



Process Optimization and Characterization of Gamma Irradiation Induced Variations in Functional Properties of Maize (*Zea mays* L.) Flour

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Authors' contributions

This work was carried out in collaboration among all authors. Author HN designed the study protocol and performed the statistical analysis of the data. Author MAS supervised the study and proof read the manuscript. Author SS performed the experimental work and wrote the first draft of the manuscript and author RJ also supervised the experimental work and write up of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

The effect of particle size and high dose gamma irradiation on functional properties of maize flour was studied. The maize flour at three levels of particle size (-177, -250 and -420 μm obtained from sieves of 80, 60 and 40 meshes respectively) was irradiated in transparent glass bottles at three levels of gamma radiation (25, 50 and 75 kGy) using ^{60}Co gamma source. A significant linear increase in water holding and swelling capacities and bulk density values and significant linear decrease in emulsifying stability was observed in response to an increase in particle size of the flour. Gamma radiation dose was found to show a significant linear positive effect on water holding capacity and solubility but a significant linear negative effect on oil holding capacity, protein solubility and bulk density values. These observed trends in functional properties of maize flour with the application of gamma irradiation suggests that quality of maize flour, regarding the manufacture and packaging of food products, may be improved by irradiation process.

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1. INTRODUCTION

Gamma irradiation is a successfully used method to achieve commercial sterilization applicable for food and pharmaceutical grade products [1]. It prevent the growth of microorganisms hence maintain the hygiene and nutritional quality of these products. This method has been also used to improve the hygienic and nutritional quality of foods [2,3]. The structural and functional changes in food materials depend on the strength and duration of the gamma-irradiation [4]. High dose gamma irradiation of food material has been reported to cause denaturation of proteins and exposure of masked functional groups of proteins which in turn affect the functional characteristics of food material [5]. However, an average dose of 10 kGy of gamma radiation of food products has been found to be safe and exerts no hazardous effects on food quality [6].

The commercial utilization of seed flours in various food products significantly depend on their functional properties such as water absorption, oil absorption, gelation, emulsifying, foaming and swelling capacities. The functional properties of food materials depend upon the charge, hydrophilic nature, hydrophobic character and hydrogen bonding of starch and proteins [7]. These functional properties play a significant role in the maintenance of flavor, taste, texture and consistency of the food products [8]. The processing and preservation treatments are the important factors which may affect the functional properties of food materials [9]. High dose gamma irradiation of flours has significant effect on the structural arrangement of starch and proteins which may affect the stability and quality of food products [10–12].

Maize flour and its blends with other seed flours have been frequently used in various food formulations all over the world [13,14]. The increasing utilization of maize flour in food products has urged the researchers to optimize the processing and preservation conditions to improve the nutritional as well as the functional quality of maize flour. Previously, studies have been performed to evaluate the effect of various factors on nutritional composition and functional properties of maize flour and its blends with other seed flours [14–18]. However, no data is available regarding the accumulative effect of

gamma radiations and particle size on the functional properties of maize flour. Therefore, the present study was designed to optimize the said parameters using Response Surface Methodology (RSM). The data would provide useful information for the food manufacturers regarding the choice of suitable particle size and minimal dose of gamma radiation for the preservation of their maize products.

2. MATERIALS AND METHODS

The maize grains were collected from Maize and Millet Research Institute (MMRI), Yousaf Wala, Sahiwal, Pakistan. The mature and immature grains were separated manually. The mature grains were dried, cleaned and ground to fine flour using an electric grinder. The flour was packed in air tight glass bottles and subjected to sieving and gamma irradiation processes according to the developed experimental design.

2.1 Experimental Design

A bi-factorial face centered central composite design (CCD) was developed to study the functional properties of maize flour as affected by two independent variables as given below:

X_1 : Particle size of flour

Three levels of particle sizes, <420, <250 and <177 μm were obtained from the sieves of 40, 60 and 80 meshes respectively. The negative sign shows the particle size below the respective value [19].

X_2 : Radiation dose

Three levels of gamma radiation dose were selected as 25, 50 and 75 kGy.

