Research Article

Effect of Gamma Radiation on Microflora, Proximate Analysis and Sprouting of Garlic

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Abstract | Garlic (*Allium sativum* L.) belongs to the genus *Allium* and family *Alliaceae* is an important and widely cultivated plant. It is used as flavor in various dishes due to its constiuents.composition. Gamma radiation is one of the sterilization technique involves total demolition of all microorganisms and their spores in foods. In recent study garlic samples were treated with three different doses of gamma radiation such as 0.5, 1 and 2 kGy. Effects of gamma radiation were analyzed for different aspects like microflora, moisture, fat, ash, fiber, phenolic content, sprouting inhibition, physiological weight loss, pyruvate analysis and sugar contents. Gamma radiation affected these parameters at higher doses but at 0.5 kGy no significant change was observed. 0.5 kGy prouting was inhibited and microflora was reduced at this dose without affecting its nutritional and sensory qualities. So, it was proved to be the best optimized dose was 0.5kGy.

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1. Introduction

Garlic is herbaceous plant belongs to family *Alliaceae* comprises of 280 genera and 4000 species including onions, leeks, shallots, rakkyo and chives which can be used as a vegetable or medicinal herb. There are two main subspecies of garlic named as Softneck garlic and Hardneck garlic. Garlic is best known medical plant that is used to treat infection, cold, diabetes and heart diseases, lowering of blood pressure, glucose concentration, cholesterol as well as the prevention from arteriosclerosis and tumors (Tasi *et al*., 2012). For the treatment of various ailments garlic is employed as folk medicine all over the world (Ali *et al*., 2000). The composition of garlic bulbs greatly depends upon the soil fertility, agronomic practices, climate, postharvest storage conditions and these factors determine the flavor intensity, quality and nutritional values (Zhou *et al*., 2012). Garlic contains proteins (17.35%), carbohydrates (73.3%), moisture (4.88%) and fiber contents (0.68%) (Niwunki *et al*., 2005). It also contains Sulphur containing compound allicin which is largely responsible for pungent odor of the garlic (Block *et al*., 2010). Garlic plant is infected by a large number of bacteria, fungus, viruses and nematodes. Different species cause rots of garlic bulbs. White rot is caused by *Sclerotium cepivorum* when the temperature is low. Basal rot is caused by *Fusarium culmorum* infect the stem plate of garlic. Blue and green spores of *Penicillium spp*. can cause blue mold rot of garlic bulbs (Tucker, 2000).

Gamma radiation is a process of food sterilization in which food is treated with certain ionizing radiation

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to kill microorganisms such as bacteria, viruses, or insects hence this technique is of great significance (Farkas, 2011).

2. Materials and Methods

2.1 Plant materials

Hard neck fresh garlic were collected from local market of Lahore in Pakistan. All the samples were packed in brown paper and send to PARAS (Pakistan Atomic Radiation and Services) for irradiation at different doses such as 0.5, 1 and 2 kGy. All the radiated and un-irradiated samples were kept at room temperature (37^0C) in dark.

2.2 Sample preparation

For proximate analysis, garlic cloves were peeled and sliced in half longitudinally and homogenized in waring blender at the ratio of 1 ml of water per gram of garlic. The homogenate was allowed to stand at room temperature for 10 min then an aliquot of the sample was transferred to 2 ml centrifuge tube and centrifuged at 10,000 rpm for 10 min. Pellet was discarded and supernatant was taken for further test analysis.

2.3 Chemical analysis

Sensory evaluation was done by Hedonic 9-point scale that ranging from "Like extremely" to "Extremely dislike" given in the table (Nicolas *et al*., 2010).

Physiological loss in weight was determined by following formula: (Jamali *et al*., 2012).

$$PLW\ (\%) = [P_1 - P_2 \div P_1] \times 100$$

Where;
P_1 = Initial weight and P_2 = Weight after n days.

AOAC (2005) method was used for determination of moisture content. Abut of sample were placed in a pre-weighed, dried crucibles and exposed to 120 ˚C for 2 h and 30 min in oven. Then cooled in a desiccator and finally weighed. The moisture content in the sample was determined by this formula.

$$\text{Moisture (\%)} = \frac{(\text{Wt. of original sample} - \text{Wt. of dried sample})}{\text{Wt. of original sample}} \times 100$$

Total sugar was determined by Phenol Sulphuric acid method. Extract (0.2 ml) was mixed with 1.8ml Con.H_2SO_4 and 3ml of 5% phenol. After 20 min of incubation period at room temperature, the optical density was taken at the wavelength of 480nm by spectrophotometer. The concentration was measured by the standard curve of glucose (Nielsen, 2009).

