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Response surface optimization of gamma radiation-induced variation in functional quality of *Nelumbo nucifera* rhizome flour at various particle sizes

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Summary

The cumulative effect of particle size (PS), gamma radiation dose (GRD), and suspension period (SP) on the functional quality of *N. nucifera* rhizome flour was optimized. A tri-factorial central composite design was constructed at five levels of each of PS, GRD, and SP. The *N. nucifera* rhizome flour at the selected particle sizes (50, 100, 150, 200, and 250 μm) was subjected to gamma irradiation at the selected levels of GRD (5, 10, 15, 20, and 25 kGy) followed by the analysis of its functional properties at different levels of SP (6, 12, 18, 24, and 30 h). The response-surface analysis showed a statistically significant linear positive effect of PS on the water absorption capacity (WAC) and swelling capacity (SC) and a linear negative effect on protein solubility index (PSI) ($p=0.000-0.028$). The GRD showed a significant linear positive effect on oil absorption capacity (OAC) and PSI while a linear negative effect on WAC and SC ($p=0.000-0.008$). The PS and GRD showed significant quadratic negative effects on WAC, OAC, and SC. However, the SP showed no significant linear effect on the studied functional properties of the flour.

Keywords: Central composite design, Functional properties, Gamma radiation, *Nelumbo nucifera*, Lotus, Particle size, Process optimization, Response surface methodology

Introduction

Nelumbo nucifera Gaertn. is an ornamental plant species also cultivated as a nutritional crop in East Asia. The *N. nucifera* rhizome, a rich source of protein and carbohydrates mainly starch and fiber is widely used as food in China and the Subcontinent (SHAD et al., 2011). The *N. nucifera* rhizome flour is used to improve the nutritional composition and functional properties of wheat and maize flour-based bakery products (SUN et al., 2016; THANUSHREE et al., 2017). The *N. nucifera* rhizome is also a good source of minerals and medicinally important bioactive phytochemical compounds (CHEN et al., 2019; HUANG et al., 2011; JANG et al., 2017). Based on the presence of these compounds, it possesses anti-hypercholesterolemic, antioxidant, anti-allergy, antidiabetic, and anti-inflammatory activities (CHEN et al., 2019; CHEN and TENGKU, 2020; KANEYASU et al., 2019; KAUR et al., 2019, 2019; NAWAZ et al., 2021; SHOWKAT et al., 2021). The application of *N. nucifera* rhizome flour in various food formulations entirely depends on its functional properties (CHANDRA and SAMSHER, 2013). The functional properties depend on the structure, type, and ratio of the protein and carbohydrates present in the flour. The hydrophobic and hydrophilic nature and hydrogen bonding in protein and carbohydrates significantly contribute to the consistency, texture, taste, and flavor of the food products (BALJEET et al., 2010).

The protein and carbohydrates possessing more hydrophilic functional groups in their structure show relatively higher values of water absorption, swelling capacities and solubility index (AWUCHI et al., 2019). However, relatively higher oil absorption capacities have been shown by those with more hydrophilic groups in their structures. Any change in the structure and composition of the protein and carbohydrates directly affects the functional behavior of the flour (IYENAGBE et al., 2017; NAWAZ et al., 2018a).

The preservation and processing methods are reported to affect the nutritional attributes of food materials to extend their shelf life (GUILLARD et al., 2010). Heat, pH change, microwave and gamma-ray treatments are some of the physical factors that have been reported to modify the structure and properties of the flours (ARINOLA and AKINGBALA, 2018; NAWAZ et al., 2019; PÉREZ et al., 2017; SUN et al., 2020; TORBICA et al., 2021). Gamma irradiation is frequently used for the sterilization of food materials and other industrial products. As high-energy radiation, gamma rays can kill microorganisms and protect food products from spoilage. However, the process is not safe for temperature-sensitive products as high doses of gamma radiation may result in the denaturation and degradation of the protein and carbohydrates present in food materials. Studies have shown that high dose gamma irradiation significantly affect the structural arrangement of starch and protein molecules resulting in the modifications in the functional quality and stability of foods (ONEH ABU et al., 2006). The particle size of the flour and duration of suspension of flour in water or oil for the determination of functional properties are the associated factors that may affect the functional quality of the flours (NAWAZ et al., 2021).

The nutritional and functional quality of *N. nucifera* rhizome flour may be improved by optimizing the processing and preservation factors for its utilization in food formulations. Previously, the literature has been reported on the nutritional and functional quality of *N. nucifera* rhizome flour and its blends with other seed flours (HUSSAIN et al., 2017; NAWAZ et al., 2018b; M. SHAD et al., 2011). However, no literature has been found on the combined effect of various process variables on the functional quality of *N. nucifera* rhizome flour. The present study was designed to optimize the effect of gamma irradiation dose on the functional quality of *N. nucifera* rhizome flour at different particle sizes. The optimization was done by response surface methodology, a statistical technique successfully applied to optimize the relationship among the input and response variables in a multivariate study (MONTGOMERY, 2017).

