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Research Article

Effect of High Dose Gamma Radiation on Free Radical Scavenging Capacity of Maize (*Zea mays* L.) Flour at Various Levels of Particle Size

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ABSTRACT

Gamma radiation induced variation in free radical scavenging capacity of maize flour was optimized by response surface methodology. Central composite design was constructed 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). A significant increase (*p<0.05*) in IC_{50} value for 2, 2-Diphenyl-1-picrylhydrazyl radical scavenging capacity (DPPH RSC) and hydroxyl radical scavenging capacity (HRSC) was observed in response to an increase in gamma radiation dose. Increase in mesh No. (decrease in particle size) resulted in a significant increase in IC_{50} for DPPH RSC and decrease in IC_{50} for HRSC. Increase in IC_{50} value indicates a decrease in free radical scavenging activity and vice versa. Optimum levels of radiation dose to achieve minimum value of IC_{50} for DPPH RSC and HRSC were found to be 25 and 37 kGy respectively. Optimum level of mesh number No. to achieve minimum levels of IC_{50} for DPPH RSC and HRSC were found to be 60 and 70 meshes respectively.

Key words: Central composite design, Free radical scavenging capacity, Gamma irradiation, Maize flour, Particle size, Response surface methodology, *Zia mays* L.

INTRODUCTION

Free radicals are reactive oxygen and nitrogen species which may lead to protein denaturation, lipid per-oxidation, DNA lesions and finally diseased conditions if not captured effectively[1, 2]. 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[3-5]. 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[3]. 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[6, 7]. Foods rich in antioxidant compounds have been proved to be effective in decreasing risk of cardiovascular mortality, destruction of cancer cells and preventing the oxidative lung damage[8-10].

Maize, botanically known as *Zia mays* L., 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, tochopherls, phenolic acids, flavonoids and anthocyanins[11, 12].

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 and technology to improve the nutritional and functional quality of various food materials[13, 14]. However, high dose irradiation may have some adverse effects on the biochemical, phytochemical and antioxidant quality of food materials.

Previously, a few data have been reported regarding the gamma radiation induced variations in starch structure and nutritional quality of maize grains[15, 16]. Unfortunately no investigations have been reported on the effect of particle size and high dose gamma irradiation on free radical scavenging capacity of maize flour. Therefore, the present study was designed to optimize the effect of particle size and gamma radiations on free radical scavenging capacity of maize flour of different particle sizes using response surface methodology.

MATERIALS AND METHODS

The maize grains were collected from Maize and Millet Research Institute (MMRI), Yousafwala, Sahiwal, Pakistan. 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 radiations on free radical scavenging capacity of maize flour 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 free radical scavenging capacity. 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:

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

where ξi is the specific location of independent variable, is the centre point and is the scale factor i.e. the difference between ξ_i and . Xi is the coded value of an independent variable ( ).

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 free radical scavenging capacity of maize flour with particle size and gamma radiations dose. 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 level of 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.

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.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity (RSC):

DPPH radical scavenging capacity of each sample was determined by following the reported method[17] with some modifications. Methanolic extract (1 mL) was mixed with freshly prepared 40µM DPPH solution (3ml) and allowed to stand at 25±5°C for 30 min. Absorbance was recorded at 517 nm and percentage inhibition of DPPH radical was calculated as:

$$DPPH\,RSC\,(\%) = \left[\,(Abs_0 - Abs_{30}) \div Abs_0\,\right] \times 100$$

where Abs_0 is the absorbance of control (DPPH· solution without sample or standard) and Abs_{30} is absorbance of the reaction mixture after 30 minutes. RSC was expressed in terms of IC_{50} (minimum concentration required for 50% inhibition) values for DPPH radical. Relatively low value of IC_{50} of sample shows high radical scavenging ability. IC_{50} was calculated from regression equations obtained by the linear curve ($R^2=0.9835$) of percentage inhibition of DPPH radical by various dilutions of the methanolic extracts.