The coded levels of these variables were calculated as:

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

Where ξ_i is the specific location of independent variable, $\bar{\xi}_i$ is the center point and S_i is the scale factor *i.e.* the difference between ξ_i and $\bar{\xi}_i$. X_i is

the coded value of an independent variable ξ_i ($i = 1, 2, \dots, k$). 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 was found by using the procedure of sequential experimentation. A response surface polynomial quadratic model was developed to find the levels of independent variables in new region where optimal response is achieved. The developed model determines the relationship of functional properties against the particle size of maize flour and the dose of gamma radiations. The study was done in phases using CCD which consists of 11 points with $n_f = 4$ factorial points, $n_a = 4$ axial point and $n_c = 3$ center points.

2.2 Sieving

The flour was sieved successively through micro sieves of different mesh number (80, 60 and 40 to obtain different levels of particle size.

2.3 Gamma Irradiation of Flour

The maize flour of each particle size was irradiated in transparent glass bottles at three levels of gamma radiation dose (25, 50 and 75 kGy) 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. All of the gamma irradiated samples of maize flour were stored at 25±5°C in sterile environment throughout the study period. The non-irradiated flour of each particle size (taken as control) was also preserved in sealed glass bottles at the similar laboratory conditions.

2.4 Functional Properties

Water holding capacity (WHC), oil holding capacity (OHC), loose bulk density (LBD), packed bulk density (PBD), emulsifying capacity (EA) and emulsifying stability (ES) of the gamma irradiated and non-irradiated samples of maize flour were determined by reported method [20].

Swelling capacity (SC) and protein solubility (PS) of flour in water were determined by the reported method [21] with some modifications. The flour (1 g) was dispersed in distilled water (50 mL) and heated at 60°C for 30 minutes in a thermally controlled water bath. The mixture was then

allowed to cool at room temperature and centrifuged at 2200 × g for 15 minutes. The residue obtained from the centrifugation along with the water it retained was reweighed. The percent SC was calculated as:

$$SC (\%) = W_{rw}/W_s \times 100$$

where W_s is weight of the sample while W_{rw} is weight of the residue along with the water it retained.

The supernatant was allowed to evaporate and the residue obtained after evaporation was subjected to the measurement of the percent protein solubility of flour in water as:

$$PS (\%) = W_r/W_s \times 100$$

where W_s is weight of the sample while W_r is weight of the residue obtained after evaporation.

2.5 Statistical Analysis

The results for the functional properties of non-irradiated maize flour of different particle size were expressed as means ± standard deviation of three parallel replicates. The data were statistically analyzed by one way analysis of variance (ANOVA) using statistical software (SPSS, version 14.0) and the means were separated by applying Tukey's multiple range tests at a confidence interval $p \leq 0.05$.

The prediction of variation in functional properties of maize flour as a function of particle size and gamma radiation dose and the optimization of independent variables to achieve the desired values of functional properties were achieved by creating response-surface models. The quadratic polynomial models for the functional properties were predicted by determining the regression coefficients using least-squares technique. The generalized polynomial model for predicting the variation of the response variables is given below:

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

where Y_i is the predicted response, β_0 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 the interaction effect of independent variables.

The significance of the estimated regression coefficient for each functional property was assessed by lack of fit test (F -ratio) at a

Table 1. Coded and actual levels of independent variables as per chosen by central composite design

Experimental runs	Coded levels of variables		Actual levels of variables			
	X_1	X_2	ξ_1 Particle size (meshes)	ξ_2 Radiation dose (kGy)		
1	-1	-1	40	25		
2	-1	1	40	75		
3	-1	0	40	50		
4	0	-1	60	25		
5 ^a	0	0	60	50		
6 ^a	0	0	60	50		
7 ^a	0	0	60	50		
8	0	1	60	75		
9	1	0	80	50		
10	1	-1	80	25		
11	1	1	80	75		
Coded level				-1	0	1
ξ_1 : Radiation dose (kGy)				25	50	75
ξ_2 : Particle size in terms of mesh number (meshes)				40	60	80

^a Center points

probability (p) of 0.05. The lack of fit is a measure of failure of a model to fit the data in experimental domain particularly for reduced points in a randomized experiment. The reduced model contained only those terms which were found statistically significant ($p < 0.05$). The corresponding variables with larger F -values and smaller p -values were considered more significant. 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. Adequate precision measures the signal to noise ratio. A ratio greater than 4 indicates an adequate signal. The statistical software, Design Expert 8.0.4.1 (Stat-Ease, Inc.) was used for the development of experimental design, data analysis and optimization procedure.

