

## Research Article

# Effect of gamma irradiation and ethylene oxide treatment on proximate analysis and microbial count of basmati rice variety from Pakistan

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**Abstract** | Rice (*Oryza sativa* L.) is a monocotyledonous angiosperm belonging to the genus *Oryza* and is considered the world's third largest crop after maize and wheat. The premium long grain variety of rice is basmati that is exclusively grown in certain parts of the Punjab. It has distinct flavor and aroma. Basmati rice is non-glutinous, non-waxy rice having intermediary gelatinization temperature and amylose content. It is a major source of energy and an important one for protein. It also contains considerable amounts of niacin and zinc. However rice is low in calcium, iron, thiamine and riboflavin and has virtually no beta-carotene (Vitamin A). Rice has many production constraints including pests and diseases. It may be contaminated with pathogenic and harmful microbes which may result in spoilage and contamination. For sterilization of rice, gamma irradiation at dose 8 kGy and Ethylene oxide was used. Current study was conducted to check the nutritional and microbial contents of simple basmati rice, whether Cobalt 60 gamma radiation and ethylene oxide treatments effect the nutritional and microbial contents of rice or not. The results were indicative of the fact that 8kGy dose was effective in reducing the microbial load in rice and a slight difference was noted in the nutritional content. The sensory properties of treated rice were also satisfactory. It was concluded that irradiation treatment can be effectively used to protect decay losses and enhance shelf life.

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**Keywords** | Gamma irradiation, Basmati Rice, Ethylene oxide and proximate analysis

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## 1. Introduction

Rice (*Oryza sativa* L.) is the seed of the monocot plant of the family Poaceae which incorporates twenty wild species and two developed ones, *O. sativa* (Asian rice) and *O. glaberrima* (African rice).

Today throughout the whole world *O. sativa* is the most commonly grown species (Bachir *et al.*, 2022). *O. sativa* is the staple food of an approximately 3.5 billion people around the world and is the

most widely grown rice (Samal *et al.*, 2022).

As indicated by the Food and Agriculture Organization (FAO, 2009), the overall rice production is anticipated to be 68501.3 million tons. China, India, Indonesia, Bangladesh, Myanmar, Thailand and Vietnam are the main rice producing countries (Lai *et al.*, 2015). 90% of the world's total rice production is accounted by Asian countries together with Indonesia, Bangladesh, Vietnam, Myanmar, Thailand, the Philippines, Japan, Pakistan, Cambodia, the Republic of Korea, Nepal, and Sri Lanka (Gomez *et al.*, 2022).

Pakistan's yearly rice trade remains at around 2.5 million tons, which increase total of 513.0 million dollars for the country (Sekhar, 2018). Rice export was about one billion during the year 2005–2006 US\$ (Bhatia *et al.*, 2021). During 2004-05 the yield of rice was 5024.8 thousand tons from area of 2519.6 thousand hectares and it occupies about 11% of the total cropped area in the country (GOP, 2005-06). Pakistan contributes around 11% in all out world rice send out and on a normal 1/3rd of the aggregate national rice generation is sent out each year which represents 5.7% of the aggregate esteem included farming and 1.3% to GDP (Dunaway and Macabuac, 2022).

Twelve countries are specified for the export of rice namely, Thailand, Vietnam, Pakistan, the United States, India, Italy, Uruguay, China, the United Arab Emirates, Benin, Argentina, and Brazil, which represent for more than 90% of the global rice. Import of rice is mainly concentrated in the following countries. Philippines, Saudi Arabia, the United Arab Emirates, Malaysia, Iran, Iraq, Cote d'Ivoire, South Africa, Cameroon, Mexico, the United States, and Brazil (Sallah, 2017; Muthayya *et al.*, 2014).

Rice contains significant measures of zinc and niacin. Calcium, iron, thiamine and riboflavin are available in low amount in rice and it has for all intents and purposes no beta-carotene (Vitamin A). There will be most minimal the level of proteins, vitamins and minerals in the last item if there is most elevated level of cleaning (Zelig *et al.*, 2022).

