*Available online at* www.ijpab.com

DOI: http://dx.doi.org/10.18782/2320-7051.2116

**ISSN: 2320 – 7051**

*Int. J. Pure App. Biosci.* **3 (5):** 14-20 (2015)

International Journal of Pure & Applied Bioscience

*Research Article*

OPEN ACCESS

# Optimization of Gamma Radiation Induced Variation in Phytochemical Composition of Maize (*Zea mays* L.) Flour at Various Levels of Particle Size

**Haq Nawaz[1]*, Muhammad Aslam Shad[1], Ayesha Ramzan[2]**

[1]Department of Biochemistry, Bahauddin Zakariya University, Multan-60800, Pakistan

[2]Institute of Chemical Sciences, Bahauddin Zakariya University, Multan-60800, Pakistan

*Corresponding Author E-mail: haqnawaz@bzu.edu.pk

## ABSTRACT

*Gamma radiation induced variation in phytochemical composition of maize flour was optimized by response surface methodology using central composite design at three levels of each of particle size, in terms of mesh number (40, 60 and 80 meshes), and gamma radiation dose (25, 50 and 75 kGy). Response surface analysis of data indicated significant increase ($p<0.05$) in phytochemical composition in response to a decrease in particle size and increase in gamma radiation dose. Optimum level of radiation dose to achieve maximum value of responses was found to be 75 kGy for total phenolic acids and total tannins and 25 kGy for ascorbic acid content. However, optimum level of mesh number No. to achieve desired levels of each parameter was found to be 80 meshes.*

***Key words:*** *Zia mays L., Maize flour, Phytochemical composition, Particle size, Gamma irradiation, Response surface methodology, Central composite design*

## INTRODUCTION

Maize, botanically known as *Zia mays* L., is the third most important cereal crop after wheat and rice in Pakistan. It is widely used as a source of starch, protein, vitamins and minerals in various food supplementations all over the world. It possesses great medicinal importance due to the presence of considerable amounts of phytochemical compounds including ß-carotene, tochopherls, phenolic acids, flavonoids and anthocyanins[1,2]. These phytochemicals have been reported to possess antioxidant properties. Antioxidants are the substances which have ability to prevent the oxidation of easily oxidizable substances. These substances show their action by trapping endogenous free radicals produced as a result of different metabolic processes and protect the lipids, proteins and nucleic acids from the oxidative damage[3].

Gamma-irradiation is an extensively used method in industries for the sterilization of food materials and pharmaceutical products. It is also being used in food science technology to improve the nutritional and functional quality of various food materials[4-6]. Studies have also shown that gamma irradiations significantly affect the phytochemical composition of plant and other food materials[7,8].

Previously, a few data have been reported regarding the high dose gamma radiation induced variations in starch structure and nutritional quality of maize grains[9,10].

**Cite this article:** Nawaz, H., Shad, M.A., and Ramzan, A., Optimization of Gamma Radiation Induced Variation in Phytochemical Composition of Maize (*Zea mays* L.) Flour at Various Levels of Particle Size, *Int. J. Pure App. Biosci.* **3 (5):** 14-20 (2015). http://dx.doi.org/10.18782/2320-7051.2116

However, no investigations have been reported on the effect of particle size and gamma irradiation on phytochemical and antioxidant profile of maize flour. Therefore, the present study was designed to optimize the effect of particle size and gamma radiations on phytochemical composition and antioxidant activity of maize flour of different particle sizes using response surface methodology. The data would provide useful information regarding the processing and utilization of maize flour in pharmaceutical and food formulations.

## MATERIALS AND METHODS

The maize grains were collected from Maize and Millet Research Institute (MMRI), Yousafwala, Sahiwal, Pakistan. The mature grains were separated manually, dried in air under shade to remove moisture and ground to fine flour. The grinding was performed discontinuously using a low speed (1000 rpm) electric grinder in order to maintain the temperature at 35±5°C. The flour was packed in air tight glass bottles and stored in dark at standard laboratory conditions until further processing.