3. RESULTS AND DISCUSSION

The experimental values of functional properties of non-irradiated maize flour at selected levels of particle size are presented in Table 2. Statistically significant difference ($p < 0.05$) was observed in all functional properties among flours of different particle sizes. A statistically significant decrease in WHC, OHC, SC, EC and ES was observed in response to an increase in the

particle size of flour. The decrease in these functional properties in large size particles may be attributed to the small surface area and less availability of hydrophilic and hydrophobic functional groups of protein molecules for absorption, swelling and emulsification. LBD, PBD and PS were found to be increased in response to increase in particle size. The variation in WHC and OHC is in accordance with that reported earlier [17]. The EC value of maize flour was found to be comparatively low than that reported earlier for maize flour [13].

3.1 Response Surface Analysis and Optimization of Results

RSM is a set of statistical and mathematical tools which has been successfully applied for designing, developing, improving and optimizing the process under study [22]. It has been extensively used in food technology and chemical engineering to create response-surface models for the prediction of changes in response variables as a function of changes in input variables [23,24]. It has been also used previously for the optimization of preparation conditions to study the effect of preparation variables on functional properties and product characteristics of different flours [25,26].

In present study RSM was used to predict an optimum level for each of the independent variables. The non-significant terms were dropped in the initial model and the experimental data were fitted again only to the significant

Table 2. Functional properties of non-irradiated maize flour at different levels of particle size

Particle size (meshes)	Functional properties							
	WHC (%)	OHC (%)	PS (%)	SC (%)	LBD (g/dm ³)	PBD (g/dm ³)	EC (%)	ES (%)
80	175.20±1.06 ^a	123.53±2.20 ^a	65.00±1.40 ^c	273.67±3.79 ^a	0.325±0.018 ^b	0.450±0.027 ^b	45.77±0.32 ^a	97.30±1.13 ^a
60	161.66±2.08 ^b	96.20±1.06 ^b	96.17±0.76 ^b	204.83±3.33 ^b	0.360±0.010 ^a	0.528±0.019 ^a	42.53±0.50 ^b	85.57±0.51 ^b
40	146.17±1.76 ^c	57.93±3.41 ^c	103.30±2.08 ^a	197.17±4.65 ^b	0.390±0.010 ^a	0.560±0.020 ^a	39.77±1.66 ^c	83.73±0.87 ^b

Table 3. The observed values of functional properties of maize flour at random levels of experimental conditions as per chosen by central composite design

Runs	Particle size (meshes)	Radiation dose (kGy)	WHC (%)	OHC (%)	PS (%)	SC (%)	LBD (g/dm ³)	PBD (g/dm ³)	EC (%)	ES (%)
1	80	25	150.2	186.60	10	190	0.397	0.509	2.22	98.00
2	40	25	175.8	135.20	15	247	0.304	0.599	4.88	82.70
3	60	25	156.9	142.80	25	232	0.392	0.524	2.60	40.00
4	80	50	135.4	110.20	85	210	0.417	0.495	8.69	98.00
5	60	50	146.8	112.20	95	265	0.360	0.488	6.66	66.60
6	60	50	146.8	112.20	95	265	0.360	0.488	6.66	66.60
7	60	50	146.8	112.20	95	265	0.360	0.488	6.66	66.60
8	40	50	154.5	120.20	85	267	0.340	0.413	2.20	98.00
9	60	75	129.1	103.40	85	230	0.647	0.951	6.52	33.30
10	80	75	123.4	93.60	90	168	0.830	1.053	2.50	99.00
11	40	75	114.5	111.20	80	290	0.338	0.442	11.11	60.00

parameters to obtain the final reduced model. However, the non-significant linear terms were included in the final reduced model if the quadratic or interaction terms containing these variables were found to be significant.