Materials and methods

The fresh *N. nucifera* rhizomes were collected from the marshes located near River Indus in district Muzaffargarh, Punjab, Pakistan, washed in distilled water, crushed into small chunks, and dried under

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shade in air. The dried samples were ground to a fine flour using an electric grinder. The flour was preserved in an airtight container till further processing.

Experimental design

A tri-factorial response-surface central composite design (CCD) was constructed at five levels of each of the particle size (PS: 50, 100, 150, 200, and 250 μm), gamma radiation dose (GRD: 5, 10, 15, 20, and 25 kGy), and suspension period (SP: 6, 12, 18, 24, and 30 h). The design selected 20 combinations of the selected input variables including eight factorial, six axial, and six center points. The flour processed at the suggested combinations of the selected input variables was analyzed for water absorption, oil absorption, swelling capacities, and protein solubility index. The process of optimization to find out the optimal response was done by sequential experimentation procedure using a polynomial quadratic model.

Sieving

The rhizome flour was sieved consecutively to obtain different levels of particle size (50, 100, 150, 200, and 250 μm) using the micro sieve of different sieve numbers (60, 70, 100, 140, and 270 meshes/inch) as selected by the experimental design.

Gamma irradiation of flour

The rhizome flours of the selected particle sizes were irradiated in transparent airtight glass containers at five levels of GRD (5, 10, 15, 20, and 25 kGy) keeping 1.5 m sample to source distance using ^{60}Co (32000 curies) gamma radiation source at Pakistan Radiation Service (PARAS), Lahore, Pakistan. The absorbed dose was measured using Ceric-cerous dosimeters. After gamma irradiation, all the samples were kept at $25\pm 5^\circ\text{C}$ in sterile conditions. The flour of each particle size without gamma radiation treatment was also preserved at similar laboratory conditions.

Determination of functional properties

Water absorption capacity (WAC), oil absorption capacity (OAC), swelling capacity (SC), and protein solubility index (PSI) of the native and gamma-irradiated flours were determined by the previously described methods with slight modifications (SHAD et al., 2011) (BISHNOI and KHETARPAUL, 1993). For the determination of WAC, the gamma-irradiated flour (1 g) was suspended in distilled water (100 ml) for different suspension periods (6, 12, 18, 24, and 30 h) as selected by CCD. The suspension was centrifuged and weighed. The WAC was calculated as:

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

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

The gamma irradiated flour (1 g) was suspended in canola oil for the selected levels of suspension period followed by centrifugation at $3000 \times g$ and weighed for the calculation of WAC as:

$$\text{OAC (\%)} = W_{ro} / W_s \times 100$$

where W_{ro} is the weight of the residue along with the retained oil and W_s is the weight of the sample.

The gamma irradiated flour (1 g) was suspended for the selected levels of SP followed by heating in a water bath at 60°C for 30 min. After cooling to room temperature, the suspension was centrifuged at $2200 \times g$ for 15 min. The supernatant was discarded, the residue obtained with the retained water was weighed and SC (%) was calculated as:

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

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

The supernatant obtained after centrifugation was allowed to evaporate and the residue obtained was weighed for calculation of PSI (%) as:

$$\text{PSI (\%)} = W_r / W_s \times 100$$

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

Statistical Analysis

The optimization of the effect of process variables on the response was done by response-surface methodology using a polynomial response-surface quadratic model. The suggested model generated the following generalized polynomial equation to show the relationship of the selected input variables and the studied responses.

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{23} X_2 X_3$$

where β_0 is a constant showing the main effect and Y_i is the predicted response. $\beta_1, \beta_2,$ and β_3 are the linear, $\beta_{12}, \beta_{13},$ and β_{23} are the interactions while $\beta_{11}, \beta_{22},$ and β_{33} are the quadratic effects of the input variables.

The significance of variation in the functional properties under the influence of input variables was determined by the lack of fit test (F-ratio) at a probability $p \leq 0.05$. The coefficient of determination (R^2) and adjusted R^2 were also determined to evaluate the adequacy of the suggested model. The coefficient of variance (CV) was used to determine the reliability and precision of experiments. The optimum levels of independent variables to achieve an optimal response were found by a numerical optimization technique. The construction of the experimental model, data analysis, and optimization of variables was done using statistical software, Design Expert 10.0 (Stat. Ease, Inc. USA).

Results

The experimental values of the functional properties of the non-irradiated flour of *N. nucifera* rhizome at different particle sizes are presented in Tab. 1. The WAC, OAC, SC, and PSI of the flour ranged from 274.40 ± 8.82 to 300.67 ± 9.85 , 123.67 ± 7.21 to 160.0 ± 12.80 , 108.13 ± 9.96 to 114.75 ± 10.04 , and 8.93 ± 0.35 to $11.02 \pm 0.41\%$ respectively. The WAC and PSI of the native flour at different PS were found to be statistically different ($p < 0.05$) while no significant variation was observed in OAC and SC. The experimental data of the studied functional properties of the gamma-irradiated flour at different combinations of PS, GRD, and SP as selected by CCD are presented in Tab. 2. The WAC, OAC, SC, and PSI ranged from 203 to 416, 85 to 190, 103 to 111, and 8 to 15% with mean values of 365.10 ± 15.81 , 152.25 ± 10.80 , 107.50 ± 1.62 , and $12.05 \pm 1.36\%$ respectively.