Rice faces numerous generation imperatives, including nuisances and ailments like rice sheath rot disease. Fungi such as *Sarocladium oryzae* and *Fusarium sp.* belonging to the *Fusarium fujikuroi* complex and the bacterial pathogen *Pseudomonas fuscovaginaeare* the major pathogens associated with rice sheath rot. A variety of other pathogens are also responsible for rice sheath rot (Nayak *et al.*, 2017).

Contingent upon the reason for the sterilization and the material that will be disinfected there are a wide range of cleansing strategies. We can change the sterilization strategy relying upon materials and gadgets for giving no mischief (da Silva Aquino, 2012). Gamma irradiation eliminates microscopic organisms by separating bacterial DNA. It is a physical method for cleaning. By the self-breaking down of Cobalt-60 (<sup>60</sup>Co) or Cesium-137 (<sup>137</sup>Cs) sources, gamma beams are framed. Two gamma producers utilized for radiation preparing are <sup>137</sup>Cs and <sup>60</sup>Co (da Silva Aquino, 2012). Gamma Irradiation helps a lot in food safety to healthy and compromised consumers (pregnant mothers, immune-compromised patients, people on medication and ageing persons). The use of gamma irradiation is a promising technology that could be applied to the end product for the sterilization of food. This physical, safe, environmentally clean and efficient technology is used to enhance the shelf life of fresh, frozen or cooked products (Bashir and Aggarwal, 2016).

More than 50 nations at present permit food irradiation, and the volume of nourishment treated is assessed to surpass 500,000 metric tons every year around the world (Campanile and Campanile, 2022). Shelf life of food can be increased by using this process along with increasing storage time and it also improve microbiological and parasitological safety of foods (Tantala *et al.*, 2022 ; Kim *et al.*, 2022). Each sterilization process had its preferences and burdens and its productivity relies upon a wide range of variables including kind of microorganisms, their number, type and amount of organic matter that is ensuring them and so on (Tietjen *et al.*, 2003).

## **2. Materials and Methods**

### **2.1. Sample collection and Experimental site**

Locally available variety of basmati rice were collected from Metro Store, Lahore and divided into three groups, viz. control, for gamma radiation and for ethylene oxide treatment. Experiments were conducted in laboratories of Biotechnology Department of Lahore College for Women University, Lahore.

### **2.2. Sample packaging and treatments**

Both samples were packed in transparent low-density polythene (LDP) bags for radiation and packed for respective groups. One group was carried out to PARAS (Pakistan Radiation Services) Multan Road, Lahore where Gamma irradiation was carried out in a Co-60 Food Package Irradiator. Other groups of samples were sent for ethylene oxide treatment. Both control and irradiated samples were used for microbial and proximate analysis.

### **2.3. Sensory evaluation**

Sensory evaluation was conducted on irradiated and control samples of basmati rice. These include the comparison of texture, color and visual defects of irradiated and control samples.

## **2.4. Microbial analysis**

### **2.4.1. Microbial count**

The growth media used for the enumeration and identification of bacteria and fungi were Nutrient Agar, MacConkey Agar, and Potato Dextrose Agar.

### **2.4.2. Bacterial count**

Bacterial analysis of basmati rice was done by spreading on nutrient agar (for bacterial isolation) and MacConkey agar medium (for gram-negative enteric bacilli isolation). One ml of the sample was serially 10-fold diluted in saline water. 0.1 ml from each serially diluted sample (10<sup>-3</sup>) was inoculated on nutrient agar and MacConkey agar medium by spreading in triplicates. The samples were incubated at 37°C for 24 hours. After incubation of samples, the colonies were counted, observed under microscope by gram staining and colony characteristics were recorded.

