BJPS

Brazilian Journal of
Pharmaceutical Sciences
vol. 52, n. 4, oct./dec., 2016
http://dx.doi.org/10.1590/S1984-82502016000400022

Article

Influence of high-dose gamma radiation and particle size on antioxidant properties of Maize (*Zea mays* L.) flour

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

[1]Department of Biochemistry, Bahauddin Zakariya University, Multan, Pakistan, [2]Institute of Chemical Sciences, Bahauddin Zakariya University, Multan, Pakistan

Influence of high-dose gamma radiation and particle size on antioxidant properties of maize (*Zea mays* L.) flour was studied using response surface methodology. A central composite design based on 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) was constructed. A statistically significant dose-dependent decrease ($p<0.05$) in antioxidant properties of gamma irradiated flour was observed. However, an increase in the mesh number (decrease in particle size of flour) resulted in an increase in antioxidant properties. The optimum level of radiation dose to achieve maximum value of responses was found to be 50 kGy for Trolox equivalent total antioxidant activity (TETAOA), 25 kGy for iron chelating ability (ICA), 25 kGy for reducing power (RP) and 75 kGy for linoleic acid reduction capacity (LARC). However, the optimum level of mesh number to achieve desired levels of TETAOA, ICA, RP and LARC was found to be 80 meshes.

Uniterms: Maize flour/antioxidant properties. Maize flour/gamma irradiation/effects. Central composite design. Maize flour/particle size. Response surface methodology. *Zea mays* L.

INTRODUCTION

Antioxidants are a unique class of substances that are able to protect the biomolecules from oxidative damage caused by endogenous free radicals such as superoxide and hydroxyl radicals produced during various metabolic processes (Nimse, Pal, 2015; Lobo *et al*., 2010; Sisein, 2014). These free radicals may lead to protein denaturation, lipid per-oxidation, DNA lesions and finally diseased conditions if not captured effectively (Kabel, 2014; Valko *et al*., 2007). The antioxidants perform their action by trapping the free radicals, reducing the metal ions and preventing the initiation of free radical chain, hydrogen abstraction and peroxide decomposition (Nimse, Pal, 2015). A large number of natural compounds such as ascorbic acid, tocopherols, phenolic acids, flavonoids, anthocyanins, proanthocyanidins and other phytochemical compounds present in food materials have been reported to possess antioxidant properties due to the presence of hydrogen donating groups in their chemical structures (Goufo, Trindade, 2014; Shahidi, 2000). Foods rich in antioxidant compounds have been proved to be effective in decreasing the risk of cardiovascular mortality, destruction of cancer cells and preventing the oxidative lung damage (Miranda-Vilela *et al*., 2011; Khan *et al*., 2014).

The processing and preservation techniques significantly influence the nutritional and antioxidant properties of food materials. 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 (Abdelwhab, Nour, Fageer, 2009; Hassan *et al*., 2009; Gani *et al*., 2013). Studies have also shown that gamma irradiations significantly affect the antioxidant composition of plant and other food materials (Kortei *et al*., 2014; Hussein *et al*., 2011; Harrison, Were, 2007).

Maize, botanically known as *Zea 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 naturally occurring antioxidant compounds including ß-carotene, tocopherols, phenolic acids, flavonoids and anthocyanins (Mboya *et al*.,

***Correspondence:** H. Nawaz. Department of Biochemistry. Bahauddin Zakariya University, 60800 - Multan, Pakistan. E-mail: haqnawaz@bzu.edu.pk

2011; Nawaz, Shad, Batool, 2013). Previously, a few data have been reported regarding the gamma radiation induced variations in starch structure and nutritional quality of maize grains (Roushdi *et al*., 1981; Roushdi *et al*., 1983). However, no investigations have been reported on the effect of particle size and gamma irradiation on the 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.

MATERIAL 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 airtight glass bottles and stored in dark at standard laboratory conditions until further processing.

