Research Article

Comparative Study of Ethylene Oxide Treatment and Gamma Irradiation on Nutritional Content and Shelf Life Enhancement of *Lentil Germplasm*

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Abstract | Lentils (family: *Leguminaceae*) is a yearly sown cool season food legume crop. Long periods of storage and pathogenic fungi reduce the quality and quantity of the harvested crop. A lack of proper storage conditions along with several other problems result in yield losses, which in turn cause economic losses in Pakistan. Current research was conducted to enhance the shelf life and decrease the microbial flora of lentils by using ethylene oxide (EtO) sterilization and gamma irradiation (Cobalt-60) without affecting the nutritional value. Treated samples were evaluated at three intervals. Outcomes are indicative of the fact that irradiation dose (8kGy) was more effective in reducing microbial count than EtO sterilization. Slight difference in nutritional content was observed in gamma irradiated lentils whereas the difference in nutritional content observed for EtO sterilized lentils was significant. It was concluded that gamma irradiation can be used efficiently to eliminate microbial flora and increase shelf life of lentils.

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Keywords | Ethylene oxide sterilization, Gamma irradiations, Lentil, Microbial load, Proximate analysis

1. Introduction

Lentil (*Lens culinaris*), also known as masser or massur belongs to the *Leguminoseae* (Janghel and Sharma, 2022). It is among the oldest vegetation originated foods within the fertile crescent of the Middle East. It is a yearly sown cool season food legume crop which has ample protein content (25-30%) in its grains (Heidecker, 2022). The total global cultivated areas for lentil production is around 5.4 million hectares producing 6.3 million tons of seeds with a mean production of 11,523 kg/ha (FAO, 2016). Australia, USA, Canada, Nepal, Ethiopia, India, China and Turkey are the most important lentil producing nations (Knez *et al*., 2023). The global import and export of lentil is about 1.71 mt worth 1.55 million

USD and 1.79 mt worth 1.48 million USD, respectively (Malik *et al*., 2022). \In Pakistan, the province of Punjab is foremost in lentil development with 65% of the overall national production and area. Khyber Pakhtunkhwa, Sindh and Baluchistan make a contribution of 14%, 14% and 7% of the area, respectively (Hossain *et al*., 2022). In Pakistan, the overall area and production of *Lens culinaris* is lowering at an alarming pace. Lentil plants are damaged by an extensive variety of pathogens and the most vigorous are fungal diseases including fusarium wilt (*Fusarium oxysporum f. sp. lentis*), ascochyta blight (*Ascochyta lentis*), rust (*Uromyces viciae-fabae*), botrytis grey mould (*Botrytis cinerea* and *B. fabae*), anthracnose (*Colletotrichum truncatum*) and stemphylium blight (*Stemphylium botryosum*) (Taylor *et al*., 2007). PSbMV (pea seed-borne mosaic virus) is also common in Pakistan (Gheshlaghi *et al*., 2019). *B. lentis*, *Bruchus ervi*, *C. maculatus* and *Callosobruchus chinensis* are the most severe and frequently found insect pests of the stored grain (Vega *et al*., 2022).

In Pakistan, annual production of lentil is only 36 thousand tones and the demand is fulfilled by import, specifically from Canada, Australia or Mediterranean countries. Therefore, to fulfil domestic demand a huge quantity of lentil is imported which puts a massive burden on the national finances. The reason of low per capita consumption is that the market value remains comparatively high (Popkin and Ng, 2022). In Pakistan, the reason of low yield can be related to constant development of cultivars with extreme vegetative development and low yield capacity, thin flexibility, low balance of yield, and susceptibility to stress factors and insufficient nutrition (Rahim *et al*., 2010). Generally preservation, processing or value addition is done to prevent the food item to protect them from the development of micro-organisms and to avoid spoilage during storage. To compete with the production and export challenges there is a need to work on local lentil cultivars by using radiation treatment. The objective of present study was to examine the effect of ethylene oxide sterilization and γ-irradiation on microbial count, nutritional value and the consequence of γ radiation on sensory parameters of lentils of Pakistan.