Reducing sugar was examined by DNS method. 0.2 ml extract was taken and makeup the volume up to 1ml and 2ml DNS solution was added. Then heated the mixture at water bath for 5min. and after cooling made the volume up to 10ml by adding 7ml distilled? water. The optical density was taken at 540nm by spectrophotometer and concentration was measured by standard curve of glucose (Macias *et al*., 2001). Non- reducing sugar was obtained by subtracting the reducing sugar from total sugar (Revathy *et al*., 2007).

Pyruvic acid determined by 0.0125% 2, 4-dinitrophenylhydrazine (DNPH) solution. 1 ml of a 0.0125% solution of 2, 4-dinitrophenylhydrazine in 2N HCl was added to 2ml sample of diluted and filtered homogenates. 5 ml of 0.6 N NaOH added after 15 min in a water bath at 37 ˚C and the absorbance was measured instantaneously at 420 nm by spectrophotometer. Sodium pyruvate (10 μM/ml) used as standard.

For the determination of ash AOAC (2011) method was used. Three grams of control and radiated sample were weighed, grinded in a piston mortar and put into pre-weighed crucibles. Samples were then placed in Muffle furnace at 500˚– 550˚C temperature for 4 to 6 hours till the samples became ash. Weight of ash was calculated by:

Weight of ash= weight of crucible+ash–weight of crucible
% of ash was calculated as Ash %= wt. of ash (g)/wt. of sample × 100

For the determination of crude Fat AOAC (2011) method was used. Three grams of de-moisture sample of each un-radiated and radiated samples were added into pre-weighed thimbles. In a Soxhlet apparatus, 6 hours were required for extraction with 300 ml of ethanol. Loss of weight was calculated as.

Loss in weight = wt. of thimbles + de-moisture sample – (weight of thimbles – fat free sample)
Fat % = loss in weight (g)/wt. of sample × 100

Crude fiber is the loss of ignition of dried residue remaining after digestion of sample with H_2S0_4 and NaOH. 4g of the sample whose fat was removed

was taken in reflux flask and 100ml of 1.25% H_2SO_4 was added in sample and refluxed for half an hour. The sample solution was filtered with silky cloth and washed with 200ml of hot distilled water. Then the filtrate was again reflux with 1.25% NaOH for half an hour. Sample solution was washed with 200 ml of hot distilled water on pre-weighted whatman filter paper. The filtrate will be then dried in an oven and weighed; the sample was C1. After drying the filtrate was placed in muffle furnace at 500 °C to 550°C where filtrate became ash. The ash sample was C2.

Percentage of crude fiber= C1-C2/Original Sample × 100

Protein content was estimated by Kjeldahl Method by AOAC (2011).

Digestion of sample: 0.4g of sample and 0.4g of digestion mixture was taken in digestion flask. 15 to 20ml of concentrated H_2SO_4 was added. Then the flask was heated till solution became clear. After digestion the flask was cooled. Clear solution was added into 100ml of volumetric flask and diluted with distilled water up to the mark.

10ml of digested sample was taken in distilled flask and was placed under receiving end of condenser tube. Then 5ml of 2% boric acid was taken into a beaker of 200ml and dip the mouth of condenser into it to avoid the release of ammonia gas. The mixture was heated, just after boiling 15 to 20ml of 40% NaOH solution was added. The red color of boric acid solution was changed due to conversion of ammonia into ammonium borate. Boric acid solution became colorless. Allowed the reaction to continue till volume of (boric acid beaker) raised up to 25ml.

Titration: Titrated the boric acid solution by using standard solution of 0.7N HCl till the original color of boric acid solution restore. The amount of 0.7N HCl used.

The total Phenolic content was examined with an improved method with slight modifications in Folin Ciocalteau (Ghafoor *et al.*, 2012). For the determination of phenolic compound, samples were prepared by adding 0.25ml Folin Ciocalteau reagent, 0.25 ml garlic extract in dilute form and then added 3.5 ml of distilled water. When Folin Ciocalteau reagent was added then sample became more diluted with distilled water to get the final volume of 4.6 by mixing carefully. When final sample preparation was made then they kept at room temperature for the ten minute and after that added 1ml of 20% Na_2CO_3 solution followed by vortex mixing. The optical density was taken at 765nm on spectrophotometer. On a spectrophotometer absorbance was taken at 765nm.