### **2.4.3. Fungal count**

Potato dextrose agar (PDA) was used for fungal analysis. One ml of the sample was serially 10-fold diluted in saline water. 0.1 ml from each dilution (10<sup>-3</sup>) of the serially diluted sample was spread on PDA in triplicates. The plates were incubated at 30°C for 2 days. After incubation colonies were observed by methyl blue staining, counted and their characteristics were recorded.

## **2.5. Proximate analysis**

The proximate analysis of basmati rice was done by performing tests for moisture, ash, crude fat, fiber, protein and carbohydrates. The proximate composition of basmati rice was determined by using methods of AOAC (Association of Official Analytical Chemists official methods 2005).

### **2.5.1. Determination of moisture content**

Thermal drying method was used in the determination of moisture content of the

samples. 1.0g of dried samples was grinded, weighed and placed in washed, dried and weighed crucible. They were placed in hot air oven and dried at 105 °C for three hours. The samples were allowed to cool in desiccators and then reweighed. The percentage moisture content was calculated by computing or expressing the loss in weight on drying as a fraction of the initial weight of sample used and multiplied by 100.

Percentage of moisture was calculated by given formula

$$\text{M.C} = ((W_w - W_d) / W_w) \times 100$$

#### **2.5.2. Determination of ash content**

The ash content was determined by using ignition method in muffle furnace. Two to three grams of samples were grinded and weighed into pre-weighed crucibles. Samples were first ignited and then placed in Muffle furnace at 500 –550 °C for 3 hours till the samples became ash.

Weight of ash was calculated by

$$\text{Weight of ash} = \text{Weight of crucible} + \text{Ash} \\ - \text{Weight of crucible}$$

#### **2.5.3. Determination of crude fat**

Soxhlet apparatus was used for the calculating the percentage of crude fat. About 2-3grams of dried samples were taken and then added into thimble which was pre-weighed. The extraction was done in a Soxhlet apparatus for 6 hours and the quantity of ethanol used was 500 ml.

Decrease in the weight of sample was determined by using the formula

$$\text{Loss in weight} = \text{wt. of thimbles} + \text{de} \\ \text{moisture sample} - (\text{weight of thimbles} - \\ \text{fat free sample})$$

$$\text{Percentage of Fat} = \text{loss in weight (g)/wt.} \\ \text{of sample} \times 100$$

#### **2.5.4. Determination of fiber**

Crude fiber is the loss of ignition of dried residue remaining after digestion of sample with H<sub>2</sub>SO<sub>4</sub> and NaOH. 1g of fat free sample was added in the reflux flask and the sample was mixed with 100ml of 1.25% H<sub>2</sub>SO<sub>4</sub> and refluxed for half an hour. The filtration of the sample solution was done with the help of silky cloth and 200ml of hot distilled water was used for washing it. Then the filtrate is again refluxed with 1.25% NaOH for half an hour. Sample solution was washed with 200ml of hot distilled water on pre-weighed Whatman filter paper. The filtrate was dried in an oven and weighted. After drying, filtrate was ignited at low flame & then placed in muffle furnace at 500 to 550 °C where filtrate becomes ash. % of fiber was calculated by

$$\text{Loss in weight} = \text{wt. of oven dried sample} \\ - \text{wt. of ash}$$

$$\% \text{ of fiber} = \text{wt. of sample (g) - loss in} \\ \text{weight (g)/wt. of sample} \times 100$$

#### **2.5.5. Determination of protein content**

Protein was estimated by Kjeldahl apparatus. The samples were digested in the presence of digestion mixture by heating with concentrated H<sub>2</sub>SO<sub>4</sub>. As a result, mixture was made alkaline. The ammonium sulphate formed and released from ammonia that was collected in 2% solution of boric acid and titrated with standard HCl. Total protein was calculated by the amount of nitrogen multiplied by appropriate factor and the amount of protein was calculated.