### *Experimental Design*

The cumulative effect of particle size and gamma radiation dose on phytochemical composition of maize flour of different particle sizes was studied using response surface methodology (RSM). A face centred central composite design (CCD) was employed to optimize the effect of two independent variables on phytochemical composition of maize flour. The selected levels of input variables are as under:

$X_1$: Particle size of flour in terms of sieve mesh number.

Three levels of particle size in terms of sieve mesh number were selected as 40, 60 and 80 meshes.

$X_2$: Radiation dose

Three levels of radiation doze were selected as 25, 50 and 75 kGy. The coded levels of the variables were calculated as:

$$X_i = \left( \frac{\xi_i - \bar{\xi}_i}{S_i} \right) \qquad i = 1, 2, ..., k$$

where $\xi_i$ is the specific location of independent variable, $\bar{\xi}_i$ is the centre point and $S_i$ is the scale factor *i.e.* the difference between $\xi_i$ and $\bar{\xi}_i$. $X_i$ is the coded value of an independent variable $\xi_i$ ($i = 1, 2, ..., k$). The combination of coded and actual levels of input variables as per chosen by CCD is shown in Table 1.

The optimum point of response variables was searched by performing sequential experimentation. A response surface polynomial quadratic model was developed to find the levels of input variables in region of optimal response. The developed model determines the relationship of phytochemical composition of maize flour against the particle size and the dose of gamma radiations. The study was done in phases based on CCD which consists of 13 points with nf = 4, factorial points, na-4 axial point and nc = 5 centre points.

### *Sieving*

The flour was sieved successively through micro screens of mesh No. 40, 60 and 80 meshes to obtain required levels of particle size. The distribution of particle size was done on the basis of sieve mesh number. Particle size is inversely proportional to sieve mesh number. A gradual increase in mesh number is associated with a respective decrease in particle size. The range of particle size obtained from selected sieves was as under (Sigma Aldrich 2014):

| Sieve No. (meshes) | Particle size (µm) |
|---|---|
| 40 | 250-420 |
| 60 | 177-250 |
| 80 | <1-177 |

The flour of each particle size was processed for gamma irradiation.

### *Gamma irradiation of flour*

The maize flour at selected levels of particle size was subjected to gamma irradiation in transparent glass bottles at selected levels of radiation dose at a dose rate of 0.26 kGy/h, sample to source distance 1.5 m and average temperature 30°C using $^{60}Co$ (32000 Curies) gamma radiation source at Pakistan Radiation Services (PARAS), Lahore, Pakistan. Ceric-cerous dosimeters were used to measure the absorbed dose. The gamma irradiated samples and non-irradiated flour at each level of particle size (taken as control) were stored at 25±5°C in sterile laboratory environment to minimize the chances of microbial contamination and growth throughout the study period. The exposure of the samples to direct sunlight was prevented throughout the study period in order to minimize the chances of photo-oxidation of antioxidant compounds.

***Preparation of Extracts***

The seed flour was soaked in 75% methanol at 1:10 solid to solvent ratio for 24 h at 25±5°C with occasional shaking. The contents were filtered through Whatman filter paper (40), volume of the filtrate was made up to 100 mL with 75% methanol and used for analysis.

***Phytochemical analysis***

Total phenolic acids (TPA), total tannins (TT) and ascorbic acid (AA) content of methanolic extracts were determined using the previously described methods[11,12].

***Statistical Analysis***

The results were expressed as means of three parallel replicates. The prediction of optimum levels of response variable as a function of input variables was achieved by creating polynomial quadratic response-surface models. The generalized polynomial model for predicting the variation in response variables is given below:

$$Y_i = B_0 + B_1X_1 + B_2X_2 + B_{12}X_1X_2 + B_{11}X_1^2 + B_{22}X_2^2$$

where $Y_i$ is the predicted response, $\beta_o$ is a constant, $\beta_1$ and $\beta_2$ are the regression coefficients for the main variable effects, $\beta_{11}$ and $\beta_{22}$ are quadratic effects and $\beta_{12}$ is interaction effect of independent variables.