The experimental values of functional properties of gamma irradiated maize flour at various levels of particle size and radiation dose as per chosen by the experimental design are given in Table 3. The application of RSM yielded the regression equations (Table 4) which showed that the relationship between particle size, radiation dose and functional properties of maize flour could be explained significantly by second order polynomial regression models. The polynomial regression equation includes the coefficient for intercept, main 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 functional properties of maize flour as determined by analysis of variance (ANOVA) are given in Table 5. The significance and adequacy of the model was measured in terms of *F*-value and *p*-value at 5% significance level ($p \leq 0.05$). The measurement of *F*-value and *p*-values indicated that radiations dose has a significant positive linear effect on WHC and PS but a significant negative linear effect on OHC, LBD and PBD of maize flour. Particle size has a significant linear positive effect on WHC, SC, LBD and PBD and significant negative linear effect on ES of the flour. The interaction effects were found to be significant on each of the functional properties except EC and ES. Quadratic effects of both variables were found to be significant on PS, SC and ES. However, the quadratic effect of radiation dose was also found to be significant on OHC, LBD and PBD of the flour.

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 values of R^2 (0.9281-0.9950) indicated that more than 92% of the variability in the functional properties could be explained by the suggested model. The values of adjusted R^2 (0.8843-0.9915) for these responses also advocate the significance of the model. The relatively low values of CV (2.95-12.42%) showed a better precision and reliability of the experimental runs. The relatively high values of adequate precision (15.072-40.671) provides an

adequate signal which indicates that the suggested model can be used to navigate the design space.

The variations in functional properties in response to a change in radiation dose and particle size have been explained in three dimensional (3D) response surface plots (Fig. 1a-h). To test the applicability of the model, predicted values of functional properties calculated from the polynomial regression equations were plotted against the experimental values (Fig. 2a-h). A good agreement between the experimental and predicted values of responses was observed with high values of coefficients of determination ($R^2 = 0.903-0.995$). The higher values of R^2 prove the applicability of proposed model with greater accuracy to study the effect of suggested variables on functional properties of maize flour.

The results for numerical optimization of radiation dose and particle size to achieve the desired levels of functional properties are presented in Table 6. The optimum levels of each variable were selected in range at maximum desirability by suggesting the desired goals of functional properties. The optimum levels of particle size in terms of sieve mesh No. and radiation dose to achieve the desired levels of response variables were found to be 25 kGy and 40 meshes for WHC, 75 kGy and 80 meshes for OHC, 25 kGy and 80 meshes for PS, 59.83 kGy and 40 meshes for SC, 75 kGy and 80 meshes for both the LBD and PBD, 69.22 kGy and 40 meshes for EC and 50 kGy and 80 meshes for ES respectively.

The increase in WHC and decrease in OHC may be attributed to the exposure of more hydrophilic groups of proteins in response to an increase in radiation dose. It suggests that gamma irradiation is an important factor in improving the quality of fat free diet formulations [18]. The flour possessing high WHC and low OHC are considered more suitable for the preparation of viscous and fat free foods such as soups, gravies and bakery products. The favorable range of WAC for the preparation of viscous foods such as soups, gravies and bakery products has been found to be 149.1-471.5% [27]. The reduction in the bulk density of flours helps in preparation of supplementary food and packing of raw or processed foods at large scale. The increase in EC and ES of maize flour makes it better to be used in food formulations like snacks, pastries, coffee whiteners and frozen desserts.