Experimental Design

The cumulative effect of particle size and gamma radiations on antioxidant activity of maize flour of different particle sizes was investigated using response surface methodology (RSM). A bi-factorial face-centered central composite design (CCD) was employed to optimize the effect of two independent variables on antioxidant properties of maize flour. The selected levels of input variables are as follows:

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 dose were selected as 25, 50 and 75 kGy.

The codes for the selected levels of the two input variables were calculated using following generalized equations:

$$X_i = \left(\frac{\xi_i - \xi_i^-}{S_i} \right) \quad i = 1, 2, \dots k$$

where X_i is the coded value of an input variable, ξ_i is the specific location of independent variable, ξ_i^- is the center point and is the scale factor i.e. the difference between ξ_i and ξ_i^-. The specific codes for the selected input variables were calculated by putting the values of ξ_i, ξ_i^- and S_i in the generalized equation as:

$$X_1(Mesh\ No.) = \frac{[Mesh\ No.(meshes) - 60]}{20}$$

$$X_2(Radiation\ dose) = \frac{[Radiation\ dose(kGy) - 50]}{25}$$

The calculated codes and combination of coded and actual levels of input variables as per chosen by CCD are shown in Table I.

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 a region of optimal response. The developed model determines the relationship of antioxidant properties 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 center 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 follows (Sigma-Aldrich, 2011):

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

Gamma irradiation of flour

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

Antioxidant analysis

Total antioxidant activity (TAOA) by phospho-molybdenum assay

Trolox equivalent total antioxidant activity of extracts was evaluated by Phospho-molybdenum assay described earlier (Prieto, Pineda, Aguilar, 1999). The methanolic extract (0.1 mL) was mixed with reagent solution (600 mM sulphuric acid, 4mM ammonium molybdate, 0.05M sodium phosphate buffer). The contents were incubated at 95 °C for 90 min, cooled to room temperature and absorbance was recorded at 695 nm against blank (without extract). Total antioxidant capacity was calculated as Trolox equivalent g/100g dry weight using regression equation obtained from the standard curve ($R^2 = 0.9897$).

Reducing power (RP)

The ferric reducing antioxidant power of the samples was estimated according to the method reported earlier (Oyaizu, 1986). The methanolic extract (2.5 mL) was mixed with phosphate buffer solution of pH 6.6 (2.5 mL) and 1% potassium ferricyanide solution (2.5 mL). The mixture was incubated at 50 °C for 20 min followed by the addition of 10% trichloroacetic acid solution (2.5 mL). The mixture was centrifuged at 2000 rpm for 10 min. The supernatant (5 mL) was mixed with distilled water (5 mL) and 0.1% ferric chloride solution (1 mL) and absorbance was recorded at 700 nm. The reducing power was expressed in terms of decrease in absorbance of the reaction mixture.

Linoleic acid reduction capacity (LARC)

The linoleic acid reduction capacity of methanolic extracts was determined by using the method described earlier (Osawa, Namiki, 1981). The methanolic extract (2 mL) was mixed with 2.5% ethanolic solution of linoleic acid (2 mL), 0.05 M sodium phosphate buffer of pH 7.0 (2 mL) and distilled water (2 mL). The reaction mixture was incubated at 40 °C for 24 h. An aliquot (0.1 mL) from above mixture was mixed with 75% methanol (9.7 mL), 20mM $FeCl_2$ solution (0.1 mL) and 30% ammonium thiocyanate solution (0.1 mL) and allowed to stand for 3 min. The absorbance was recorded at 500 nm and LARC was calculated in terms of percent inhibition of lipid peroxidation in linoleic acid emulsion by following equation:

$$LARC(\%) = \left[1 - \left(\frac{A_s}{A_c}\right)\right] \times 100$$

where A_c is the absorbance of control (solution without extracts or standards) and A_s is the absorbance in the presence of sample or standard.