#### **2.5.6. Determination of carbohydrates**

The carbohydrate content was determined by using values of all tests. It was calculated by adding all percentages of moisture, crude protein, fat crude, fiber and ash then subtracted from 100. This gave the quantity of carbohydrate

otherwise known as extract free from nitrogen.

$$\% \text{ carbohydrate} = 100 - (\% \text{ Moisture} + \% \text{ Ash} + \% \text{ Fat} + \% \text{ Crude protein} + \% \text{ Crude fiber})$$

### 2.6 Statistical analysis

The data generated from the study was analysed through one-way analysis of variance (ANOVA) and the treatment, means were compared for significance by Duncan's New Multiple Range test at 0.05% using SPSS computer software.

## 3. Results

In current study, the effects of gamma irradiation (8kGy) and ethylene oxide treatment were studied on the sensory, microbial and proximate analysis of basmati rice. No noteworthy effect was observed on the sensory properties of rice. It was observed that color and texture did not change in gamma treated sample. At start of work there were no insects in control sample, when it was stored insects were developed in control sample and they damaged it but gamma irradiated basmati rice did not show any insect and remained undamaged.

The total bacterial count on control, EtO and irradiated sample was calculated. No growth was observed for radiated sample on first interval (day 0), while total bacterial count (cfu/ml) for controlled sample was  $6.5 \times 10^5$ . Whereas on second interval  $1.15 \times 10^6$  (cfu/ml) was obtained whereas count reduces to  $2 \times 10^4$  on radiated samples. However on third interval bacterial count for controlled sample was  $2.72 \times 10^6$  (cfu/ml) while that of the irradiated sample was  $1.3 \times 10^5$ . Total Bacterial Count of EtO sterilized samples was  $3.4 \times 10^5 \pm 0.01528$ bcfu/ml. Bacterial count was highest in the non-treated samples. Furthermore, all colonies that were isolated were gram positive.

No growth was observed on MacConkey agar medium in all the three samples of basmati rice.

Fungal count was observed on PDA. Total fungal count (cfu/ml) obtained on first interval (day 0) for controlled samples of rice was  $6.8 \times 10^5$ , while that of radiated samples was  $1.2 \times 10^4$ cfu/ml, Whereas on second interval (day 30)  $8.2 \times 10^5$ cfu/ml was obtained on controlled samples, whereas count reduced to  $1.6 \times 10^5$ cfu/ml on radiated samples. However, on 3rd interval (day 60) fungal count for controlled samples was  $1.6 \times 10^6$  (cfu/ml), while that of the irradiated samples was  $5.2 \times 10^5$  (cfu/ml). Total fungal count of EtO sterilized samples was observed to be  $4.4 \times 10^5 \pm 0.01528$ bcfu/ml. Fungal count was highest in the non-treated samples.

Furthermore in the current study, the proximate analysis was also studied in the control, gamma irradiated and EtO sterilized samples of basmati rice. In rice, the moisture content in control sample was 9.550g/100g, 9.3133g/100g and 8.3033g/100g at Day 0, Day 30 and Day 60 respectively. The moisture content in irradiated sample was 9.2467g/100g, 9.1133g/100g and 7.9500g/100g at Day 0, Day 30 and Day 60 respectively. The moisture content of EtO sterilized sample was 9.66g/100g. Thus moisture content decreased after exposing to gamma irradiation and increased in EtO sterilized sample. The ash content in control sample was 0.4633g/100g, 0.4833g/100g and 0.4867g/100g at Day 0, Day 30 and Day 60 respectively. The ash content in irradiated sample was 1.1667g/100g, 1.2667g/100g and 1.5000g/100g at Day 0, Day 30 and Day 60 respectively. The ash content of EtO sterilized sample was 1.6676g/100g. By exposing rice to radiations, ash content increases. The levels of ash in the control sample of rice in the present study (approximately 0.41%). The fat content in control sample was 0.3200g/100g, 0.3367g/100g and



