analysis: Non-irradiated and irradiated samples were analyzed on day 1, day 7 and day 14 after their storage. Microbial analysis of the treated and untreated samples was done in the BS Biotechnology General laboratory at Lahore College for Women University, Lahore (LCWU).

Microbial Analysis: After irradiation, samples were tested for the reduction in microbial count. Bacteria were analyzed using Nutrient Agar, MacConkey Agar, Mannitol Salt Agar, Blood Agar while yeast and fungi were analyzed using Potato Dextrose Agar (PDA).

Homogenate Preparation for Microbial Analysis: Homogenized mixtures of control and radiated samples were prepared by taking 1g of sample and mixing it in 2ml of sterile saline solution in an eppendorf. It was mixed properly in a centrifuge at 1000rpm for 3 min. 10^1 dilutions were prepared in sterile water and they were used for further analysis.

Assessment of Microbial Load: The study was carried out to identify bacteria and fungi of non-irradiated and irradiated samples. Bacterial identification included Total Aerobic Plate Count, Spore count, gram negative bacterial count, and *Staphylococcus spp.* count. Acid resistant bacteria, antibiotic resistance bacteria and hemolytic bacteria were also detected by using various selective media, identification of fungus included yeast and mold count.

For identification of microbial load in untreated and irradiated samples, media were prepared, autoclaved, and poured in sterile plates and solidified. 100µl of the sample was poured and spread over the media used for identification of bacteria and fungi. Replicates for these samples were spread and incubated. Following incubation, colony morphology and characteristics including shape, texture, margins, color, elevation, size, pigmentation and optical properties were noted. For each plate, the cfu/g was calculated. Various general purpose media as well as selective media were used to detect and quantify microbial variety in control and irradiated samples. General purpose media used include Nutrient Agar and PDA for evaluation of Total Aerobic Plate Count, and yeast and fungal count, respectively. MacConkey Agar is a selective and differential media and was used for isolation of coliforms. Other selective media included Mannitol Salt Agar for *staphylococcus aureus* and blood agar for hemolytic bacteria.

Results

Coliform count: MacConkey agar is a differential media which is used to isolate lactose fermenting gram negative bacteria from a sample. Analysis was performed and results are summarized in Table 1. Subsequent radiation treatments eliminated coliforms from samples. A similar trend was seen when these samples were analyzed after 7 days of storage and sample was made coliform free at a dose of 0.5 kGy.

Staphylococcus aureus Count: Mannitol Salt agar was used for the evaluation of presence of *staphylococcus aureus i*n control and irradiated samples. After 7 days of storage at -18°C, there was a reduction in *Staphylococcus aureus* count as compare to control sample. Day 14 did not witness any of the *S. aureus* colonies in either control or irradiated samples. Results are given in Table 1.

Hemolytic Bacterial Count: For the enumeration of hemolytic bacteria, blood agar supplemented with 5% sheep blood was used. Hemolytic bacteria were isolated by observing clear zones around colonies and results were shown in Table 1. After irradiation procedure, this number of hemolytic bacterial count was lowered to a significant rate at doses 0.5 kGy and 1.0 kGy, respectively and eventually procedure led to complete elimination of hemolytic bacteria at 1.5 kGy.

Acid Resistance: In order to check whether bacteria present in sample understudy can grow on acidic pH or not, control and irradiated samples were spread on nutrient agar and blood agar plates after adjustment of their pH to 2 and 3. 10^1 dilutions of these samples were spread. On nutrient agar plates, after radiation there was a significant decrease in acid resistant bacteria at pH 2 and pH 3 respectively. Acid resistant bacterial count was also reduced on blood agar plates after irradiation at 1.5kGy dose. Results are shown in Table 2.

Table 1. Total count of Coliform, *Staphylococcus* and Hemolytic bacteria in control samples and samples irradiated at 0.5, 1 and 1.5kGy at three intervals.