2. Materials and Methods

2.1. Sample collection

Masoor whole (desi) and destoned were taken from hyperstar market in Lahore. Seeds were apparently of good quality and without any physical injury.

2.2. Gamma sterilization and EtO treatment

The lentil samples were packaged in polythene bags and were carried to PARAS (Pakistan Radiation Services) radiation unit, for treatment with 8kGy dose of γ-irradiation with cobalt-60 and ethylene oxide sterilization. Both the control as well as the treated lentil seeds were stored at room temperature till further analysis.

2.3. Sensory evaluation

Sensory evaluation was performed on control and gamma irradiated lentil seed samples. Sensory parameters such as variation in texture, colour, odour and visual defects were observed at three intervals, every 30 days (Iqtedar *et al*., 2015).

2.4. Microbial analysis

Three different media were utilized for the identification of fungi and bacteria linked with lentils. For bacterial isolation, gram-negative enteric *bacilli* isolation and fungi isolation, nutrient agar, MacConkey agar and potato dextrose agar were used respectively. Both the control and radiated samples were tested for the microbial load. One gram of each sample was soaked in 10ml of distilled water for 1 hour to get

stock solution of surface washed micro flora. About 1 ml of each sample was 10 fold diluted through serial dilution in distilled sterilized H_2O. About 0.1 ml of sample (10-2) was inoculated on MacConkey agar and nutrient agar medium by spread-plate technique. The samples were kept in incubator for 24 hours at 37°C. Colonies were counted after incubation and colony characteristics were recorded (Gultie and Sahile, 2013). The same procedure was repeated for enumeration of mold and yeast. Petriplates were placed in incubator at 30°C for 2 days and appearing colonies were counted. Bacterial and fungal living count was measured by standard formula (Gent and Schwartz, 2005).

CFU/ml = Number of colonies on plate / volume plated × dilution factor

2.5. Proximate analysis

Lentils were also examined to calculate the ash, moisture content, fiber, fat, protein and carbohydrates. Standard methods of analysis (AOAC, 2005) were utilized for proximate analysis of lentils.

2.6. Statistical analysis

Means of several different growth parameters were compared using standard error. The results obtained were analysed by using SPSS version 20.0. The mean values were compared by using Duncan's New Multiple Range test at $p \leq 0.05$ with three replicates each.

3. Results

3.1. Sensory evaluation

Lentils were assessed for sensory attributes like color, texture, odor and visual defects (Table 1 and Table 2). Odor was pleasant and no visual defects were observed initially, but after 30 days of storage, unpleasant odor was observed in control sample of lentils and in irradiated sample at day 60.

Table 1: Sensory evaluation of γ-irradiated and non-irradiated whole masoor.

Sample analyzed	Sensorial Properties	Treatments	Days of Storage		
			0	30	60
Masoor Whole	Color	Control	Light brown	Light brown	Light brown
		Irradiated	Light brown	Light brown	Light brown
	Texture	Control	Soft	Soft	Soft
		Irradiated	Soft	Soft	Soft
	Odor	Control	Pleasant	Unpleasant	Unpleasant
		Irradiated	Pleasant	Pleasant	Unpleasant
	Visual defects	Control	No	No	No
		Irradiated	No	No	No

Table 2: Sensory evaluation of γ-irradiated and non-irradiated destoned masoor.

Sample analyzed	Sensorial Properties	Treatments	Days of Storage		
			0	30	60
Masoor Destoned	Color	Control	Orange	Orange	Orange
		Irradiated	Orange	Orange	Orange
	Texture	Control	Hard	Hard	Hard
		Irradiated	Hard	Hard	Hard
	Odour	Control	Pleasant	Unpleasant	Unpleasant
		Irradiated	Pleasant	Pleasant	Unpleasant
	Visual defects	Control	No	Sprouting	Sprouting
		Irradiated	No	No	Sprouting

Sprouting occurred in masoor destoned control sample initially during storage whereas gamma irradiated lentil showed sprouting after 60 days. There was no change observed in color or texture in both gamma irradiated and control sample of lentils. The results showed that these attributes were not as much affected by gamma irradiation but were affected with the passage of time in control sample of lentils.