**Table 1:** Sensory evaluation

Intervals	Characteristics	Control	Irradiated (8kGy)
Day 0	Texture	Hard	Hard
	Color	Light Yellow	Light Yellow
	Visual Defects	No	No
	Insects produced	No insects	No insects
Day 30	Texture	Hard	Hard
	Color	yellow	Light yellow
	Visual Defects	No	No
	Insects produced	Some insects	No insects
Day 60	Texture	Hard	Hard
	Color	Dark Yellow	Light Yellow
	Visual Defects	No	No
	Insects produced	More insects	No insects

**Table 2:** CFU/ml on Nutrient Agar

Time Intervals	Treatments	
	Control	Irradiated
Day 0	$6.5 \times 10^5 \pm 0.0208^c$	Nil
Day 30	$1.15 \times 10^6 \pm 0.0200^b$	$2 \times 10^4 \pm 0.0208^b$
Day 60	$2.72 \times 10^6 \pm 0.0264^a$	$1.3 \times 10^5 \pm 0.0208^a$

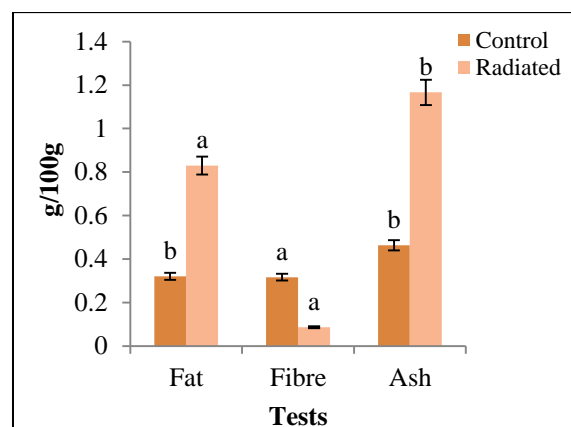
**Table 3:** CFU/ml on Potato Dextrose Agar

Time Intervals	Treatments	
	Control	Irradiated
Day 0	$6.8 \times 10^5 \pm 0.0200^c$	$1.2 \times 10^4 \pm 0.0208^c$
Day 30	$8.2 \times 10^5 \pm 0.0152^b$	$1.6 \times 10^5 \pm 0.0264^b$
Day 60	$1.6 \times 10^6 \pm 0.0264^a$	$5.2 \times 10^5 \pm 0.02517^a$

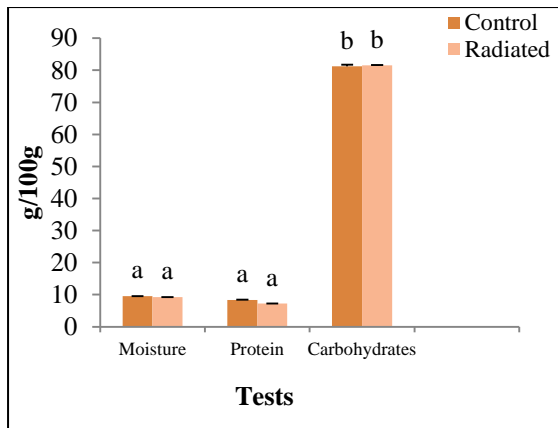
0.3600g/100g at Day 0, Day 30 and Day 60 respectively. The fat content in irradiated sample was 0.8000g/100g, 0.6667g/100g and 0.2333g/100g at Day 0, Day 30 and Day 60 respectively. The fat content of EtO sterilized sample was 0.7g/100g. Fat content increased in the irradiated sample and was decreased in EtO sterilized sample as a compared to the radiated sample.

The fibre content in control sample was 0.3167g/100g, 0.2933g/100g and 0.2600g/100g at Day 0, Day 30 and Day 60 respectively. The fibre content in irradiated sample was 0.0867g/100g, 0.0700g/100g and 0.0600g/100g at Day 0, Day 30 and Day 60 respectively. The fibre

content of EtO sterilized sample was 0.0667g/100g.