Significance of estimated regression coefficient for each response was assessed by lack of fit test (*F*-value) at a probability (*p*) of 0.05. The coefficient of determination ($R^2$) and adjusted coefficient of determination ($R^2_{adj}$) were also determined to check the adequacy of the response surface models and to measure the fairness of fit of regression equation respectively. The precision and reliability of experiments was checked by determining the coefficient of variation (*CV*). A low value of *CV* suggests a better precision and reliability of the experiments. A ratio greater than 4 indicates an adequate signal. The statistical software, Design Expert 9.0 (Stat-Ease, Inc.) was used for the development of experimental design, data analysis and optimization procedure.

For graphical optimization of particle size and dose of gamma radiation, the three-dimensional plots were constructed between response and independent variables. The adequacy of the response-surface models was verified by plotting the experimental values versus those predicted by the final reduced models. The optimum levels of input variables at which the desired goals of responses may be achieved were found by numerical optimization of data at maximum desirability.

## RESULTS AND DISCUSSION

Maize is a widely used cereal after wheat and rice to fulfil the nutritional requirements in Pakistan and many other developing countries of the world. Maize flour has been found to be a rich source of carbohydrates and protein. It also constitutes some non nutritional components such as phenolic compounds, flavonoids, tannins, anthocyanins and cardiac glycosides generally known as phytochemicals[2].

The experimental values of phytochemical composition of non-irradiated flour at different levels of particle size and gamma irradiated flour at various levels of particle size and gamma radiations dose as per chosen by the experimental design are presented in Table 1. TPA, TT and AA content ranged from 0.397 to 1.017, 0.163 to 0.363 and 0.020 to 0.072 g/100g dw respectively. Statistically significant difference ($p<0.05$) in phytochemical composition was observed among non-irradiated maize flours of different particle sizes.

***Response surface analysis and optimization of results***

The cumulative effect of particle size and gamma radiation on phytochemical composition of maize flour was optimized by response surface methodology. The prediction of an optimum level of each of the independent variables was carried out by using central composite design (CCD).

The polynomial regression equations were yielded by RSM in order to show an empirical relationship between the response and input variables. These equations include the coefficient for intercept, main (linear) effects, interaction terms and quadratic effects. The influence of each factor on the response is shown by the sign and magnitude of the main effect. The RSM indicated that the relationship between process conditions and the antioxidant properties of maize flour could be explained significantly by second order polynomial regression models. The following polynomial regression equations were obtained to show the relationship between the process variables and responses including phytochemical composition:

$$\text{TPA (g/100g dw)} = 0.609 - 6.42X_1 - 0.012X_2 + 1.90E^{-005}X_1X_2 + 1.26\ E^{-004}X_1^2 + 1.69\ E^{-004}X_2^2$$

$$\text{TT (g/100g dw)} = 0.311 - 6.04\ E^{-003}X_1 - 1.195\ E^{-004}X_2 + 2.30\ E^{-005}X_1X_2 + 5.85\ E^{-005}X_1^2 + 4.66E^{-006}X_2^2$$

$$\text{AA (g/100g dw)} = 0.09 + 0.122\ E^{-003}X_1 - 1.105\ E^{-003}X_2 - 6.50E^{-006}X_1X_2 + 1.71E^{-005}X_1^2 + 1.015\ E^{-005}X_2^2$$

The main, quadratic and interaction effects of particle size and gamma radiation dose on phytochemical composition of maize flour as obtained by analysis of variance (ANOVA) are given in Table 2. Three dimensional (3D) response surface plots were drawn to show the main and interaction effects of particle size and gamma radiation dose on phytochemical composition of maize flour (Figure 1A-C).

The significance and adequacy of the response surface model was measured in terms of *F*-value (lack of fit) and *p*-value (probability) at 5% significance level ($p \leq 0.05$). The *F*-value is a measure of failure of a model to fit the data in experimental domain particularly for reduced points in a randomized experiment.