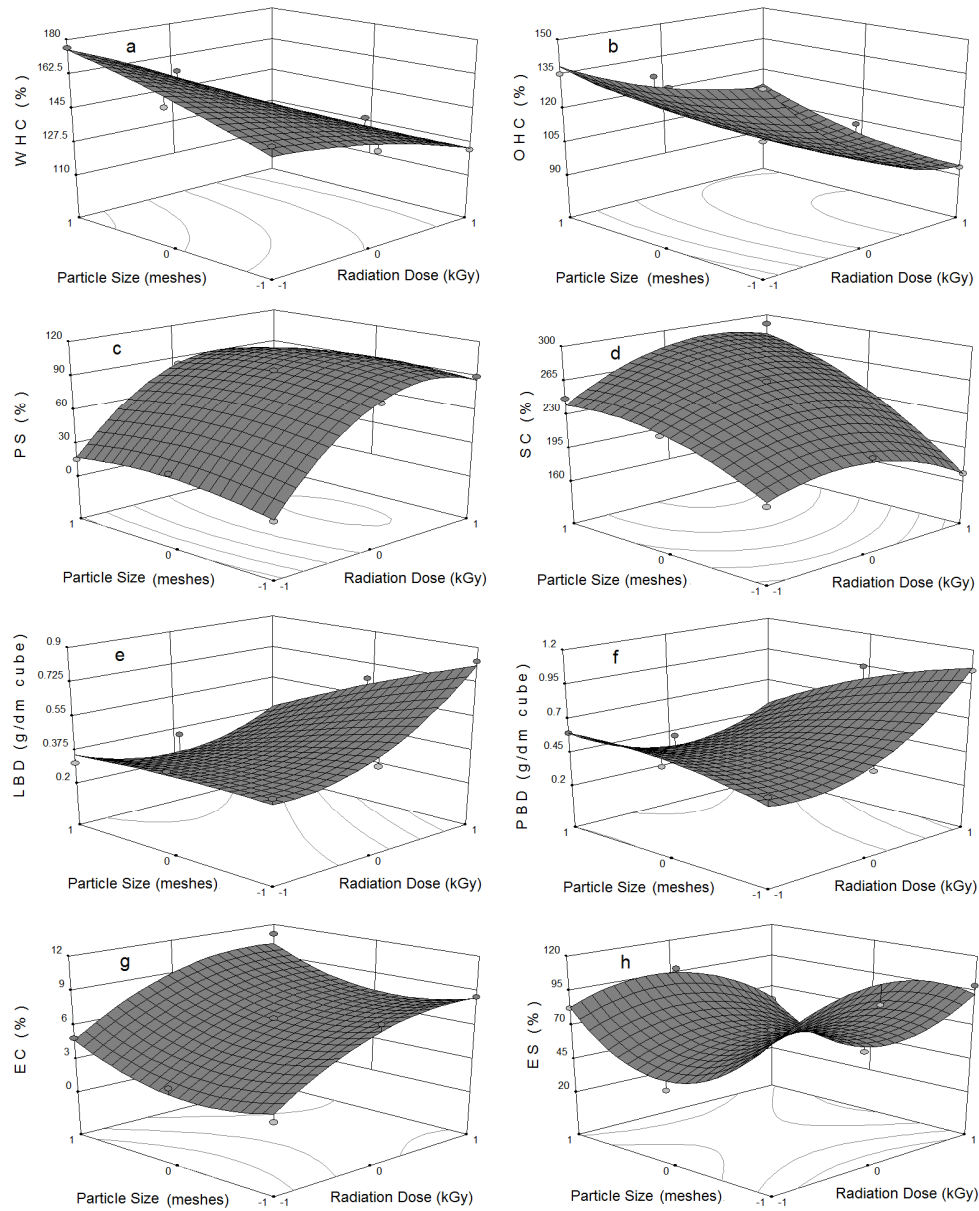


Fig. 1(a-h). 3D response surface plot of functional properties of maize flour as a function of gamma radiation dose and particle size

Table 4. Polynomial regression equations as obtained by response surface models

Functional property	Regression equation
WHC (%)	$Y_1 = 146.82 + 5.97X_1 - 19.32X_2 - 1.93X_1^2 - 3.88X_2^2 - 8.62X_1X_2$
OHC (%)	$Y_2 = 106.81 + 2.53X_1 - 19.57X_2 + 4.35X_1^2 + 12.25X_2^2 + 7.00X_1X_2$
PS (%)	$Y_3 = 94.48 - 0.83X_1 + 34.17X_2 - 8.19X_1^2 - 38.19X_2^2 - 3.75X_1X_2$
SC (%)	$Y_4 = 262.34 + 39.33X_1 + 3.17X_2 - 17.21X_1^2 - 24.71X_2^2 + 16.25X_1X_2$
LBD (g/dm ³)	$Y_5 = 0.37 - 0.11X_1 + 0.12X_2 - 0.016X_1^2 + 0.13X_2^2 - 0.10X_1X_2$
PBD (g/dm ³)	$Y_6 = 0.50 - 0.10X_1 + 0.14X_2 - 0.059X_1^2 + 0.22X_2^2 - 0.18X_1X_2$
EC (%)	$Y_7 = 6.57 + 0.91X_1 + 2.74X_2 + 1.77X_1^2 - 1.77X_2^2 - 0.013X_1X_2$
ES (%)	$Y_8 = 64.41 - 9.38X_1 - 4.73X_2 + 40.06X_1^2 - 22.29X_2^2 - 5.92X_1X_2$