Hydroxyl radical scavenging capacity (HRSC):

Hydroxyl radical scavenging activity of sample extracts was assayed by reported method[18]. Methanolic extract was mixed with 9mM/L $FeSO_4$ solution (1 mL), 9mM/L salicylic acid solution (1 mL) and 8.8mM hydrogen peroxide solution (1 mL). The reaction mixture was incubated in water bath at 37°C for 30 min and absorbance was measured at 510 nm against blank. Mixture without sample was taken as control and mixture without hydrogen per oxide was as blank. HRSC was calculated as:

$$RSC\,(\%) = \left[1 - (A_s - A_b) \div A_c\right] \times 100$$

where A_s is the absorbance of sample, A_b is absorbance of blank and A_c is absorbance of control. RSC was expressed in terms of IC_{50} values for hydroxyl radical calculated from regression equations obtained by the linear curve ($R^2 = 0.9882$) of percentage inhibition of hydroxyl radical by various dilutions of the 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:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{12} X_1 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2$$

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.
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 input 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

The experimental values of free radical scavenging abilities of non-irradiated flour at different levels of particle size and gamma irradiated flour at selected levels of particle size and gamma radiations dose as per chosen by the experimental design are presented in Table 1. Free radical scavenging capacities were determined in terms of IC_{50} value of flour for DPPH radical and hydroxyl radical. IC_{50} for DPPH and hydroxyl radicals ranged from 0.195 to 0.291 and 0.207 to 0.318 mg/mL respectively. Statistically significant difference ($p<0.05$) in free radical scavenging abilities was observed among non-irradiated maize flours of different particle sizes.

DISCUSSION

Response surface analysis and optimization of results:

The cumulative effect of particle size and gamma radiation on free radical scavenging abilities 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 obtained to show the relationship between the process variables and free radical scavenging abilities of maize flour:

$$DPPH\ RSC\ (IC_{50}\ mg/mL) = 0.25 + 5.33E^{-003}X_1 + 0.013X_2 + 8.00E^{-003}X_1X_2 + 0.011E^{-003}X_1^2 + 5.707E^{-003}X_2^2$$

$$H\ RSC\ (IC_{50}\ mg/mL) = 0.24 - 7.33E^{-003}X_1 + 9.833E^{-003}X_2 - 1.50E^{-003}X_1X_2 + 6.034E^{-003}X_1^2 + 0.011X_2^2$$

These equations include the coefficient for intercept, main (linear) effects, interaction terms and quadratic effects. The effect of independent variables on free radical scavenging ability is shown by the sign and magnitude of main effect. The main, quadratic and interaction effects of particle size and gamma radiation dose on free radical scavenging capacity of maize flour as obtained by analysis of variance (ANOVA) are given in Table 2.
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 DPPH RSC, linear positive effect of radiation dose and linear negative effect of mesh No. on HRSC. The interaction and quadratic effect of both variables were found to be significant on DPPH RSC and HRSC. It is clear from RSM results that DPPH RSC scavenging capacity of maize flour is significantly increased in response to an increase in mesh number (decrease in particle size) and decreased in response to increase in gamma radiation doses.
The significance and adequacy of the applied response surface model was measured by calculating the value of coefficient of determination (R^2). A response surface model with value of R^2 closer to Unity shows better prediction and high significance. The calculated values of R^2 (0.9209-0.8634) indicated that 86-92% of the variability in free radical scavenging capacity of maize flour could be explained by the suggested model. The values of adjusted R^2 (0.7658-0.8644) for these responses also advocate the significance of the model. Relatively low values (1.92-2.38) of coefficient of variation (*CV*) and high value of adequate precision (9.10-14.599) 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 free radical scavenging capacity of maize flour (Fig. 1). To test the applicability of the model, the predicted values of free radical scavenging capacities were calculated from the polynomial regression equations and plotted against the experimental values (Fig. 2A-C). A good agreement between the experimental and predicted values of responses was observed with high values of coefficients of determination ($R^2=0.9209\text{-}0.8634$). 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 DPPH radical scavenging capacity of maize flour.
The selection criteria and results for numerical optimization of particle size and radiation dose to achieve the significant amounts of free radical scavenging capacity are presented in Table 3. Optimum levels of radiation dose to achieve minimum value of IC_{50} for DPPH RSC and HRSC were found to be 25 and 37 kGy respectively. Optimum level of mesh number No. to achieve minimum levels of IC_{50} for DPPH RSC and HRSC were found to be 60 and 70 meshes respectively.