Iron chelating Ability (ICA)

Iron chelating ability of extracts was estimated by reported method (Puntel, Nogueira, Rocha, 2005) with some modifications. Methanolic extract (1 mL) was mixed with 0. 1M hydrochloric acid solution (1.6 mL), saturated sodium chloride solution (2.18 mL) and 2 mM ferrous sulfate solution (1.5 mL) successively. The mixture was allowed to stand at 25±2 **°C** for 5 min followed by the addition of 0.25% 1,10-phenanthroline solution (1.3 mL). The absorbance of the reaction mixture was recorded at 510 nm after 5 min. The iron chelating ability was calculated in terms of their EC_{50} (effective concentration required for 50% chelation) values for iron using regression equation obtained from the linear curve ($R^2 = 0.9734$) of series of different dilutions of methanolic extracts.

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, β_o is a constant, β_1 and β_2 are the regression coefficients for the main variable effects, β_{11} and β_{22} are quadratic effects and β_{12} is interaction effect of independent variables.

The 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 the 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 were 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 flour is a source of carbohydrates and protein. It also contains some nonnutritional components such as phenolic compounds, flavonoids, tannins, anthocyanins and cardiac glycosides generally known as phytochemicals (Nawaz, Shad, Batool, 2013). These phytochemicals have been reported to possess antioxidant properties. Antioxidants are the natural or synthetic compounds which are generally known to prevent the oxidation of endogenous and exogenous biomolecules due to their hydrogen donating ability. The antioxidants 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 (Kabel, 2014).

The experimental values of antioxidant activities of non-irradiated and gamma irradiated flour at different levels of particle size as per chosen by the experimental design are presented in Table I. Trolox equivalent TAOA (g/100g dw), RP (absorbance at 700 nm) and LARC (%) ranged from 1.50 to 3.48, 0.215 to 0.354 and 25.75 to 68.88 respectively. Iron chelating ability was determined in terms of IC_{50} which ranged from 0.304 to 0.350 mg/mL of extract. Relatively low value of IC_{50} shows good chelating ability. Statistically significant difference ($p<0.05$) in antioxidant activities 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 antioxidant activities 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 following polynomial regression equations were yielded by RSM in order to show an empirical relationship between the process variables and antioxidant activities of maize flour:

$$\text{TAOA (g/100g dw)} = -2.1236 + 0.116X_1 + 0.05393X_2 + 2.25E^{-004}X_1X_2 - 8.629E^{-004}X_1^2 - 7.523E^{-004}X_2^2$$

$$\text{ICA (IC}_{50}\text{ mg/mL)} = 0.881 - 0.0173E^{-004}X_1 - 5.00E^{-003}X_2 - 3.45E^{-005}X_1X_2 + 1.489E^{-004}X_1^2 + 8.091E^{-005}X_2^2$$

$$\text{RP (Abs. at 700 nm)} = 0.691 - 0.012X_1 - 4.62E^{-003}X_2 + 2.00E^{-005}X_1X_2 + 1.07E^{-004}X_1^2 + 2.055E^{-005}X_2^2$$

$$\text{LARC (\%)} = 188.00 - 4.00X_1 - 1.558X_2 + 2.600E^{-003}X_1X_2 + 0.0358X_1^2 + 9.082E^{-003}X_2^2$$

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 main, quadratic and interaction effects of particle size and radiation dose on TETAOA, ICA, RP and LARC of maize flour as obtained by analysis of variance (ANOVA) are given in Table IIa, IIb, IIc and IId respectively. The significance and adequacy of the response surface model were 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 the 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 the significant linear negative effect of both variables on each of the studied parameters except TETAOA. Interaction effects were found to be nonsignificant on each of the studied parameters. The quadratic effect of particle size was found to be significant on each parameter while that of radiation dose was found to be significant on TETAOA and ICA. It is clear from RSM results that antioxidant activity of maize flour is significantly decreased in response to a