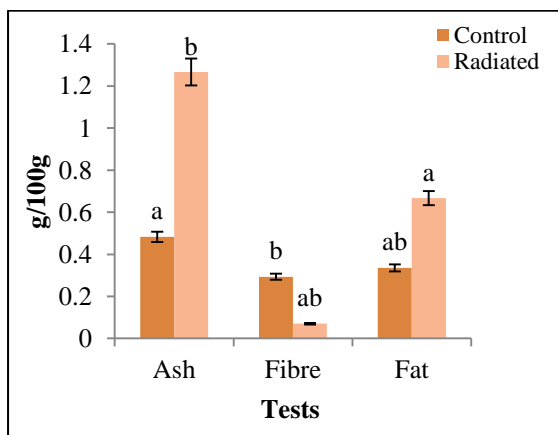


**Figure 1:** Proximate analysis of rice at day 0

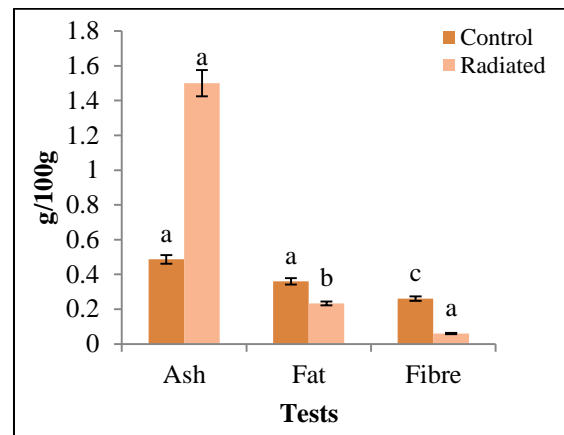


**Figure 2:** Proximate analysis of rice at day 0

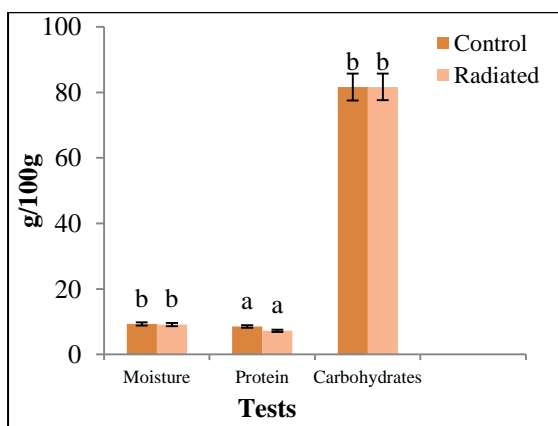
83.1867g/100g at Day 0, Day 30 and Day 60 respectively. The carbohydrate content of EtO sterilized sample was 81.69g/100g which shows that carbohydrate content is less in the EtO sterilized samples as compare to gamma radiated sample. 73.14% to 80.47% carbohydrate content. Stability in the carbohydrate content after irradiation is benefit as it has a lot of health benefits. The increase in the carbohydrate content in the radiated sample was due to the breakdown of oligosaccharides in sample by gamma irradiation.



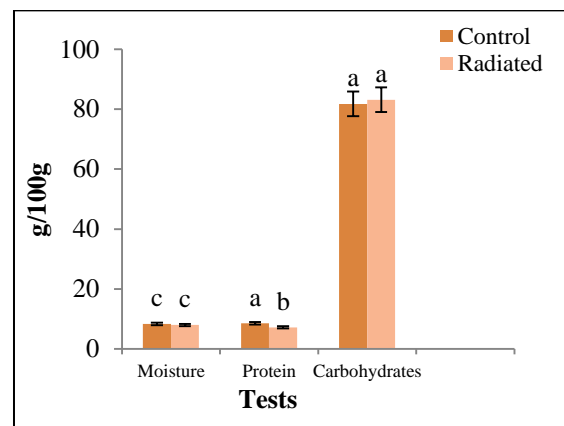
**Figure 3:** Proximate analysis of rice at day 30