The corresponding variables with relatively larger *F*-values ($F>3.69$) and smaller *p*-values ($p<0.05$) were considered more significant. The measurement of *F*-value and *p*-values indicated significant linear positive effect of both variables on each of the studied parameters. The interaction effects were found to be significant on TT content. The quadratic effects of both the particle size and gamma radiation dose were found to be significant on TT and AA content. It is clear from RSM results that extraction of phytochemical components of maize flour is significantly increased in response to an increase in mesh number (decrease in particle size) and gamma radiation doses. Present results are in agreement with those reported earlier for soy bean[8,13].

The correlation coefficient ($R^2$) measures the variability of the model in the observed response values. A value of $R^2$ closer to unity gives better prediction of the response and high significance of the model. The calculated values of $R^2$ (0.9284-0.9753) indicated that 92-97% of the variability in phytochemical composition of maize flour could be explained by the suggested model. The values of adjusted $R^2$ (0.8773-0.9577) for these responses also advocate the significance of the model. Relatively low values of coefficient of variation (0.25-7.66) and high value of adequate precision (1486-26.78) suggest a better precision and reliability of the experiments.

To test the applicability of the model, the predicted values of phytochemical composition were calculated from the polynomial regression equations and plotted against the experimental values (Figure 1D-F). A good agreement between the experimental and predicted values of responses was observed with high values of coefficients of determination ($R^2=0.928\text{-}0.974$). The higher values of $R^2$ prove the applicability of proposed model with greater accuracy to study the effect of particle size and radiation dose on the phytochemical composition of maize flour.

The selection criteria and results for numerical optimization of particle size and radiation dose to achieve the significant amounts of phytochemical components and desirable antioxidant properties are presented in Table 3. Optimum level of particle size in terms of sieve mesh No. to achieve maximum value of TPA, TT and AA content was found to be 80 meshes. Optimum level of radiation dose to achieve the desired levels of TPA and TT was found to be 75 kGy. However, the optimum level of radiation dose was found to be 25 kGy for AA content.

**Table 1. The experimental values of phytochemical composition of maize flour at random levels of experimental conditions as per chosen by central composite design**

| Exp. Runs | Coded levels of variables | | Actual levels of variables | | Phytochemical composition | | |
|---|---|---|---|---|---|---|---|
| | $X_1$ | $X_2$ | $\xi_1$ Particle size (Meshes) | $\xi_2$ Radiation dose (kGy) | *TPA (g/100g dw**)* | *TT (g/100g dw)* | *AA (g/100g dw)* |
| ***Non irradiated flour*** | | | | | | | |
| | | | 40 | 0 | 0.397 | 0.163 | 0.026 |
| | | | 60 | 0 | 0.474 | 0.198 | 0.059 |
| | | | 80 | 0 | 0.710 | 0.227 | 0.072 |
| *Gamma irradiated flour* | | | | | | | |
| 1* | 0 | 0 | 60 | 50 | 0.545 | 0.233 | 0.028 |
| 2 | -1 | 1 | 40 | 75 | 0.632 | 0.255 | 0.02 |
| 3* | 0 | 0 | 60 | 50 | 0.545 | 0.233 | 0.028 |
| 4 | 0 | -1 | 60 | 25 | 0.496 | 0.211 | 0.05 |
| 5 | 0 | 1 | 60 | 75 | 0.905 | 0.27 | 0.031 |
| 6 | -1 | -1 | 40 | 25 | 0.397 | 0.178 | 0.04 |
| 7 | -1 | 0 | 40 | 50 | 0.434 | 0.218 | 0.032 |
| 8 | 1 | 1 | 80 | 75 | 1.017 | 0.363 | 0.035 |
| 9 | 1 | -1 | 80 | 25 | 0.744 | 0.24 | 0.068 |
| 10* | 0 | 0 | 60 | 50 | 0.545 | 0.233 | 0.028 |
| 11* | 0 | 0 | 60 | 50 | 0.545 | 0.233 | 0.028 |
| 12* | 0 | 0 | 60 | 50 | 0.545 | 0.233 | 0.028 |
| 13 | 1 | 0 | 80 | 50 | 0.856 | 0.304 | 0.05 |