TABLE I - The experimental values of antioxidant activity 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		Antioxidant activity			
	X_1	X_2	ξ_1 Particle size (Meshes)	ξ_2 Radiation dose (kGy)	TETAOA (g/100g dw)	ICA (IC_{50} mg/mL)	RP (Abs. at 700 nm)	LARC (%)
Non irradiated flour								
			40		2.70	0.250	0.323	45.76
			60		3.25	0.246	0.349	56.28
			80		3.48	0.215	0.360	68.88
Gamma irradiated flour								
1*	-1	-1	40	25	2.28	0.296	0.310	54.34
2	0	0	60	50	3.20	0.231	0.225	27.55
3*	1	-1	80	25	3.03	0.295	0.340	65.87
4	-1	1	40	75	1.50	0.420	0.210	25.75
5	0	-1	60	25	2.86	0.292	0.270	50.26
6	1	1	80	75	2.70	0.354	0.280	42.48
7	-1	0	40	50	2.50	0.321	0.235	36.73
8	0	0	60	50	3.20	0.230	0.225	27.55
9	1	0	80	50	3.41	0.256	0.320	58.98
10*	0	0	60	50	3.20	0.231	0.225	27.55
11*	0	0	60	50	3.20	0.231	0.225	27.55
12*	0	0	60	50	3.20	0.231	0.225	27.55
13	0	1	60	75	2.80	0.266	0.225	28.18
Input variable			Actual levels			Respective coded level		
Mesh No.(meshes)			40, 60, 80			-1, 0, 1		
Radiation dose (kGy)			25, 50, 75			-1, 0, 1		

*Center points, **dw: dry weight

decrease in mesh number (increase in particle size) and increase in gamma radiation doses.

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 a better prediction of the response and high significance of the model. The values of R^2 (0.8819-0.9653) indicated that 88-96% of the variability in antioxidant properties of maize flour could be explained by the suggested model. The values of adjusted R^2 (0.7975-0.9406) for these responses also advocate the significance of the model. Relatively low values (4.45-11.34) of the coefficient of variation (*CV*) and high value of adequate precision (9.937-20.74) suggest a better precision and reliability of the experiments.

Three-dimensional (3D) response surface plots were drawn to show the main and interaction effects of particle size and gamma radiation dose on antioxidant activity of maize flour (Figure 1). To test the applicability of the model, the predicted values of antioxidant activities were calculated from the polynomial regression equations and plotted against the experimental values (Figure 2A-D). A good agreement between the experimental and predicted values of responses was observed with high values of coefficients of determination ($R^2=0.8819-0.9653$). 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 antioxidant properties of maize flour.

The selection criteria and results for numerical optimization of particle size and radiation dose to achieve the desirable values of antioxidant ability are presented in Table III. The optimum level of particle size in terms

TABLE IIA - Analysis of variance (ANOVA) from the final reduced models for Trolox equivalent total antioxidant activity of gamma irradiated maize flour

Source	Sum of Squares	df	Mean Square	F Value	p-Value
Model	2311.93	5	462.39	24.27	0.0003
A-Mesh No. (meshes)	425.21	1	425.21	22.32	0.0021
B-Radiation dose (kGy)	914.15	1	914.15	47.98	0.0002
AB	6.76	1	6.76	0.35	0.5702
A^2	565.69	1	565.69	29.69	0.0010
B^2	89.00	1	89.00	4.67	0.0675
Mean			2.85		
Std. Dev.			0.13		
C.V. %			4.45		
R-Squared			0.9653		
Adjusted R-Squared			0.9406		
Predicted R-Squared			0.6778		
Adequate Precision			20.074		

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

TABLE IIB - Analysis of variance (ANOVA) from the final reduced models for for iron chelating activity of gamma irradiated maize flour