Table 5. Analysis of variance (ANOVA), correlation coefficient (R²), adjusted R² and probability values for functional properties of gamma irradiated maize flour

	Model	X ₁	X ₂	X ₁ X ₂	X ₁ ²	X ₂ ²	CV (%)	R ²	R ² (adj)	Adequate precision
Water holding capacity (%)										
F-value	31.30	11.81	123.82	16.46	0.57	2.30	2.95	0.9572	0.9266	19.345
P-value	0.0001 ^a	0.0109 ^a	<0.0001 ^a	0.0048 ^a	0.4743	0.1728				
Oil holding capacity (%)										
F-value	39.86	2.39	142.66	12.17	3.25	25.75	3.51	0.9661	0.9418	19.491
P-value	<0.0001 ^a	0.1659	<0.0001 ^a	0.0101 ^a	0.1145	0.0014 ^a				
Solubility (%)										
F-value	279.78	0.460	767.70	6.17	20.30	441.51	4.13	0.9950	0.9915	40.671
P-value	<0.0001 ^a	0.521	<0.0001 ^a	0.042 ^a	0.0028 ^a	<0.0001 ^a				
Swelling capacity (%)										
F-value	30.50	98.49	0.64	11.21	8.68	17.89	4.00	0.9561	0.9248	17.657
P-value	0.0001 ^a	<0.0001 ^a	0.4505	0.0123 ^a	0.0215 ^a	0.0039 ^a				
Loose bulk density (g/cm³)										
F-value	18.08	26.79	31.86	14.60	0.25	15.91	12.42	0.9281	0.8768	15.958
P-value	0.0007 ^a	0.0013 ^a	0.0008 ^a	0.0065 ^a	0.6336	0.0053 ^a				
Packed bulk density (g/cm³)										
F-value	19.81	13.81	25.17	28.00	2.23	31.59	11.60	0.9340	0.8869	16.354
P-value	0.0005 ^a	0.0075 ^a	0.0015 ^a	0.0011 ^a	0.1793	0.0008 ^a				
Emulsifying capacity (%)										
F-value	21.29	8.49	76.62	1.064E ⁻⁰⁰³	14.69	14.78	11.67	0.9383	0.8942	15.642
P-value	0.4796	0.4951	0.1603	0.3089	0.8453	0.4806				
Emulsifying stability (%)										
F-value	19.35	9.47	2.41	2.52	79.49	24.60	10.28	0.9325	0.8843	15.072
P-value	0.0006 ^a	0.0179 ^a	0.1645	0.1566	<0.0001 ^a	0.0016 ^a				

^a Significant

Table 6. 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	
Radiation dose (kGy)	in range	25.00	75.00				
Particle size (meshes)	in range	80.00	40.00				
WHC (%)	maximize	114.50	175.80	25.00	40.00	174.914	0.986
OHC (%)	minimize	93.60	146.60	75.00	80.00	94.32	0.986
PS (%)	minimize	10.00	95.00	25.00	80.00	11.02	0.988
SC (%)	maximize	168.00	290.00	59.83	40.00	288.29	0.986
LBD (g/dm ³)	maximize	0.30	0.83	75.00	80.00	0.30	1.00
PBD (g/dm ³)	maximize	0.41	1.05	75.00	80.00	0.41	1.00
EC (%)	maximize	2.22	11.11	69.22	40.00	10.30	0.908
ES (%)	maximize	33.30	100.00	50.00	80.00	113.857	1.00