**Figure 5:** Proximate analysis of rice at day 60



**Figure 4:** Proximate analysis of rice at day 30



**Figure 6:** Proximate analysis of rice at day 60

The carbohydrate content in control sample was 81.2700g/100g, 81.6600g/100g and 81.7867g/100g at Day 0, Day 30 and Day 60 respectively. The carbohydrate content in irradiated sample was 81.5200g/100g, 81.6800g/100g and

The protein content in control sample was 8.3767g/100g, 8.5333g/100g and 8.5667g/100g at Day 0, Day 30 and Day 60 respectively. The protein content in irradiated sample was 7.2267g/100g,

7.2200g/100g and 7.1800g/100g at Day 0, Day 30 and Day 60 respectively. The protein content of EtO sterilized sample was 7.1867g/100g. Protein content decreased in the irradiated sample and was the lowest in EtO sterilized sample.

The values are calculations of average of three parallel triplicates,  $\pm$  indicates standard deviation between the triplicates. Average in the columns varies significantly  $P \leq 0.05$  according to the Duncan's

Thus this study proved that, gamma irradiation is a better technique as compared to ethylene oxide treatment in suppressing the microbial load in basmati rice and very slight difference was noted in the nutritional properties of rice.

#### 4. Discussion

Darfour *et al.* (2013) reported that no significant difference in the moisture content of control and radiated rice was observed. At the higher radiation doses, there is a loss in the moisture content. The moisture content in control sample was found near to the findings of (Fasahat *et al.*, 2012). The observed increase in the moisture may be due to environmental conditions such as humidity and temperature. The levels of ash in the control sample of rice in the present study were similar to those observed in other studies of (Guimaraes *et al.*, 2015). Almost same amount of fat content (0.4%) has been reported by (Rosniyana *et al.*, 1995). The decrease in the fat content may be due to auto-oxidation and action of high energy radiation on lipid molecules. These results are very similar with the results of Bhat *et al.* (2008) who reported that crude fibre showed a significant decrease at higher doses. Thus, fibre content decreases after exposing to gamma radiations. Tang *et al.* (2012) also reported that fibre content decreases after exposing to gamma

radiations. This might be due to the fact that gamma irradiation affects the bonds of lignin with other components as well as the bonds within the molecule. Devindra and Longvah (2011) also found carbohydrate content. The protein level of the polished white rice samples was consistent with the average values found in the literature of (Fasahat *et al.*, 2012). Bhat *et al.* (2008) reported that gamma irradiation resulted in a significant increase in crude protein at all the irradiated doses. According to Guimaraes *et al.* (2015) rice grains undergo protein, vitamin, and mineral losses during processing because the fractions overlying the endosperm of the seed and the germ are removed. Storage period is responsible for the reduction of protein content of both control and irradiated sample. This decrease is also due to higher metabolic activity.

#### 5. Conclusion

It was concluded that the effect of gamma irradiation and Ethylene oxide treatment was studied on the sensory, microbial and nutritional properties of rice. The microbial load was highest in the non-irradiated samples and reduced in EtO sterilized sample and was the lowest in the gamma irradiated samples. No significant change was observed in the nutritional and sensory properties of rice after gamma irradiation. Thus, 8kGy dose was effective in reducing the bio burden and enhancing shelf life of basmati rice and also increases the export value and provides the world with better quality rice.

#### 6. Acknowledgments

Authors acknowledge Department of Biotechnology, Lahore College for Women University, Lahore.

#### 7. Author's Contribution



All authors have contributed equally.

## 8. Conflict of Interest

No author conflict.

## 9. Novelty Statement

Storage losses due to contamination, pests and diseases needs to be tackled using cheap and safe sources of disinfection without compromising the quality of rice itself. Radiation technology is one such source which can be used to irradiate rice with different doses. Present study explored the effects of radiation dose on properties of rice protein.

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