*Center points,

**dw: dry weight

**Table 2. Regression coefficient (Rc), Correlation coefficient ($R^2$), adjusted $R^2$, lack of fit and probability values from the final reduced models for phytochemical composition and antioxidant activity of γ-irradiated maize flour**

| | Model | $X_1$ | $X_2$ | $X_1X_2$ | $X_1^2$ | $X_2^2$ | CV (%) | $R^2$ | $R^2$ (Adj) | AP* |
|---|---|---|---|---|---|---|---|---|---|---|
| ***TPA*** | | | | | | | | | | |
| *F*-value | 35.91 | 94.91 | 59.93 | 0.15 | 2.99 | 13.21 | 7.66 | 0.9625 | 0.9357 | 21.01 |
| *P*-value | <0.0001** | <0.0001 | 0.0001 | 0.706 | 0.128 | 0.0083 | | | | |
| ***TT*** | | | | | | | | | | |
| *F*-value | 55.29 | 122.74 | 125.64 | 5.94 | 17.01 | 0.26 | 3.83 | 0.9753 | 0.9577 | 26.78 |
| *P*-value | <0.0001 | <0.0001 | 0.0001 | 0.0449 | 0.0044 | 0.6235 | | | | |
| ***AA*** | | | | | | | | | | |
| *F*-value | 19.86 | 32.17 | 44.82 | 2.19 | 6.78 | 5.77 | 12.25 | 0.9343 | 0.8871 | 14.863 |
| *P*-value | 0.0005 | 0.0008 | 0.0003 | 0.1823 | 0.0359 | 0.0476 | | | | |