2.3 Microbial analysis

Irradiated samples were analyzed after 20 days to determine the microbial count on Nutrient agar (for isolation of bacteria), MacConkey agar (for isolation of Gram negative enteric bacilli), and PDA (for isolation of fungi). Bacteria and fungus were identified by different staining methods.

Isolation of microorganisms: Isolation of microflora was carried out by serial dilution method. Dilutions were prepared aseptically by washing the garlic thoroughly with 100 ml amount of 0.9% sterile saline water and serial dilutions were prepared. 100 µl from dilution was transferred on agar plates. Then they were incubated at 37°C for 24 hours.

Recording results: The colonies were counted after incubation period. The arithmetic mean of all replicates was calculated and viable bacterial count of bacteria was determined by standard formula of Colony Forming Unit per ml (CFU/ml) (Fuselli et al., 2003).

CFU/ml= No. of colonies × Dilution factor/Amount plated

Bacterial morphology: The morphological characters of bacterial colony such as forms, elevations and margins were observed. The structural and functional attributes of bacteria were examined under light microscope. Following staining techniques and tests were performed (Benson, 2001).

Endospore Staining: Bacterial cultures (24 hours old) were tested for the presence of endospores by staining with malachite green. The smear was passed over flame and a piece of blotting paper was placed on it. The blotting paper was saturated with Malachite Green stain. Then this slide was passed over steam for 5 minutes recover the slide with Malachite Green as required. After cooling and washing with water, it was counterstained with safranin for 30 seconds. It was rinsed again and blot dried and observed under light microscope (Benson, 2001).

Gram staining technique: This gram staining technique was performed to determine Gram

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characteristics of bacteria. After heat fixing the bacterial smear, Crystal violet was first applied for 1 minute and rinsed with water. It was followed by addition of mordant iodine for 1 minute which fixed the stain. After rinsing with water, the slide was washed with alcohol for 30 seconds. The bacteria were subsequently stained with the safranin dye, the counterstained for 1 minute and rinsed again. The slide was blot dried and observed under light microscope.

3. Results and Discussion

Sensory analysis was done periodically after 20 days of interval. The sensory qualities were badly affected at higher doses but acceptable results were observed at low doses (0.5 kGy) (Figure 1). The sensory properties were affected in terms of ? at high doses due to the reason that temperature become so high so that they do not resist the change. The physiological weight in loss was higher at 1 and 2 kGy after 40 days as compared to 0.5 kGy. The maximum weight values? loss was observed in non-radiated sample (Figure 2). Chachin and Iwata (1998) mentioned that weight loss occurred due to increase in respiration rate and also change in membrane function of cell and delay in wound healing.

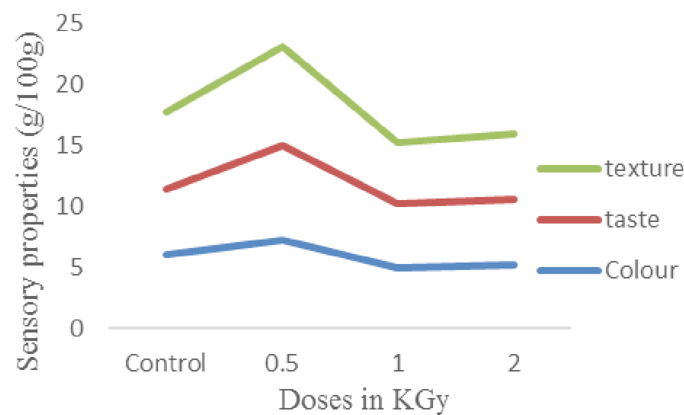

Figure 1: Effect of gamma radiation on sensory properties of garlic after 40 days.

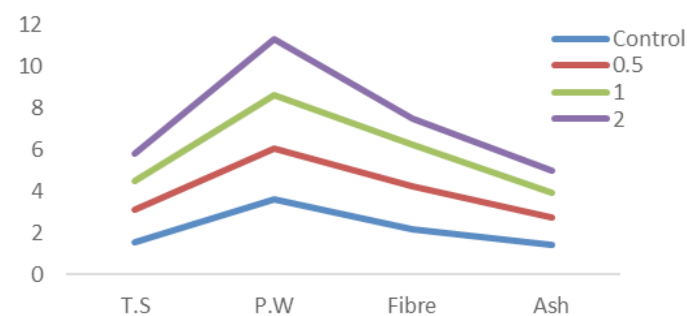

Figure 2: Gamma radiation effect on total sugar, physiological weight loss, fiber and ash contents.

