REDUCTION OF MICROFLORA AND FREQUENCY OF SPROUTING IN GAMMA IRRADIATED POTATOES

ROHEENA ABDULLAH[1], UNEEB ATHAR[1], MEHWISH IQTEDAR[1], AFSHAN KALEEM[1], MAHWISH AFTAB[1] AND SHAGUFTA NAZ[1]

[1]Department of Biotechnology, Lahore College for Women University, Lahore, Pakistan
**Corresponding author e-mail: roheena_abdullah@yahoo.com*

Abstract

The potato (*Solanum tuberosum*) is an important food crop, grown largely as a carbohydrate staple and also for industrial purposes. Of the various physiological factors which affect potato storage, sprouting is the most obvious manifestation of deterioration. In the present study potatoes were irradiated at 0.05, 0.10, 0.15 kGy to inhibit sprouting and evaluate microflora. The experimental and control group of potatoes were analyzed for the microbial load and growth of sprouts. The remarkable reduction in bioburden was noted when potatoes were subjected to 0.15kGy irradiation. The irradiated potatoes showed less viable microbial count than the non-radiated ones even after three weeks of irradiation. This study indicated that higher irradiation levels proved to be most effective in retarding the sprouting of potatoes stored at ambient conditions and enhancing the shelf life up to 14 days.

Introduction

Potato (*Solanum tuberosum*), known as "King of the vegetables", ranks as forth most important crop after wheat, rice and maize. It is a starchy tuberous crop belongs to family *Solanaceae*. Potato is basically an English word derived from Spanish *Patata* (CIP *et al.*, 2006). Pakistan is rewarded with huge agricultural assets. The annual production of fruit and vegetables in Pakistan is estimated to about 13.7 million tons where the potatoes hold a share of 2 million tons, varying from year to year.

Post-harvest losses of potato account for about 40-50% of total losses per year causing reduction in their prices. Environmental conditions increase the physiological losses while pathogenic losses are caused by the attack of fungi, bacteria and insects etc. The main reasons of storage losses are the respiration, sprouting, water loss from potato tubers, pathogenic invasion, alterations in chemical composition and damage caused due to temperature fluctuation (Thompson, 2008). Sprout development stage begins with several eyes sprouting when the tuber is in storage. Sprouting may result in the loss of marketable weight, shrinkage and softening of tubers and reduction in the nutritional contents. Peeling losses had also increased due to sprouting, and the sprouts of potatoes contained toxic alkaloid called solanin. These changes are proved to have negative effects on the processing quality (Suttle, 2004).

Due to close proximity of potatoes with soil, the exterior of the potatoes are normally contaminated with bacteria and fungi. Fungi are a common invasive pathogen of potatoes includes *Collectotrichum coccodes, Alternaria alternata, Alternaria solani, Fusarium* spp. *Phoma exigua* etc. (Bryan *et al.*, 1992). The reported pathogenic bacteria associated are *Ralstonia solanacearum, Streptomyces scabeii, Clostridium botulinum, Listeria monocytogenes, Pectobacterium carotovorum* etc. (Heisick *et al.*, 1989). Microbial safety of potato tubers and products had become vital due to the expanding demands of consumers (Doan *et al.*, 2000).

Fresh potatoes are not available throughout the year so the storage of potatoes becomes necessary. Drying, freezing, smoking, salting, preservatives, canning and pesticides are some of the methods used for the preservation of potatoes but radiation treatment can be used as an alternative procedure for storage. Irradiation is a batch process that can be used to increase the shelf life of potato. Irradiation causes the inactivation of organism due to DNA damage and to a lesser extent protein denaturation. The use of irradiation proved to be effective on both the cells and spores so its use is increasing to ensure microbiological safety of fruits and vegetables (Corbo *et al.*, 2009). The application of irradiation may be an alternative treatment for controlling undesirable changes in potatoes during long-term storage. Appropriate use of irradiation can extend shelf life, reduce the requirement of chemicals for preservation, pest control, and produce sterilized products (controlling the microorganisms) that can be stored without refrigeration, delay the ripening of fruits and vegetables and limit quality deterioration of stored tuber and bulb crops by preventing postharvest sprouting (Rezaee *et al.*, 2013)

The aim of the study is to increase the export value of potatoes of Pakistan which is declining due to post harvest losses, as well as enhancement of storage life of potatoes.