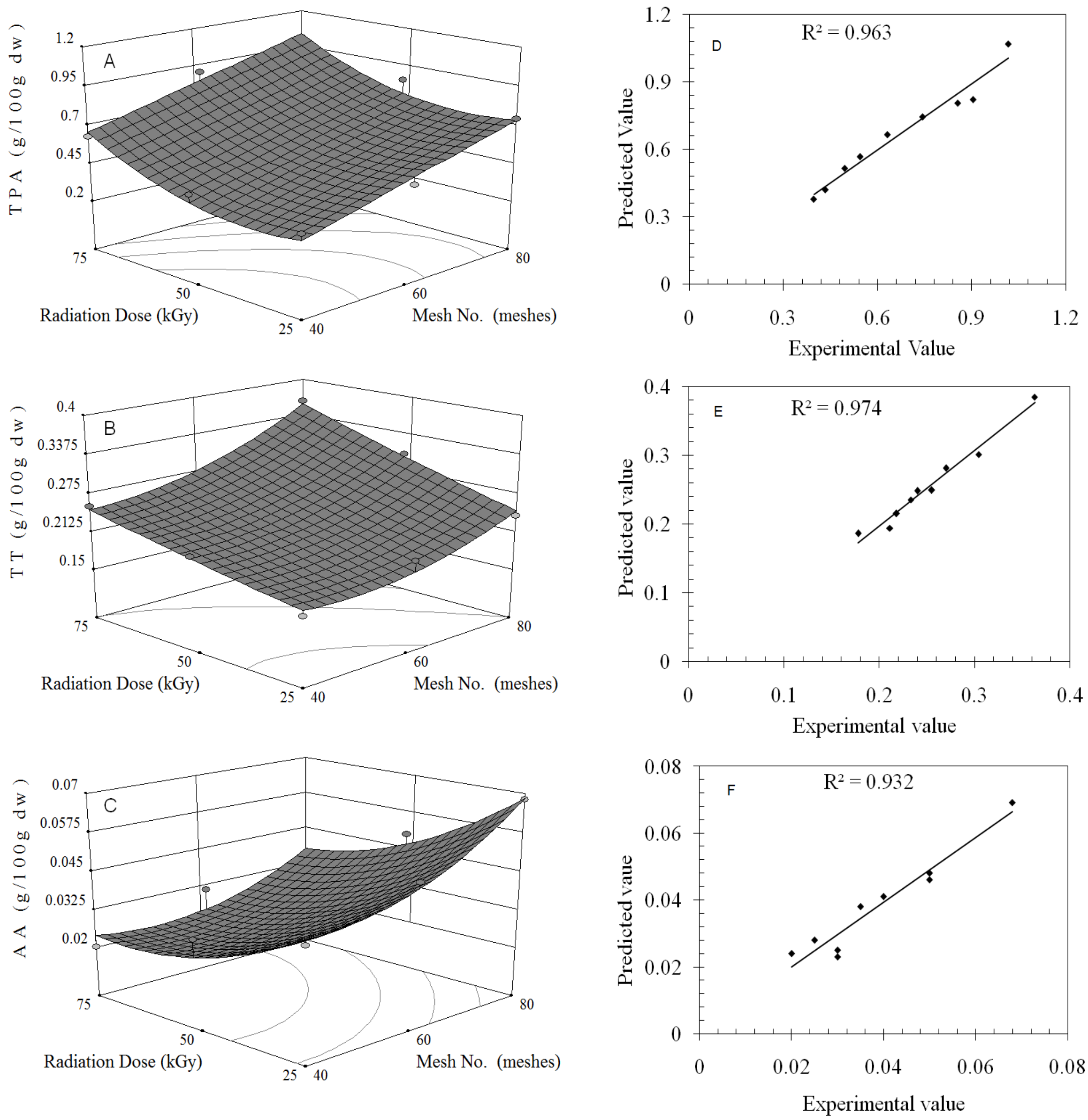
*AP: Adequate Precision

** $p \leq 0.05$ indicates significant variation at 95% confidence level.

**Table 3. Optimum levels of input variables to achieve the desired goals of response variables with maximum desirability**

| Variables | Goal | Lower limit | Upper limit | Optimum level | | | Desirability |
|---|---|---|---|---|---|---|---|
| | | | | $X_1$ | $X_2$ | $Y$ | |
| *Particle size (Mesh no.)* | in range | 40.00 | 80.00 | | | | |
| *Radiation dose (kGy)* | in range | 25.00 | 75.00 | | | | |
| *TPA (g/100g dw)* | maximize | 0.397 | 1.017 | 80.00 | 75.00 | 1.070 | 1.000 |
| *TT (g/100g dw)* | maximize | 0.178 | 0.363 | 80.00 | 75.00 | 0.358 | 0.973 |
| *AA (g/100g dw)* | maximize | 0.02 | 0.068 | 80.00 | 25.00 | 0.068 | 1.000 |

**Figure 1**




A-C: 3D response surface plots of phytochemical components of maize flour at various levels of particle size and radiation dose
A: Total phenolic acids, B: Total tannins, C: Ascorbic acid
D-F: Agreement between experimental values and predicted values phytochemical components of maize flour
D: Total phenolic acids, E: Total tannins, F: Ascorbic acid

## CONCLUSION

The present study shows an inverse correlation between particle size and phytochemical components and direct correlation between gamma radiation dose and the studied parameters. It is concluded that sieve mesh number and gamma irradiation of flour are the important factors affecting the extraction of phytochemical compounds from plant material. This increase may be due to gamma radiation assisted release and extraction of bound phytochemical compounds. The present results suggests that sieving of flour through fine sieve with higher mesh number and treatment of flour with gamma radiation increases the extraction efficiency and bioavailability of compounds from maize flour.

## Acknowledgments

The authors are grateful to Pakistan Radiation Services (PARAS), Lahore, Pakistan for providing the facility of gamma radiation source.