	Doses											
	Day 1				Day 7				Day 14			
	control	0.5	1	1.5	Control	0.5	1	1.5	Control	0.5	1	1.5
Coliform count	$2.4\times10^6 \pm 1.26^{a*}$	$1.5\times10^6 \pm 0.894^{b}$	0	0	$2.0\times10^6 \pm 0.6324^{a}$	$1.2\times10^6 \pm 0.894^{b}$	0		0	0	0	0
Staphylococcus count	$8.04\times10^6 \pm 0.489^{a*}$	$7.1\times10^6 \pm 0.632^{b}$	$1.1\times10^5 \pm 0.894^{c}$	0	$7.2\times10^6 \pm 0.8^{a}$	$4.5\times10^6 \pm 1.16^{b}$	$1.0\times10^5 \pm 1.09^{c}$	0	0	0	0	0
Hemolytic count	$0.7\times10^5 \pm 0.89^{b*}$	$1.1\times10^5 \pm 0.6^{a}$	$0.1\times10^5 \pm 0.063^{c}$	0	$1.0\times10^5 \pm 0.89^{b}$	$1.4\times10^5 \pm 0.75^{a}$	$0.4\times10^5 \pm 0.94^{c}$	0	$0.4\times10^5 \pm 0.95^{a}$	$0.1\times10^5 \pm 0.063^{b}$	0	0

* Result are represented as mean ±SEM followed by alphabets representing significant difference within group at $p < 0.05$determined by Duncan's Multiple Range Test.

Table 2. Total acid resistant, hemolytic acid resistant and antibiotic resistant bacterial count in control and samples irradiated at 0.5, 1 and 1.5kGy.

Doses							
Total acid resistant bacteria			Total acid resistant hemolytic bacteria		Total antibiotic resistant bacteria		
	pH 3	pH 2	pH 3	pH 2	Ampicillin	Gentamicin	Vancomycin
Control	$0.6\times10^4 \pm 0.7^{a*}$	$0.1\times10^4 \pm 0.089^{a}$	$2.4\times10^4 \pm 1.09^{a*}$	0	$6.7\times10^5 \pm 0.9^{a*}$	0	0
0.5	$0.4\times10^4 \pm 0.89^{b}$	0	$1.6\times10^4 \pm 0.89^{b}$	0	$6.8\times10^4 \pm 1.17^{b}$	0	0
1	$0.15\times10^4 \pm 0.063^{c}$	0	$0.6\times10^4 \pm 1.44^{c}$	0	$1.4\times10^4 \pm 1.01^{c}$	0	0
1.5	0	0	$0.3\times10^4 \pm 1.32^{c}$	0	0	0	0

* Result are represented as mean ±SEM followed by alphabets representing significant difference within group at $p < 0.05$ determined by Duncan's Multiple Range Test.

Antibiotic Resistance: To check for the antibiotic resistant bacteria, these microbes were tested against three broad spectrum antibiotics namely ampicillin, gentamicin and vancomycin. Control and irradiated samples were prepared beforehand and 10^1 dilutions were made. These samples were then spread on nutrient agar and blood agar plates which had been injected with these three antibiotics. After irradiation at 0.5, 1 and 1.5kGy, no growth was observed on plates containing vancomycin and gentamicin, whereas plates injected with ampicillin showed growth to a lesser extent on nutrient agar. It was concluded that bacteria were resistant to ampicillin since they were able to grow on ampicillin injected nutrient agar plates. These findings have been summarized in Table 2.

No hemolytic bacterial growth was observed in any of these three antibiotic added blood agar plates indicating that hemolytic bacteria were all susceptible to ampicillin, gentamicin and vancomycin.

Discussion

Meat is a readily persihable commodity and is vulnerable to microbial contamination like many other foods. Microorganisms that can contaminate meat include *E. coli*, *Salmonella spp*, *Staphylococcus spp*, *Listeria monocytogenes* and *Campylobacter* j*ejuni*. Consuming meat that carries these microorganisms can lead to the development of a number of gastro intestinal ailments including dysentry and blood diarrhea. *Escherichia coli* 0157:H7 is a rapidly emergent food-borne pathogen that can produce a clinical illness characterized by an acute grossly bloody diarrhea that is supplemented by severe, crampy abdominal pain. A few patients go on to develop hemolytic uremic syndrome (HUS) or thrombotic thrombocytopenic purpura. Undercooked and raw lamb meat is supposedly home to this pathogenic microorganism.