Materials and Methods

Sample collection and gamma irradiation: Fresh, un-injured, uniform size and age samples of Potatoes(white variety) were collected from local Market of Lahore. They were sealed in perforated bags and labeled with desired doses of gamma radiation (0.05 kGy, 0.10 kGy and 0.15 kGy). The sealed potatoes were divided into two groups i.e. control and experimental groups and sent to Pakistan Radiation Services (PARAS) for irradiation. After radiation, the radiated samples were stored in dry place at ambient temperature and microflora along with the occurrence of sprouting was observed up to 21 days.

Isolation of microorganisms: In order to isolate microorganisms serial dilution method was used. Dilutions were made by shaking a sample of potato in 300 mL of saline water. 100 µL of sample from every dilution was spread over the already prepared plates of different media including Nutrient agar, MacConkey agar, Salmonella-Shigella agar for isolation of different types of bacteria and potato dextrose agar for isolation of fungi. The plates containing media for bacterial growth was incubated at 37°C for 24 hours while those containing PDA were incubated at 30°C for 72 hours. Colony-forming units (CFU) was calculated according to Yousef and Carlstrom (2003)

$$CFU/mL = \frac{\text{Colony count on plate}}{\text{total dilution of tube(used to make plate)} \times \text{amount plated}}$$

Statistical analysis: All experimental data were subjected to statistical analysis using Costat 6.4. The values were compared by the use of Duncan's multiple range test. In addition to this standard mean error were also calculated. The significance is presented at the level of $P \leq 0.05$.

Results

Isolation of bacteria using nutrient agar: Table 1 revealed a great influence of radiation doses on the total viable bacterial count applied to potato tubers stored at ambient temperature. After storing potatoes at ambient conditions, the control group of potatoes showed initial bacterial count of 15.9×10^6 cfu/mL on nutrient agar. This count increased upto 32.5×10^6cfu/mL after three weeks. While the irradiated potatoes exhibited a reduction in the total viable bacterial count to great extent. The highest reduction was shown in the results of highest dose subjected to potatoes i.e. 0.15 kGy. The initial bacterial count observed were 13.5×10^6 cfu/mL, 12.9×10^6 cfu/mL and 10.3×10^6 cfu/mL for the applied doses of 0.05 kGy, 0.10 kGy and 0.15 kGy respectively. After 14 days of storage, the number of bacterial colonies increased up to 21.2×10^6 cfu/mL, 17.5×10^6 cfu/mL, 12.8×10^6 cfu/mL and 11.1×10^6 cfu/mL for Control, 0.05 kGy, 0.10 kGy and 0.15 kGy respectively. In case of irradiated potatoes, there was a gradual increase in the bacterial count week-wise as well but that was less as compared to their respective control groups. The average colonies of bacteria observed at highest dose (0.15 kGy) even at third week were 14.6×10^6 cfu/mL which was less than the viable bacterial count of control potatoes after first week of storage.

Enumeration of bacteria on MaConkey agar: Table 2 demonstrated the control group of potatoes exhibiting high growth of viable enteric bacteria on MaConkey agar as compared to experimental group of potatoes. The initial bacterial count observed in control was 8.3×10^6 which gradually increased up to 12.5×10^6 cfu/mL. Potatoes irradiated at 0.05, 0.10 and 0.15 kGy had an initial total viable bacterial count of 5.2×10^6 cfu/mL, 3.1×10^6 cfu/mL and 2.1×10^6 cfu/mL respectively. The irradiated potatoes showed less viable count than the non-radiated ones even after three weeks of irradiation. For example, the highest dose 0.15 kGy harbored the bacterial count of 4.3×10^6 cfu/mL which was less than that of control group of first week. API strip were used for the identification of bacteria.

Enumeration of bacteria on Salmonella-Shigella agar: The same trend of bacterial reduction was observed in SS agar as that observed on nutrient and MacConkey agar, however, there were much less colonies of bacteria as compared to the number of colonies on other media. The initial value of viable bacterial count of control group was 1×10^6cfu/mL after first week which increased up to 1.5×10^6 cfu/mL after 21 days of storage. Highest radiation dose (0.15 kGy) exhibited minimum viable count even after three weeks i.e. 0.1×10^6 cfu/mL. Potatoes irradiated at 0.05 kGy, 0.10 kGy and 0.15 kGy had an initial viable count of 0.7×10^6 cfu/mL, 0.4×10^6 cfu/mL and 0.1×10^6 cfu/mL, respectively. No *Salmonella* spp. and *Shigella* spp. were exhibited by the both the control and irradiated potato tubers indicating their complete absence on the potato surface. However colonies of *Escherichia coli* were seen on the SS plates of control and irradiated tubers.