Moisture content was decreased with the passage of time. It was higher in un-radiated sample as compared to radiated. After 40 days, the moisture content in non-radiated was 8.5% but in radiated sample, significant changes were observed at higher doses (1 and 2 kGy). The moisture content was lower at 0.5 kGy dose. Loss in moisture content was due to dehydration and physiological weight loss. Rao *et al*. (2000) reported that irradiation dose at 0.5 kGy has no effect on moisture content of the garlic and the study also concluded that low dose radiation has almost no effect on moisture content. It was observed the total sugar decreased with passage of time. As the radiation dose increased, the total sugar decreased. Smallest changes were observed in the samples of 0.5 kGy (Figure 3). The ash content of the garlic has the same result as that of moisture content of garlic with the increasing dose of gamma radiation the ash content goes on decreasing and the highest ash content was in control which was 2.06% which was related to the study of Rao *et al*. (2000) in which the ash content was 2.83% in a variety of garlic. Radiation dose 0.5kGy produced 1.53% ash contents that decreased by increasing the doses (Figure 2). Fiber content went on decreasing as the radiation dose was increased. The fiber content in garlic was 2.3% and then further decreased in radiated doses. Gamma irradiation resulted in considerable decrease in protein content. It was reduced from 8 % in control sample to 5% at the highest dose of 2 kGy. The protein content of garlic in control sample was closed to the study of Piątkowska *et al*. (2015) (Figure 2).

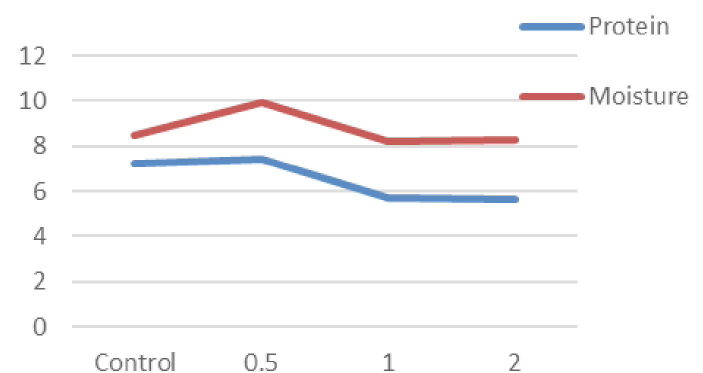

Figure 3: Protein and moisture content effected radiation after 40 days.

The fat content in garlic's composition is low. So, when the garlic was exposed to radiation the fat content decreased. When the radiation dose increased there was a prominent decrease in the fat content of garlic (Figure 4). There was decrease in phenolic content of

garlic with the increasing time period. The phenols were present in traces in control sample like 0.035% and further decreased in radiated samples. The results by Kavalcova *et al.* (2014) showed that the content of phenols in garlic is in low amount as compared to other spices (Figure 4).

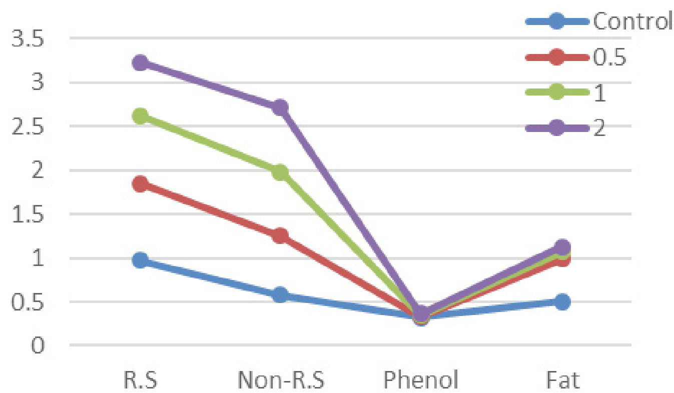

Figure 4: Reducing and non-reducing sugar, phenol Gamma fat content after 40 days in control and radiated sample.