3.2. Microbial analysis

The total bacterial count on nutrient agar for whole masoor control and irradiated sample was 5×10^4 to 4.1×10^5 and 1×10^4 to 2.3×10^5 cfu/ml during three observational intervals. In case of destoned masoor, control and irradiated sample showed total bacterial count from 1×10^5 to 2.1×10^6 and 1.5×10^5 to 5.3×10^5 cfu/ml. Bacterial count of Eto sterilized whole and destoned masoor was found to be 3×10^4 and 8×10^4 cfu/ml. No bacterial growth was observed on MacConkey agar. Observed bacteria were gram positive, rod shape and belonged to *Bacillus* species (*Bacillus subtilis*) (Figure 1).

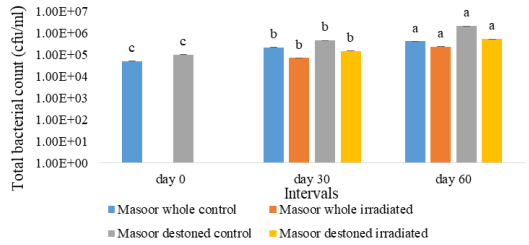

Figure 1: Total bacterial count of gamma irradiated control, whole and destoned masoor samples.

Similarly, potato dextrose agar was used for analysis of fungal count which in case of whole control and irradiated masoor sample was in range of 1.0×10^5 to 4.8×10^5 and 6×10^4 to 9×10^4 cfu/ml. For destoned control and irradiated masoor sample cfu/ml was from 2.1×10^5 to 8.1×10^5, 7×10^4 to 3.9×10^5. Fungal count of Eto sterilized whole and destoned masoor was found to be 6×10^4 and 1.5×10^5 cfu/ml. Fungal species observed were *Aspergillus flavus* and *Saccharomyces cerevisiae* (Figure 2). The microbial analysis for both samples is given in Figure 3.

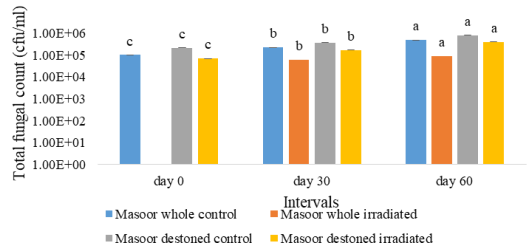

Figure 2: Total fungal count of gamma irradiated control, whole and destoned masoor samples.

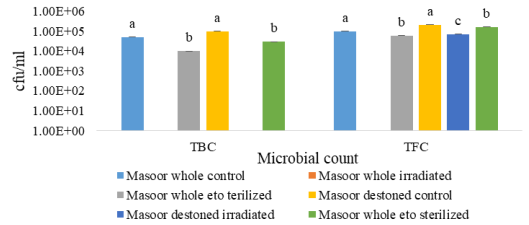

Figure 3: Total microbial count (bacterial and fungal) of gamma irradiated control, whole and destoned masoor samples.

3.3. Proximate analysis

Moisture content of control samples ranged from 9.95 to 11.04 % and 11.17 to 12.12%. For gamma irradiated whole and destoned masoor, moisture was 9.89-10.98% and 11.17-12.12% and for EtO sterilized whole and destoned masoor was 13.16 and 13.39% (Figure 4).