Source	Sum of Squares	df	Mean Square	F Value	p-Value
Model	0.035	5	7.054E-003	10.45	0.0038
A-Mesh No. (meshes)	3.038E-003	1	3.038E-003	4.50	0.0716
B-Radiation dose (kGy)	3.902E-003	1	3.902E-003	5.78	0.0472
AB	1.190E-003	1	1.190E-003	1.76	0.2259
A^2	9.801E-003	1	9.801E-003	14.52	0.0066
B^2	7.063E-003	1	7.063E-003	10.46	0.0144
Mean			0.026		
Std. Dev.			0.28		
C.V. %			9.27		
R-Squared			0.8819		
Adjusted R-Squared			0.7975		
Predicted R-Squared			-0.2022		
Adequate Precision			9.937		

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

of sieve mesh No. was found to be 70.622 for TAOA, 80 for RP and LARC and 57 for ICA. The optimum level of radiation dose at which antioxidant properties were affected to a minimum value was found to be 25 kGy for each parameter.

CONCLUSION

The present study shows an inverse correlation between particle size and gamma radiation dose and antioxidant properties. It is concluded that smaller the

TABLE IIC - Analysis of variance (ANOVA) from the final reduced models for reducing power of gamma irradiated maize flour

Source	Sum of Squares	df	Mean Square	F Value	p-Value
Model	0.022	5	4.445E-003	34.86	< 0.0001
A-Mesh No. (meshes)	7.004E-003	1	7.004E-003	54.93	0.0001
B-Radiation dose (kGy)	7.004E-003	1	7.004E-003	54.93	0.0001
AB	4.000E-004	1	4.000E-004	3.14	0.1198
A^2	5.070E-003	1	5.070E-003	39.76	0.0004
B^2	4.557E-004	1	4.557E-004	3.57	0.1006
Mean			0.011		
Std. Dev.			0.25		
C.V. %			4.46		
R-Squared			0.9614		
Adjusted R-Squared			0.9338		
Predicted R-Squared			0.6471		
Adequate Precision			17.815		

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

TABLE IID - Analysis of variance (ANOVA) from the final reduced models for linoleic acid reduction capacity of irradiated maize flour

Source	Sum of Squares	df	Mean Square	F Value	p-Value
Model	2311.93	5	462.39	24.27	0.0003
A-Mesh No. (meshes)	425.21	1	425.21	22.32	0.0021
B-Radiation dose (kGy)	914.15	1	914.15	47.98	0.0002
AB	6.76	1	6.76	0.35	0.5702
A^2	565.69	1	565.69	29.69	0.0010
B^2	89.00	1	89.00	4.67	0.0675
Mean			4.36		
Std. Dev.			38.49		
C.V. %			11.34		
R-Squared			0.9455		
Adjusted R-Squared			0.9065		
Predicted R-Squared			0.5910		
Adequate Precision			15.551		

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

particle size of flour and lower the radiation dose, the lower would be the loss of antioxidant activity. The low values of antioxidant properties of flour with large particle size may be attributed to poor extraction of bound antioxidant compounds due to small surface area. The decrease in antioxidant properties of flour treated at high radiation dose may be due to gamma rays induced oxidative degradation of antioxidant compounds. The present results suggest that sieving of flour through a sieve with higher mesh number and treatment with high-dose gamma radiation decrease