It was reported that total sugar decreased due to the activation of hydrolytic enzymes that convert polysaccharides into mono and di-saccharides (Maity *et al.*, 2004). In contrast to total sugars, reducing sugar increased with the passage of time. Maximum reducing sugar was in non-radiated sample as compared to radiated samples. Significant changes were observed at 0.5 kGy (Figure 2). The reducing sugar was increased due to the reason that fructan depolymerases hydrolyzes the fructan that convert the di-saccharides into glucose and fructose Ogata *et al.* (2007). Non-reducing sugar gradually decrease in radiated sample as compared to non-radiated sample. At higher radiation dose such as sample radiated with 1 and 2 kGy caused less decreased in non -reducing sugar contents. Similar findings were reported by Ogava *et al.* (1990). As gradual decrease occurs in non-treated sample then the sprouting commenced. Reducing sugar increase but non reducing sugar decreases. The trend of glucose and fructose are correlative but sucrose showed slightly varying were found by Chope *et al.* (2007) (Figure 4).

Current study shows that pungency is less in radiated sample as compared to non-radiated samples. The gamma radiation restricts the endogenous enzymatic reaction hence level of pyruvic acid concentration decreased. Sample radiated with 0.5 kGy showed significant effect on pungency so at low dose radiation did not effect on pungency level as reported by Pezutti *et al.* (2005) (Figure 5).

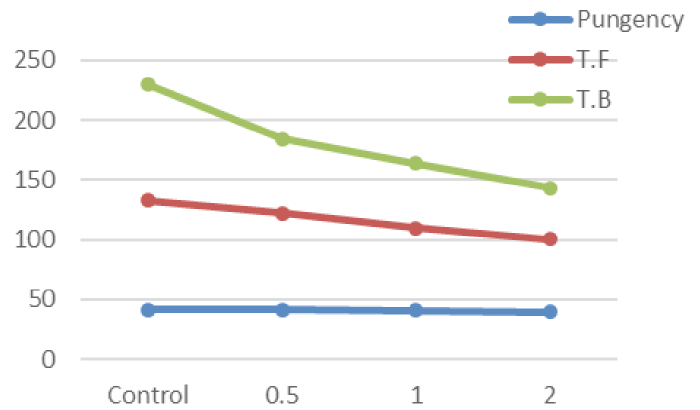

Figure 5: Gamma radiation effect on pungency, total bacteria and fungi after 40 days.

The microflora was reduced immediately after radiation. The reduction of microflora was due to the effect of gamma rays which caused the DNA damage by random strikes and chemical changes also occur which include reduction of nucleic acid synthesis etc. as reported by Grandison *et al.* (2012).

The control samples had more bacterial count (4.2 × 10^4 cfu/ml) as compared to irradiated ones (3.7×10^4 cfu/ml). Variation occurred among irradiated samples. Bacterial count was more on samples irradiated with 0.5 kGy (3.7×10^4 cfu/ml) as compared to 1 kGy (2.4×10^4 cfu/ml) and 2 kGy (2.3×10^4 cfu/ml). It may be due to the reason that at medium doses such as 1-10 kGy, inhibition of microflora occurs in food products. Similar results were found by Pezutti *et al.* (2005) (Figure 5).

In a recent study, irradiated proved to be efficient in eliminating *Esherichia coli*, *Bacillus subtilis* and *Salmonella* species found on garlic. It was similar to the study of Pezutti *et al.* (2005). Garlic was also affected greatly by infection of fungus such as *Aspergillus niger*, *Aspergillus flavus*, *Fusarium oxysporum* and *penicillium* fungus. This type of fungi was isolated and identified. Methylene blue staining was used for this purpose. These fungi were previously isolated and identified by Ghangaonkar *et al.* (2013) (Figure 5).

Bacteria were differentiated as gram positive or gram negative by gram staining technique on the basis of cell wall composition. Gram positive bacteria were stained purple because they have thick peptidoglycan layer and relation the CV-I complex. But gram positive cells do not retain CV-I complex because of

the presence of thin peptidoglycan layer. Both gram positive and gram negative bacteria were isolated from garlic. Endospore staining differentiates the vegetative cells and endospore producing cells. Malachite green stain is used which stain the endospore cells and vegetative cells are decolorized by water. The bacteria present on garlic were endospore forming. It was previously reported by Benson (2001).

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Author's Contribution

Dr. Shagufta Naz is the principal author and developed the idea of research work. Ayesha Javed woked on the sprout inhibition, Ayesha Saleem worked on microbial, Khadija Murtaza performed the proximate analysi, Rukhama Haq and Akbal Hayat worked on data analysis and Neelma Munir furnished the final draft of the manuscript.

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