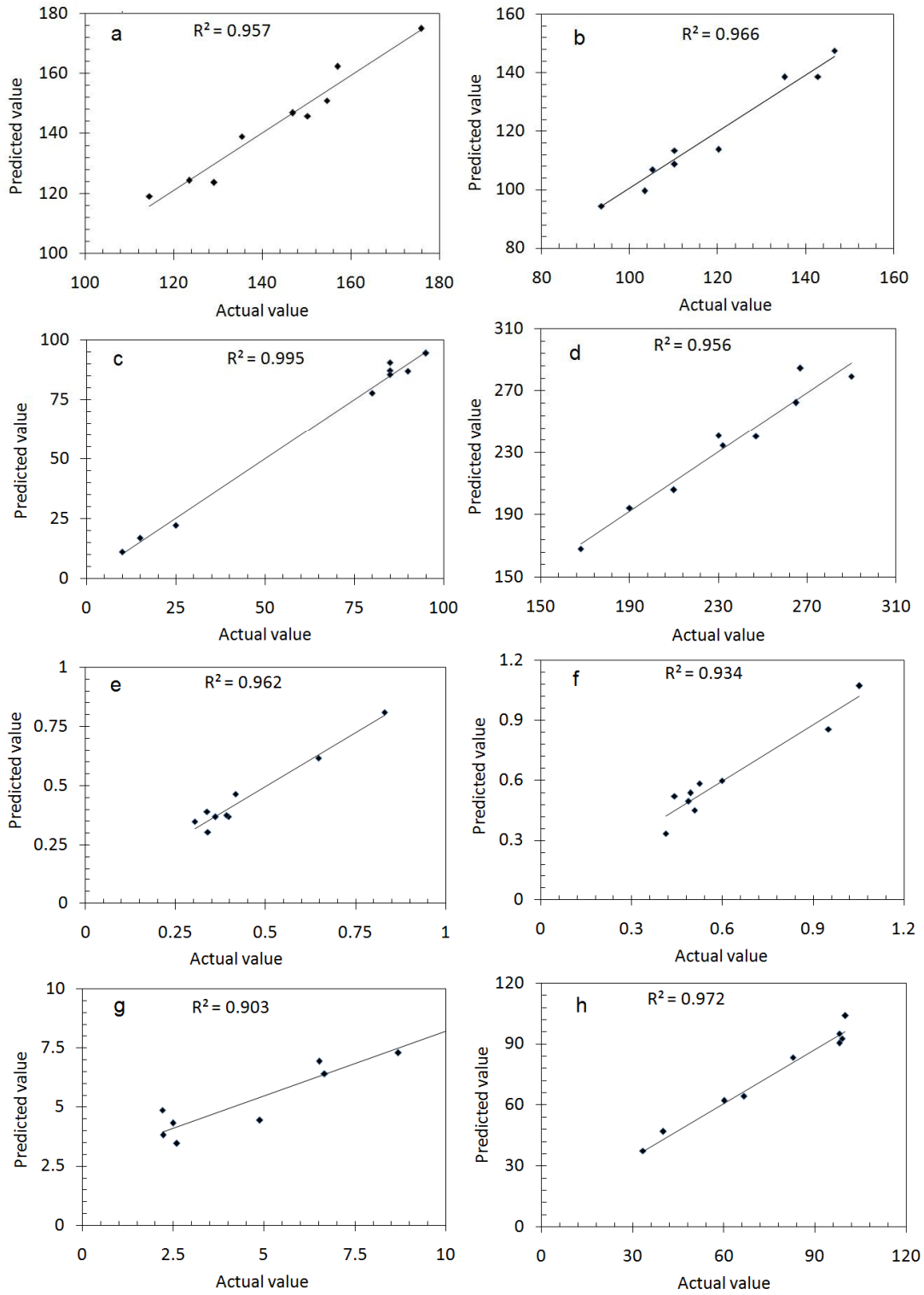


Fig. 2. Agreement between experimental and predicted values of functional properties of maize flour obtained from polynomial regression equations
a: WHC, b: OHC, c: PS, d: SC, e: LBD, f: PBD, g: EC, h: ES

4. CONCLUSIONS

In conclusion, the functional properties of maize flour have been found to be significantly affected by gamma irradiation along with a successive increase or decrease in the particle size of the flour. The particle size of the flour was found to be directly associated with the water holding capacity, swelling capacity and bulk density of flour. Moreover, gamma radiation influence favorably in improving the water and oil absorption, swelling and emulsifying capacities of maize flour.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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