Table 1. Impact of different gamma radiation doses on bacteria present on the potato using nutrient agar as testing medium.

NUTRIENT AGAR			
Gamma radiation doses (kGy)	**Days of storage**		
	7	14	21
0	$15.9 \times 10^6 \pm 0.632^{a}$	$21.2 \times 10^6 \pm 1.019^{a}$	Not-countable
0.05	$13.5 \times 10^6 \pm 1.166^{b}$	$17.5 \times 10^6 \pm 0.632^{b}$	$19.0 \times 10^6 \pm 1.532^{b}$
0.10	$12.9 \times 10^6 \pm 1.414^{c}$	$12.8 \times 10^6 \pm 1.649^{c}$	$17.2 \times 10^6 \pm 1.414^{c}$
0.15	$10.3 \times 10^6 \pm 1.565^{d}$	$11.1 \times 10^6 \pm 0.894^{d}$	$14.5 \times 10^6 \pm 1.0198^{d}$

*Each value in a column is the mean obtained from the five replicates and ± shows the square mean error among the replicates. Superscripts indicate that mean difference is significant at $p \leq 0.05$ by Duncan's New Multiple Range Test.

Table 2. Impact of different gamma radiation doses on bacteria present on the potato using MaConkey agar as testing medium

MACONKEY AGAR			
Gamma radiation doses (kGy)	**Days of storage**		
	7	14	21
0	$8.3 \times 10^6 \pm 1.532^{a}$	$11.1 \times 10^6 \pm 0.894^{a}$	$12.5 \times 10^6 \pm 1.265^{a}$
0.05	$5.2 \times 10^6 \pm 0.484^{b}$	$8.6 \times 10^6 \pm 1.356^{b}$	$9 \times 10^6 \pm 0.632^{b}$
0.10	$3.1 \times 10^6 \pm 1.72^{c}$	$5.2 \times 10^6 \pm 0.632^{c}$	$5.6 \times 10^6 \pm 0.565^{c}$
0.15	$2.1 \times 10^6 \pm 1.66^{d}$	$3.1 \times 10^6 \pm 0.489^{d}$	$4.3 \times 10^6 \pm 1.414^{d}$

*Each value in a column is the mean obtained from the five replicates and ± shows the square mean error among the replicates. Superscripts indicate that mean difference is significant at $p \leq 0.05$ by Duncan's New Multiple Range Test.

Table 3. Impact of different gamma radiation doses on bacteria present on the potato using Salmonella-Shigella agar as testing medium.

SALMONELLA-SHIGELLA AGAR			
Gamma radiation doses (kGy)	**Days of storage**		
	7	14	21
0	$1 \times 10^6 \pm 1.232^{a}$	$1 \times 10^6 \pm 1.265^{a}$	$1.5 \times 10^6 \pm 0.894^{a}$
0.05	$0.7 \times 10^6 \pm 0.632^{b}$	$0.6 \times 10^6 \pm 0.632^{b}$	$0.8 \times 10^6 \pm 0.632^{b}$
0.10	$0.4 \times 10^6 \pm 0.894^{c}$	$0.3 \times 10^6 \pm 0.894^{c}$	$0.4 \times 10^6 \pm 1.019^{c}$
0.15	$0.1 \times 10^6 \pm 0.283^{d}$	$0.1 \times 10^6 \pm 0.282^{d}$	$0.1 \times 10^6 \pm 0.282^{d}$

*Each value in a column is the mean obtained from the five replicates and ± shows the square mean error among the replicates. Superscripts indicate that mean difference is significant at $p \leq 0.05$ by Duncan's New Multiple Range Test.

Table 4. Impact of different gamma radiation doses on fungi present on the potato tubers using Potato Dextrose agar as testing medium

POTATO DEXTROSE AGAR			
Gamma radiation doses (kGy)	**Days of storage**		
	7	**14**	**21**
0	$8.5 \times 10^6 \pm 1.5^{a}$	$10.5 \times 10^6 \pm 0.984^{a}$	$14.9 \times 10^6 \pm 1.166^{a}$
0.05	$5.2 \times 10^6 \pm 1.095^{b}$	$7.4 \times 10^6 \pm 1.469^{b}$	$12.0 \times 10^6 \pm 0.84^{b}$
0.10	$4.9 \times 10^6 \pm 1.0198^{c}$	$6.6 \times 10^6 \pm 1.166^{c}$	$10.6 \times 10^6 \pm 1.649^{c}$
0.15	$3.5 \times 10^6 \pm 1.23^{d}$	$4.2 \times 10^6 \pm 1.0198^{d}$	$7.8 \times 10^6 \pm 1.0198^{d}$

*Each value in a column is the mean obtained from the five replicates and ± shows the square mean error among the replicates. Superscripts indicate that mean difference is significant at $p \leq 0.05$ by Duncan's New Multiple Range Test.