Lamb meat was brought from Zenith store and was processed further. Samples were prepared and were irradiated at doses 0.5 kGy, 1.0 kGy and 1.5 kGy.

Untreated control samples in the literature had total viable counts of 10^5 cfu/g, whereas in untreated sample under study, it came out to be 3.8×10^6 cfu/g. This microbial load was significantly lowered at dose 1.5 kGy but these microbes were not totally eliminated.

Coliform are the gram negative bacteria. In literature, total coliform count in untreated sample is reported to be 10^2 cfu/g. Total coliform count in control sample came out to be 2.4×10^6 cfu/g at day 1. This amount was lowered at dose 0.5 kGy and subsequent analysis of samples irradiated at 1.0 kGy and 1.5 kGy showed no. This results is in accordance with the findings of (Paul *et al.*, 1997).

Staphylococcus spp. count in untreated sample in literature is reported to be10^4 cfu/g. Treatment of samples 1.5 kGy resulted in effective elimination of *staphylococcus spp* from lamb meat. These results are similar to those of (Paul *et al.*, 1997), where *staphylococcus spp* were only reduced and not completely eliminated at 1kGy.

Hemolytic bacteria are those that lyse or destruct red blood cells. Blood agar supplemented with 5% sheep blood was used for the enumeration of hemolytic bacteria. Total hemolytic count in untreated sample was estimated to be 0.7×10^5 cfu/g on average at day 1. Sample irradiated at 1.5 kGy was free of any hemolytic bacteria. A similar trend was seen after 7 days of storage at -18°C. After 14 days of storage, total elimination of hemolytic bacteria occurred even at 1.0 kGy dose.

Bacteria in samples under study were checked for the acid resistance on nutrient agar and blood agar. Blood agar plates showed growth of hemolytic bacteria to a lesser extent on pH 3 whereas, no growth was observed at pH 2. As far as nutrient agar is concerned, no growth was observed at pH 2 in samples that had undergone radiation treatment. At pH 3, samples irradiated at 1.5 kGy had no acid resistant bacterial count. Bacteria were checked for acid resistance since pH of stomach is acidic (1.5 to 3.5) and it was concluded that most bacteria are killed at acidic pH of stomach.

Bacteria were tested for antibiotic resistance against ampicillin, gentamicin and vancomycin. No growth was observed in control and irradiated samples when they were incubated on nutrient agar plates injected with gentamicin and vancomycin. Non-irradiated and irradiated sample showed growth on ampicillin added plate. Sample irradiated at dose 1.5 kGy was free of ampicillin resistant bacteria. No hemolytic bacteria was seen in control and irradiated samples when they were incubated on blood agar plates injected with these three antibiotics suggesting that most hemolytic bacteria get killed by these three broad spectrum antibiotics.

Conclusion

To conclude, a dose of 1.5 kGy should be sufficient to make lamb meat pathogen free. Total viable count was reduced at 1.5 kGy; total coliform content was also lowered at 0.5 kGy and finally eliminated completely even at 1.0 kGy. Samples were made *Staphylococcus aureus* free at 1.5 kGy. Hemolytic count was lowered at 1.0 kGy and hemolytic bacteria were totally eliminated at 1.5 KGy. Yeast and fungal cells were also exterminated completely at 1.0 kGy. Acid resistant bacteria and antibiotic resistant bacteria were also eliminated at dose 1.5 kGy. However, sample understudy couldn't be made free of endospore forming bacteria at these doses. So it seemed that total microbial load was much less than that of untreated samples and a dose of 1.5 kGy should be sufficient to make lamb meat safe to consume.

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