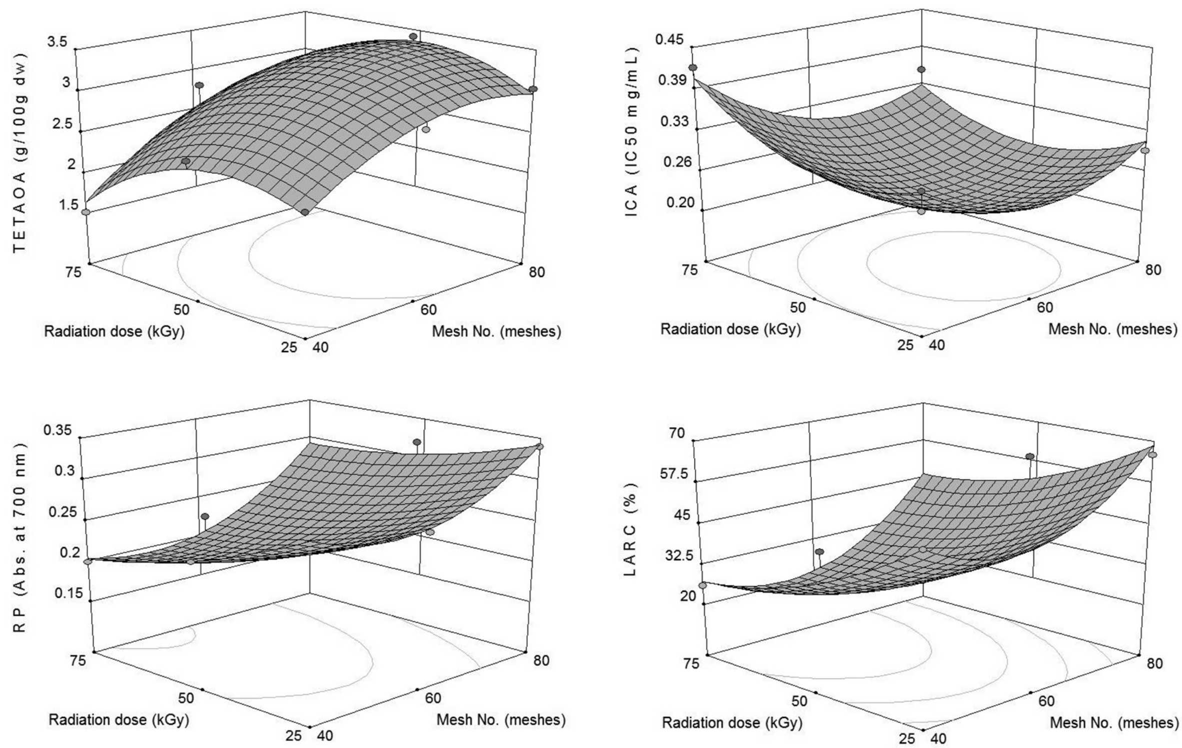

FIGURE 1 - 3D response surface plots of antioxidant properties of maize flour at various levels of particle size and radiation dose.