Table 5. Effect of different gamma radiation doses on sprout inhibition of potatoes stored at ambient temperature.

Time interval	Control	0.05 kGy	0.10 kGy	0.15 kGy
Week1	No sprouting	No sprouting	No sprouting	No sprouting
Week2	Sprouted	Sprouted	No sprouting	No sprouting
Week3	Sprouted	Sprouted	Sprouted	No sprouting

*At ambient conditions

Enumeration of fungi on potato dextrose agar: Table 4 showed the fungal count of potatoes stored at ambient conditions. Fungal load of potato tubers was considerably higher than that seen for the total bacterial load on different nutrient media. Fungi require higher doses (up to 3 kGy) for their complete elimination. However, the selected doses also exhibited reduction in fungal count. The control potatoes harbored the greatest fungal load with a total viable fungal count of 8.5×10^6cfu/mL on the 7^{th} day of storage which further exploited to 14.9×10^5 cfu/mL until 21 days. Potatoes irradiated at 0.05 kGy after three weeks showed highest fungal growth among irradiated group. A Significant reduction was noted when samples were irradiated at 0.15 kGy. The initial fungal count was 5.2×10^6 cfu/mL, 4.9×10^6cfu/mL and 3.5×10^6 cfu/mL for the selected doses of 0.05 kGy, 0.10 kGy and 0.15 kGy respectively. Fungi were identified on the basis of microand macroscopic properties. The fungi isolated from the potatoes were *Aspergillus niger, Alternaria alternata*, *Alternaria solani, Fusarium* sp. and *few* yeasts species.

Sprout inhibition: Table 1 depicted that there was a significant reduction in the sprouting as the radiation dose increased (0.15kGy). 0.15 kGy completely inhibited the sprouting up to 21 days after irradiation. While the potatoes showed occurrence of sprouting after two and three weeks. 0.05 kGy inhibited sprouting until one week, after that those potatoes began to sprout. Control group of potatoes followed the same trend in sprouting. Sprouting occurred in the potatoes of 14 days post-irradiation.

Discussion

The most important tuber crop, potato is grown largely as a carbohydrate staple and also for industrial purposes. Because of the high water content and the difficulty of storing, processing, and transportation, postharvest losses in tuber crops were potentially very high. Many processing methods were developed to prevent these losses. Food irradiation is the process of exposing food to a carefully controlled amount of energy in the form of high-speed particles or rays. In the present study a remarkable decrease in bacterial and fungal count was observed at 0.15 kGy. It was also noted all the irradiated samples at various doses showed reduction in microbial flora as compared to controlled samples. The reduction in microbial count might be due to the reason that radiation treatment inactivated the DNA of organism followed by denaturation of proteins (Corbo *et al.*, 2009). It had been observed that radiation dose of 0.1 kGy caused great damage to 2.8% of DNA (Lee, 2004). Radiations must have penetrated the core of irradiated food so that all the microbes, and their enzymes, present on surface were destroyed.

In the present study, gamma irradiation reduced the total count of pathogenic bacteria to a great extent. The identified bacteria were *Escherichia coli* and Non-fermentor spp. These strains were identified by using the Analytical profile index 20E strips. Gamma irradiation proved to be effective in controlling the number of colonies of these pathogens also. By the use of gamma irradiation various effects can be achieved resulting in reduced storage loses, extended shelf life and microbiological and parasitological safety of food. Irradiation of main commodities such as tubers had an enormous application in preventing the food poisoning through the elimination of non-spore forming pathogens. Fresh potatoes are not available all around the year because they could be cultivated best under moderate climatic conditions so their storage is required for continuous supply. In case of potatoes, sprouting is the most undesirable and it cause great economic losses. Sprouting is induced by the breakage of dormancy period. The factors which control the sprout inhibition were the temperature, water supply and photoperiod during growth and storage. Sprouts growth was reduced by subjecting potatoes to the irradiation dose of 0.15 kGy. Our findings are in accordance with Farkas (2006) who reported the dose range of 0.03-0.15 kGy for the inhibition of sprouting in potatoes.

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