CONCLUSION

The present study shows an inverse correlation between particle size and free radical scavenging capacity 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 DPPH radical scavenging capacity of plant material. The present results suggests that sieving of flour through sieve with lower mesh number and treatment of flour with gamma radiation decreases DPPH radical scavenging capacity of maize flour. The study provides information regarding the adverse effects of high dose gamma irradiation on biological activity of food materials

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Table 1
The experimental values of free radical scavenging capacity 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		Free radical scavenging capacity	
	X_1	X_2	ξ_1 Particle size (meshes)	ξ_2 Radiation dose (kGy)	*DPPH-RSC (IC_{50} mg/mL)*	*HRSC (IC_{50} mg/mL)*
Non irradiated flour						
			40		0.274	0.318
			60		0.237	0.245
			80		0.195	0.207
Gamma irradiated flour						
1*	-1	-1	40	25	0.260	0.250
2	1	-1	80	25	0.255	0.235
3*	-1	1	40	75	0.264	0.281
4	1	1	80	75	0.291	0.260
5	-1	0	40	50	0.263	0.251
6	1	0	80	50	0.273	0.243
7	0	-1	60	25	0.245	0.250
8	0	1	60	75	0.280	0.253
9	0	0	60	50	0.251	0.240
10*	0	0	60	50	0.251	0.240
11*	0	0	60	50	0.251	0.240
12*	0	0	60	50	0.251	0.240
13	0	0	80	50	0.251	0.240

*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 free radical scavenging capacity of gamma irradiated maize flour

	Model	X_1	X_2	X_1X_2	X_1^2	X_2^2	CV (%)	R^2	R^2 (Adj)	AP*
DPPH-RSC										
F-value	16.29	6.84	37.58	10.26	13.90	3.61	1.92	0.9209	0.8644	14.599
P-value	0.001	0.035	0.0005	0.015	0.007	0.10				
HRSC										
F-value	8.85	9.24	16.62	0.26	2.88	8.78	2.38	0.8634	0.7658	9.10
P-value	0.006	0.0189	0.0047	0.627	0.134	0.021				

*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				
DPPH-RSC (IC_{50} mg/mL)	minimize	0.245	0.291	60	25	0.246	0.985
HRSC (IC_{50} mg/mL)	minimize	0.235	0.281	70	37	0.236	0.975