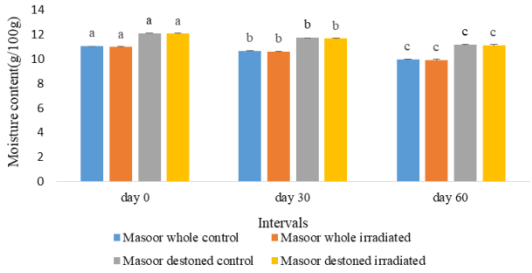

Figure 4: Proximate analysis for testing moisture content of gamma irradiated control, whole and destoned masoor.

Ash content for control of destoned masoor was varied from 4.11 to 3.53% and for control whole masoor was 3.54 to 2.85%. For EtO sterilized whole and destoned masoor ash content was 5.49% and 5.82%. Whereas for gamma irradiated whole and destoned masoor, ash content ranged within 2.85-3.54% and 3.48-4.01% (Figure 5).

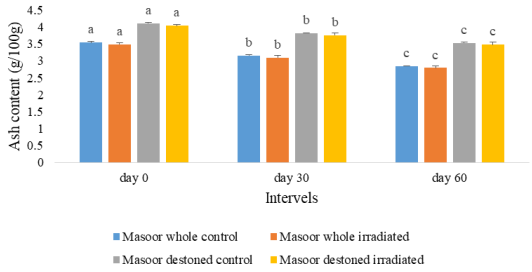

Figure 5: Proximate analysis for testing ash content of gamma irradiated control, whole and destoned masoor.

Fiber content of control both sample were in the range of 2.89 to 3.73% and 3.46 to 4.09%. For gamma irradiated and EtO sterilized whole masoor fiber content was from 2.82-3.68% and 3.03%. In case of destoned radiated and EtO sterilized masoor 3.41 to 4.02% and 3.11% fiber content was present (Figure 6).

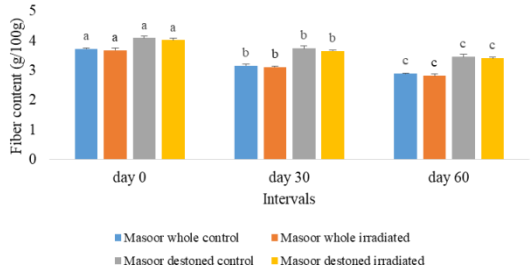

Figure 6: Proximate analysis for testing fiber content of gamma irradiated control, whole and destoned masoor.

A comparison of proximate analysis of gamma irradiated and EtO sterilized irradiated control, whole and destoned masoor for moisture, ash and fiber content is illustrated in Figure 7.

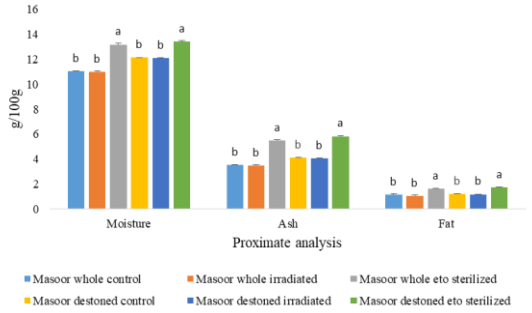

Figure 7: A comparison of proximate analysis of gamma irradiated and EtO sterilized irradiated control, whole and destoned masoor for moisture, ash and fiber content.

In case of control sample of whole and destoned masoor, fat content was 0.89 to 1.13% and 0.96 to 1.21%. Fat content of gamma irradiated whole and destoned masoor was 0.81 to 1.06% and 0.92 to 1.17%. For EtO sterilized whole and destoned masoor fat content was 1.64% and 1.72% (Figure 8).

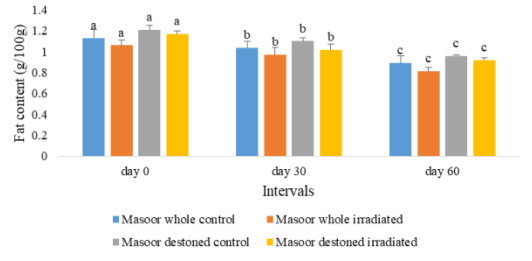

Figure 8: Proximate analysis for testing fat content of gamma irradiated control, whole and destoned masoor.