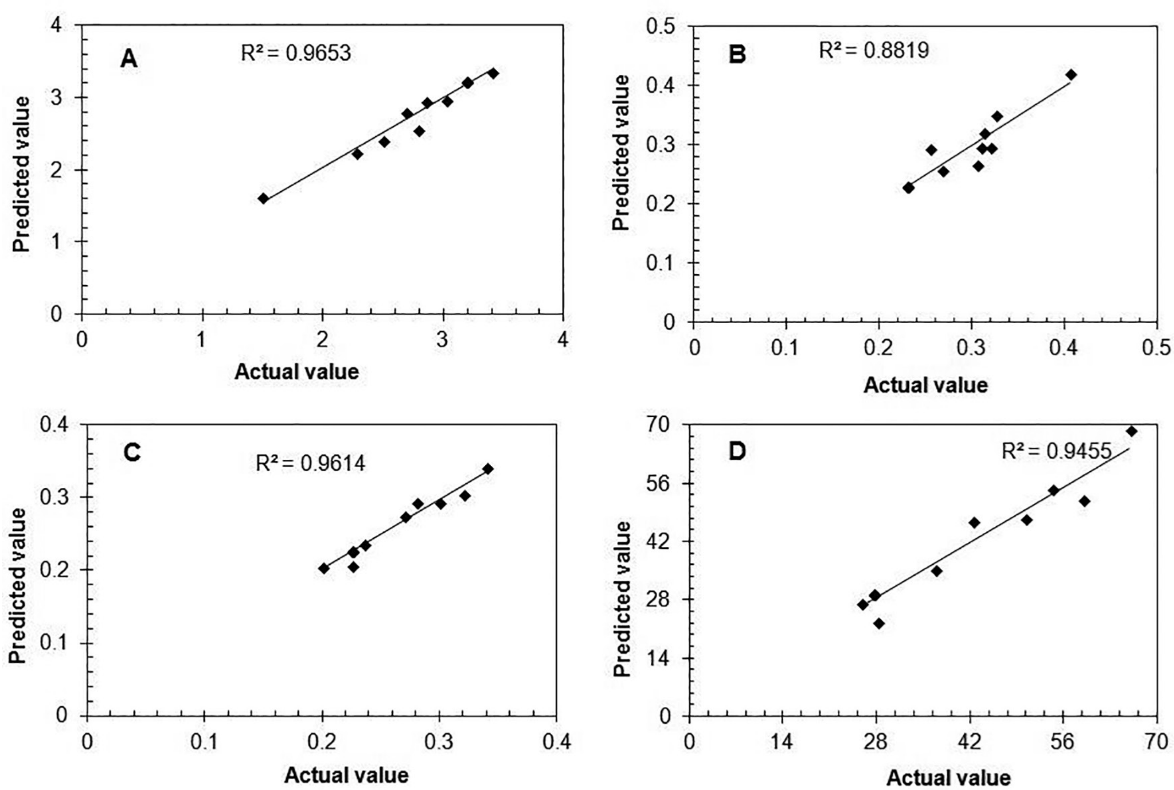

FIGURE 2A-D - Agreement between experimental values and predicted values of antioxidant properties of maize flour. A: Total antioxidant activity, B: Iron chelation activity, C: Reducing power, D: Linoleic acid reduction capacity.

TABLE III - 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	
Mesh No (meshes)	in range	40.00	80.00				
Radiation dose (kGy)	in range	25.00	75.00				
TAOA (TE g/100g dw)	maximize	1.5	3.41	70.622	25.429	3.063	0.901
ICA (IC_{50} mg/mL)	minimize	0.23	0.42	57.000	25.000	0.257	0.927
RP (Abs. at 700 nm)	maximize	0.2	0.34	80.000	25.000	0.342	1.000
LARC (%)	maximize	25.75	65.87	80.000	25.000	68.712	1.000

the extraction efficiency and bioavailability of antioxidant 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

ABDELWHAB, N.M.; NOUR, A.M.; FAGEER, A.S.M. The nutritive and functional properties of dry bean (*Phaseolus vulgaris*) as affected by gamma irradiation. *Pak. J. Nutr.*, v.8, n.11, p.1739-1742, 2009.

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. *J. Saudi Soc. Agric. Sci.*, v.12, 109-115, 2013.

GOUFO, P.; TRINDADE, H. Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, γ-oryzanol, and phytic acid. *Food Sci. Nutr.*, v.2, n.2, p.75-104, 2014.

HARRISON, K.; WERE, M.L. Effect of gamma irradiation on total phenolic content yield and antioxidant capacity of almond skin extracts. *Food Chem.*, v.102, p.932-937, 2007.

HASSAN, A.B.; OSMAN, G.A.M.; RUSHDI, M.A.H.; ELTAYEB, M.M.; DIAB, E.E. Effect of gamma irradiation on the nutritional quality of maize cultivars (*Zea mays*) and sorghum (*Sorghum bicolor*) Grains.. *Pak. J. Nutr.,* v.8, n.2, p.167-171, 2009.

HUSSEIN, S.Z.; YUSOFF, K.M.; MAKPOL, S.; YUSOF, Y.A.M. Antioxidant capacities and total phenolic contents increase with gamma irradiation in two types of malaysian honey. *Molec.*, v.16, p.6378-6395, 2011.