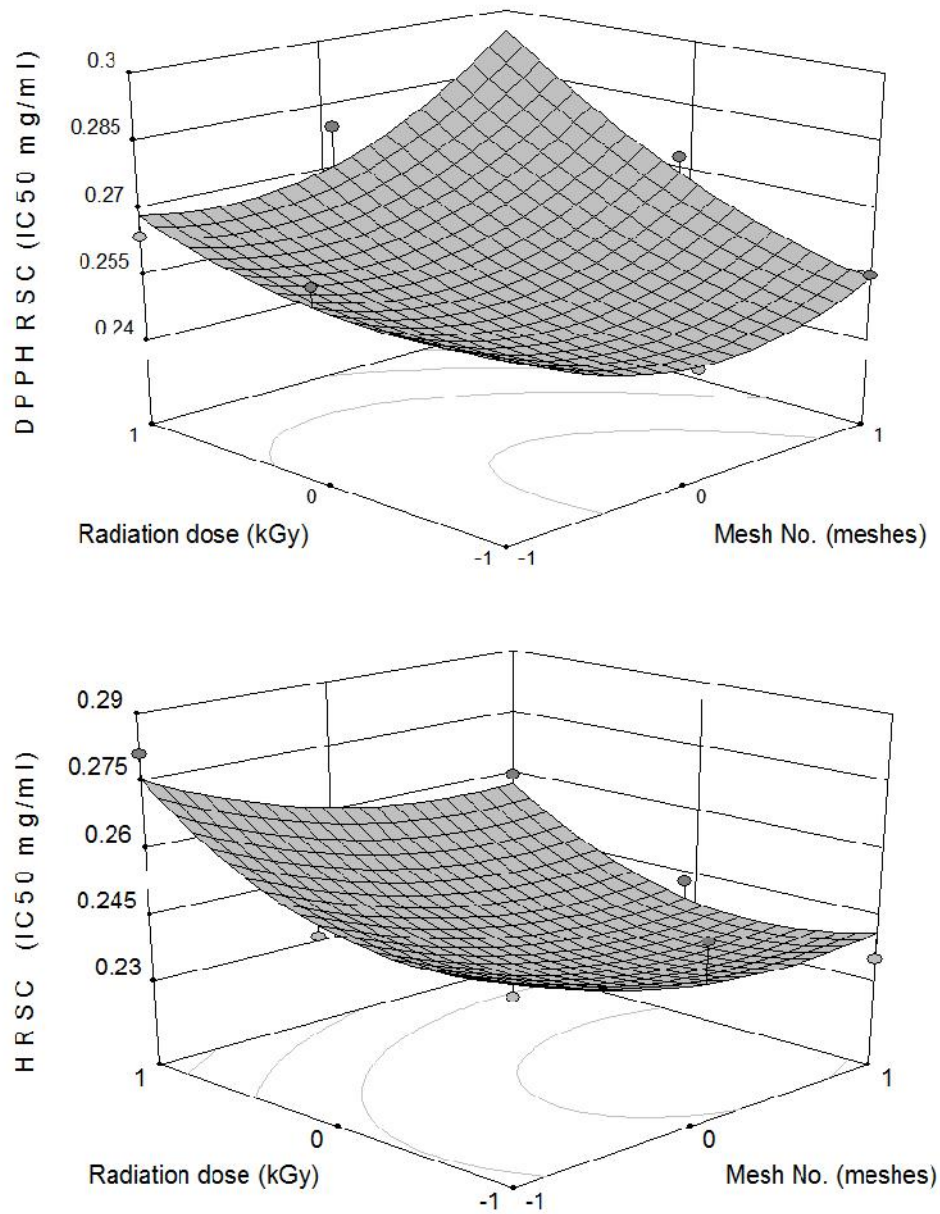

Figure 1
3D response surface plots of free radical scavenging capacity of maize flour at various levels of particle size and radiation dose