Protein content of whole control sample was 24.30 to 25.03% and for destoned control masoor it was 24.82 to 25.45%. Protein content of gamma irradiated whole masoor was 24.25 to 24.98% and destoned masoor was 24.77 to 25.37%. For ethylene oxide sterilized whole and destoned masoor protein was 23.62 and 24.07% (Figure 9).

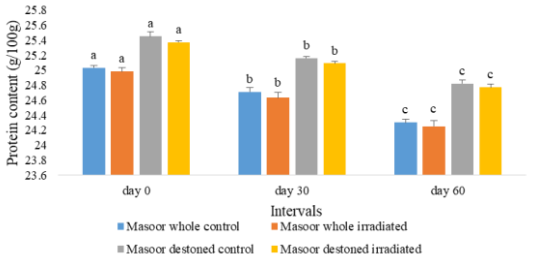

Figure 9: Proximate analysis for testing protein content of gamma irradiated control, whole and destoned masoor.

Carbohydrate content was calculated as total of results of all the other tests. Carbohydrate content of whole and destoned control masoor sample fall in range of 55.53 to 59.12% and 53.02 to 56.06% For EtO sterilized whole masoor carbohydrate content was 53.06% and for destoned masoor it was 51.88%. Carbohydrate content of gamma irradiated whole masoor was 55.81 to 59.44% and destoned masoor was 53.33 to 56.31% (Figure 10).

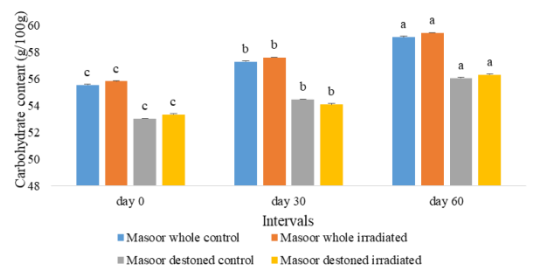

Figure 10: Proximate analysis for testing carbohydrate content of gamma irradiated control, whole and destoned masoor.

A comparison of proximate analysis of gamma irradiated and EtO sterilized irradiated control, whole and destoned masoor for fat, protein and carbohydrate content is illustrated in Figure 11.

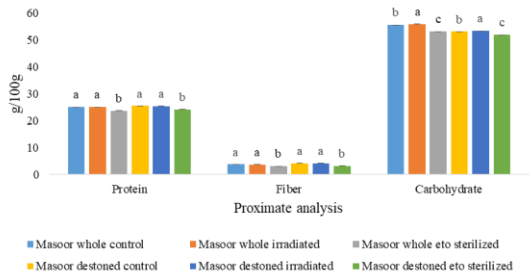

Figure 11: A comparison of proximate analysis of gamma irradiated and EtO sterilized irradiated control, whole and destoned masoor for fat, protein and carbohydrate content.

4. Discussion

In present study the sensory evaluation of lentils through attributes like color, texture, odor and visual defects of treated and control samples did not initially yield any observable changes. However, after 30 days of storage, unpleasant odor was observed in control sample of lentils and in irradiated sample at day 60. Furthermore, sprouting occurred in masoor destoned control sample initially during storage whereas gamma irradiated lentil did not show sprouting until after 60 days had passed. These moderate protective effects against sprouting are attributed to irradiation and are in accordance with results reported by Rajkowski and Bari (2012).

In current study, the microbial analysis revealed presence of gram positive bacteria belonging to *Bacillus* species (*Bacillus subtilis*) as previously reported by Haq *et al.* (2017) for chickpea. Similarly, an analysis of fungal species determined the presence of *Aspergillus flavus* and *Saccharomyces cerevisiae*. These fungal species have been discovered in stored grains such as maize (Chuck-Hernández *et al.*, 2012) and cereal grains (Abdel-Kareem *et al.*, 2019).