KABEL, A.M. Free radicals and antioxidants: role of enzymes and nutrition. *J. Nutr. Health,* v.2, n.3, p.35-38, 2014.

KHAN, M.; IQUBAL, A.; JOSHI, A.; AJAI, K. Role of antioxidants in prevention of cancer: a review. *Int. J. Curr. Res. Rev.*, v.6, n.9, p.80-88, 2014.

KORTEI, N.K.; ODAMTTEN, G.T.; OBODAI, M.; APPIAH,V.; AKUAMOA, F.; ADU-BOBI, A.K.; ANNAN, S.N.Y.; ARMAH, J.N.O.; ACQUAH, S.A. 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. *Adv. Pharm.*, v.2014, p.1-8, 2014.

LOBO, V.; PATIL, A.; PHATAK, A.; CHANDRA, N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn. Rev.*, v.4, n.8, p.118-126, 2010.

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. *Afr. J. Agric. Res.*, v.6, n.11, p.2617-2626, 2011.

MIRANDA-VILELAA, A.L.; PORTILHOA, F.A.; DE ARAUJO, A.V.; ESTEVANATOA, L.; MEZZOMOA, B.; SANTOSB, M.; LACAVAA, Z. The protective effects of nutritional antioxidant therapy on Ehrlich solid tumor bearing mice depend on the type of antioxidant therapy chosen: histology, genotoxicity and haematology evaluations. *J. Nutr. Biochem.*, v.22, n.11, p.1091-1098, 2011.

NAWAZ, H.; SHAD, M.A.; BATOOL, Z. Inter-varietal variation in biochemical, phytochemical and antioxidant composition of maize (*Zea mays* L.) grains. *Food Sci. Technol. Res.*, v.19, n.6, p.1133-1140, 2013.

NIMSE, S. B.; PAL, D. Free radicals, natural antioxidants and their reaction mechanisms. *RSC. Adv.*, v.5, p.27986-28006, 2015.

OSAWA, T.; NAMIKI, M. A novel type of antioxidant isolated from leaf wax of eucalyptus leaves. *Agric. Biol. Chem.,* v.45, p.735-739, 1981.

OYAIZU, M. Studies on products of browning reaction: antioxidative activities of products of browning reaction prepared from glucosamine. *Jap. J. Nutr. Diet.,* v.44, p.307-315, 1986.

PRIETO, M.; PINEDA, M.; AGUILAR. Spectrophotometric quantification of antioxidant capacity through the formation of phosphomolybdenum complex: specific application of vitamin E. *Anal. Biochem.*, v.269, n.2, p.337-341, 1999.

PUNTEL, R.L.; NOGUEIRA, C.W.; ROCHA, J.B.T. Krebs cycle intermediates modulate thiobarbituric acid reactive species (TBARS) production in rat brain *In vitro*. *Neurochem. Res.,* v.30, p.225-235, 2005.

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*, v.35, n.1, p.15-18, 1983.

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 Chem.*, v.58, n.2, p.110-112, 1981.

SHAHIDI, F. Antioxidant in food and food antioxidants. *Nahrung*, v.44, p.158-163, 2000.

SIGMA-ALDRICH. Aldrich Handbook. A catalog of fine chemicals and laboratory equipment. 2012-2014. St. Louis, Missouri: Sigma Aldrich Corporation, 2011.

SISEIN, E.A. Review article: biochemistry of free radicals and antioxidants. *Sch. Acad. J. Biosci.*, v.2, n.2, p.110-118, 2014.

VALKO, M.; LEIBFRITZ, D.; MONCOL, J.; CRONIN, M.; MAZUR, M.; TELSER, J. Free radicals and antioxidants in normal physiological functions and human disease. *Int. J. Biochem. Cell Biol.*, v.39, n.1, p.44-84, 2007.

Received for publication on 06th November 2015
Accepted for publication on 22th September 2016