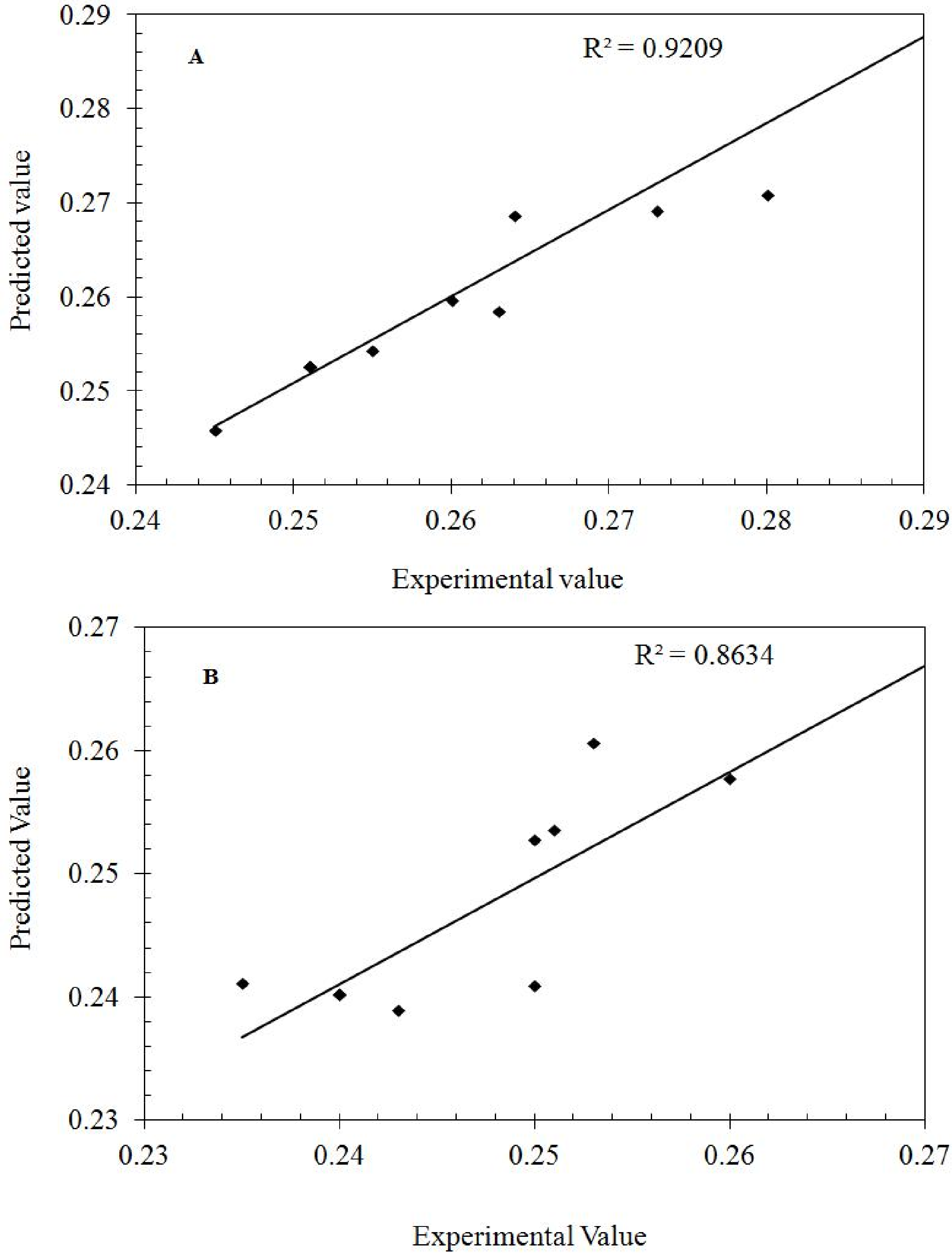

Figure 2
Agreement between experimental values and predicted values free radical scavenging capacity of maize flour
A: DPPH RSC, B: HRSC

REFERENCES

1. Kabel AM. Free Radicals and Antioxidants: Role of Enzymes and Nutrition. J. Nutr. Health, 2014; 2(3):35-38.
2. Valko M. et al. Free Radicals and Antioxidants in Normal Physiological Functions and Human Disease. Int. J. Biochem. Cell Biol, 2007; 39(1):44-84.
3. Nimse SB, Dilipkumar P. Free radicals, natural antioxidants and their reaction mechanisms. Royal Soc. Chem. Adv, 2015; 5:27986–28006.
4. Sisein EA. Review Article: Biochemistry of Free Radicals and Antioxidants. Sch. Acad. J. Biosci, 2014; 2(2):110-118.
5. Lobo V, Patil A, Phatak A, Chandra N. Free radicals, antioxidants and functional foods: Impact on human health. Phcog. Rev, 2010; 4(8):118–126.
6. Goufo P, Trindade H. Rice antioxidants: phenolic acids, flavonoids, anthocyanins, proanthocyanidins, tocopherols, tocotrienols, *γ*-oryzanol, and phytic acid. Food Sci. Nutr, 2014; 2(2):75–104.
7. Shahidi F. Antioxidant in food and food antioxidants. Nahrung, 2000; 44(3):158-163.
8. Miranda-Vilelaa AL, et al. The protective effects of nutritional antioxidant therapy on Ehrlich solid tumorbearing mice depend on the type of antioxidant therapy chosen: histology, genotoxicity and haematology evaluations. J. Nutr. Biochem, 2011; 22(11):1091-1098.
9. Khan M, Iqubal A, Joshi A, Ajai K. Role of Antioxidants in Prevention of Cancer: A Review. Int. J. Curr. Res. Rev, 2014; 6(9):80-88.
10. Alissa EM. Antioxidants and Cardiovascular Disease: A summary of the Evidence. J. Cardiovasc. Disease, 2015; 3(3):347-356.
11. Mboya R, Tongoona P, Derera J, Mudhara M, Langyintuo A. The dietary importance of maize in Katumba ward, Rungwe district, Tanzania, and its contribution to household food security. Afr. J. Agr. Res, 2011; 6(11):2617-2626.
12. Nawaz H, Shad MA, Batool Z. Inter-varietal Variation in Biochemical, Phytochemical and Antioxidant Composition of Maize (*Zea mays* L.) Grains. Food Sci. Technol. Res, 2013; 19(6):1133-1140.
13. Gani A, Gazanfar T, Jan R, Wani SM, Masoodi FA. 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. Agr. Sci, 2013; 12(2):109-115.
14. Abdelwhab NM, Nour AM, Fageer ASM. The Nutritive and Functional Properties of Dry Bean (*Phaseolus vulgaris*) as Affected by Gamma Irradiation. Pak. J. Nutr**,** 2009; 8(11):1739-1742.
15. 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, 1980; 58(2):110-112.
16. 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, 1983; 35(1):15–18.
17. Shad MA, Nawaz H, Yaqoob M, Yousuf B. Phytochemical Composition and Antioxidant Properties of Rhizomes of *Nilumbo nucifera*. J. Med. Plant Res, 2012; 6(6): 972-980.
18. Simirnoff N, Cumbes QJ. Hydroxyl radical scavenging activity of compatible solutes. Phytochem, 1989; 28(4):1057-1060.