In current study, the proximate analysis was carried out in all samples. The moisture content of control samples ranged from 9.95 to 11.04 % and 11.17 to 12.12% which is analogous to that stated by Adsule *et al.* (1989) but lower than that stated by Muehlbauer and Summerfied (1985). Results showed that moisture reduced after the gamma irradiation whereas it was increased after EtO sterilization which is in agreement with other investigators (Aziz *et al.*, 2007; Mohamed *et al.*, 2015). This might be due to hindrance of senescence and inhibition of metabolic activities that moisture content is reduced in the irradiated lentils.

Ash content of control in present work was in accordance to previously reported works on destoned masoor (Huisman and Van der Poel, 1994). However, ash content of whole masoor was in accordance to that reported by Sulieman (2007) and lower than that of Zia-Ul-Haq *et al.* (2011). Results showed that the ash content in the EtO sterilized whole and destoned masoor was decreased after gamma irradiation whereas it was increased after EtO sterilization. Bhat *et al.* (2008) also stated that ash on irradiation showed a decrease dependent on dose.

The fiber content of control samples was within the range as stated by Sulieman (2007) but lower than the range reported by Hulse (1989). Results showed that, fiber content was reduced after gamma and ethylene oxide treatment. Sandev and Karaivanov (1979) demonstrated that radiations decrease fiber content due to depolymerization.

The fat content of control sample was found to be lower than that reported by Hulse (1989) and Duke (1981). The results

demonstrated that the fat content increased by EtO sterilization while it reduced after gamma irradiation treatment in both forms of lentils which might have happened due to lipid oxidation process that was sped up by gamma radiation. Nawar (1977) mentioned that gamma irradiation causes the oxidation of unsaturated fats and phospholipids decomposed into free fatty acids.

Protein content of control sample was analogous to Hawtin *et al*. (1977) but lower than values reported by Adsule *et al*. (1989) and Sulieman (2007). It was demonstrated through results that protein content decreased after both treatments. This decrease could be due to protein denaturation, oxidation and limited activity of pectinase enzyme which was induced by gamma radiation. Fan *et al*. (2003) mentioned the reason for reduction in protein content could be due to deamination of peptide and disulfide bonds which resulted in the formation of radiolytic chemicals in radiated product.

In current work, the carbohydrate content was calculated as total of results of all the other tests. The carbohydrate content of whole and destoned control masoor samples fell in ranges which were similar to values reported by Zia-Ul-Haq *et al*. (2011) but lower than those reported by Adsule *et al*. (1989) and Muehlbauer and Summerfied (1985), while these values are greater than those stated by Sulieman (2007). Results of current study showed increase in carbohydrate content in lentils. Lu *et al*. (2012) reported that irradiation increased the glucose content. Ajlouni and Hamdy (1988) mentioned that sucrose content increased with irradiation under certain conditions.

5. Conclusions

The results of current research work revealed that gamma irradiation technique was more effective in reducing microbial count as compared to EtO sterilization. Nutritional content of gamma irradiated lentils showed a slight difference from the control sample, whereas the difference observed for EtO sterilized lentils was significant. Microbial load was significantly reduced at 8 kGy dose. So, it was concluded that 8 kGy was appropriate dose of gamma radiation that can be applied on lentils to achieve desired quality legumes for consumption and marketing.

6. Author's Contribution

Dr. Saiqa Ilyas conceived and designed the experiment. All authors contributed equally to the experimental work, data collection, data analysis and write-up.

7. Conflict of Interest

The authors have declared no conflict of interest.

8. Novelty Statement

The increasing global threat of antibiotic resistance has created a dire need for effective control measures against threats posed to stored grains. Gamma irradiation technique is a low cost and effective technique which can be used effectively in reducing microbial count. Additionally, the nutritional content of gamma irradiated lentils showed only a slight difference from the control sample.

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