**REFERENCES**


1. Mboya, R., Tongoona, P., Derera, J., Mudhara, M., and Langyintuo, A. The dietary importance of maize in Katumba ward, Rungwe district, Tanzania, and its contribution to household food security. *African Journal of Agricultural Research*, **6(11)**: 2617-2626 (2011).
2. Nawaz, H., Shad, M.A. and Batool, Z. Inter-varietal Variation in Biochemical, Phytochemical and Antioxidant Composition of Maize (*Zea mays* L.) Grains. *Food Science and Technology Research*, **19(6)**: 1133-1140 (2013).
3. Kabel, A.M. Free Radicals and Antioxidants: Role of Enzymes and Nutrition. *Journal of Nutrition and Health*, **2(3)**: 35-38 (2014)
4. Abdelwhab, N.M., Nour, A.M. and Fageer, A.S.M. The Nutritive and Functional Properties of Dry Bean (*Phaseolus vulgaris*) as Affected by Gamma Irradiation. *Pakistan Journal of Nutrition*, **8(11)**: 1739-1742 (2009).
5. Gani, A., Gazanfar, T., Jan, R., Wani, S.M., Masoodi, F.A. Effect of gamma irradiation on the physicochemical and morphological properties of starch extracted from lotus stem harvested from Dal lake of Jammu and Kashmir, India. *Journal of the Saudi Society of Agricultural Sciences*, **12**: 109-115 (2013).
6. Hassan, A.B., Osman, G.A.M., Rushdi, M.A.H., Eltayeb, M.M. and Diab, E.E. Effect of Gamma Irradiation on the Nutritional Quality of Maize Cultivars (*Zea mays*) and Sorghum (*Sorghum bicolor*) Grains. *Pakistan Journal of Nutrition*, **8(2)**: 167-171 (2009).
7. Kortei, N.K., Odamtten, G.T., Obodai, M., Appiah,V., Akuamoa, F., Adu-Bobi, A.K., Annan, S.N.Y., Armah, J.N.O., and Acquah, S.A. (2014). Evaluating the Effect of Gamma Radiation on the Total Phenolic Content, Flavonoids, and Antioxidant Activity of Dried *Pleurotus ostreatus* ((Jacq. ex. Fr) Kummer) Stored in Packaging Materials. *Advances in Pharmaceutics*, **2014**, 1-8 (2009).
8. Alothman, M., Bhat, R. and Karim, A.A. Effects of Radiation Processing on Phytochemicals and Antioxidants in Plant Produce. *Trends in Food Science and Technology*, **20**: 201-212 (2009).
9. Roushdi, M., Harras, A., El-Meligi, A. & Bassim, M. Effect of high doses of gamma rays on corn grains. I. Influence on the chemical composition of whole grains and technological process of starch and By-Product Isolation. *Cereal Chemistry*, **58(2)**: 110-112 (1980).
10. Roushdi, M., Harras, A., El-Meligi, A. & Bassim M. Effect of High Doses of Gamma Rays on Corn Grains. Part II. Influence on Some Physical and Chemical Properties of Starch and Its Fractions. *Starch*, **35(1)**: 15–18 (1983).
11. Shad, M.A., Nawaz, H., Yaqoob, M. and Yousuf, B. Phytochemical Composition and Antioxidant Properties of Rhizomes of *Nilumbo nucifera*. *Journal of Medicinal Plant Research*, **6(6)**: 972-980 (2012).
12. Shad, M.A., Pervez, H., Nawaz, H**.**, khan, H., and Aman-Ullah, M. Evaluation of Biochemical and Phytochemical Composition of Some Groundnut Varieties Grown in Arid Zone of Pakistan. *Pakistan Journal of Botany*, **41(6)**: 2739- 2749 (2009).
13. Variyar, P.S., Limaye, A. and Sharma, A. Radiation-Induced Enhancement of Antioxidant Contents of Soybean (*Glycine max* Merrill). *Food Chemistry*, **52**: 3385-3388 (2004).