Tab. 1: Functional properties of non-irradiated *N. nucifera* rhizome at different particle sizes

Particle size (μm)	WAC (%)	OAC (%)	SC (%)	PSI (%)
50	274.40 ± 8.82	143.67 ± 7.21	108.13 ± 9.96	8.93 ± 0.35
100	281.53 ± 15.06	148.0 ± 10.40	110.79 ± 12.04	9.31 ± 0.43
150	288.20 ± 6.41	154.01 ± 13.6	114.75 ± 10.04	9.91 ± 0.45
200	300.67 ± 9.85	160.0 ± 12.80	112.24 ± 9.11	10.67 ± 0.34
250	292.28 ± 12.63	148.0 ± 12.30	109.51 ± 9.48	11.02 ± 0.41
<i>p</i> -value	0.035	0.065	0.532	0.023

Response surface analysis and optimization of results

The cumulative effect of three process variables including PS, GRD, and SP on the studied functional properties of *N. nucifera* rhizome flour was optimized using RSM. The relationship between the pro-

Tab. 2: Functional properties of *N. nucifera* rhizome flour at various combinations of input variables selected by CCD.

Experimental Runs	Coded levels of variables			Actual levels of variables			WAC (%)	OAC (%)	SC (%)	PSI (%)
	X1	X2	X3	PS (μm)	GRD (kGy)	SP (h)				
1	0	0	0	150	15	18	400	185	110	14
2	-1	1	-1	200	20	12	363	115	105.32	13
3	1	1	1	100	20	24	330	170	106.02	12
4	0	0	2	150	15	30	393	135	103.76	11
5	0	0	0	150	15	18	400	185	110	14
6	0	-2	0	150	5	18	370	125	111	10
7	0	0	0	150	15	18	400	185	110	14
8	-2	0	0	250	15	18	400	100	105.42	10
9	-1	1	1	200	20	24	364	180	103.95	12
10	0	0	-2	150	15	6	375	165	103.97	9
11	0	0	0	150	15	18	400	185	110	14
12	2	0	0	50	15	18	203	85	104.15	15
13	-1	-1	-1	200	10	12	406	155	110.49	9
14	0	0	0	150	15	18	400	185	110	14
15	1	-1	1	100	10	24	300	100	105.67	11
16	1	-1	-1	100	10	12	356	130	105.92	8
17	-1	-1	1	200	10	24	416	145	109.15	8
18	1	1	-1	100	20	12	300	140	106.31	15
19	0	0	0	150	15	18	400	185	110	14
20	0	2	0	150	25	18	326	190	108.83	14
Mean \pm SD							365.10 \pm 15.81	152.25 \pm 10.80	107.50 \pm 1.62	12.05 \pm 1.36

*GRD: Gamma radiation dose, OAC: Oil absorption capacity, PSI: Protein solubility index, SC: Swelling capacity, SN: Sieve number, SP: Suspension period, WAC: Water absorption capacity

Tab. 3: Parameters of statistical analysis of the experimental data on the effect of process variables on the functional properties of *N. nucifera* rhizome flour.

Source	WAC			OAC			SC			PSI		
	CE	F-value	p-value	CE	F-value	p-value	CE	F-value	p-value	CE	F-value	p-value
Model	399.27	21.675	0.000	184.20	20.060	0.000	109.90	36.633	0.000	13.80	5.304	0.008
β_1 -PS (μm)	41.06	107.863	0.000	5.31	3.872	0.077	0.47	9.121	0.013	-0.88	6.613	0.028
β_2 -GRD (kGy)	-13.06	10.915	0.008	12.81	22.520	0.001	-0.87	31.393	0.000	1.50	19.436	0.001
β_3 -SP (h)	1.31	0.110	0.747	-0.31	0.013	0.910	-0.23	2.167	0.172	0.13	0.135	0.721
$\beta_1\beta_2$	-8.63	2.379	0.154	-10.63	7.743	0.019	-1.39	39.710	0.000	0.00	0.000	1.000
$\beta_1\beta_3$	4.63	0.684	0.427	6.88	3.242	0.102	-0.27	1.515	0.247	-0.25	0.270	0.615
$\beta_2\beta_3$	9.63	2.963	0.116	16.88	19.532	0.001	-0.01	0.002	0.969	-0.75	2.429	0.150
β_1^2	-24.99	62.772	0.000	-23.52	119.280	0.000	-1.35	118.393	0.000	-0.48	3.092	0.109
β_2^2	-13.36	17.953	0.002	-7.27	11.402	0.007	-0.07	0.318	0.585	-0.60	4.924	0.050
β_3^2	-4.36	1.914	0.197	-9.15	18.039	0.002	-1.58	162.079	0.000	-1.10	16.493	0.002
Residual		2501.13			1166.34			3.89			18.52	
Pure Error		0.00			0.000			0.000			0.000	
R^2		0.9512			0.9475			0.9706			0.8268	
Adj. R^2		0.9074			0.9003			0.9441			0.6709	
CV (%)		4.33			7.09			0.58			11.29	
AP (%)		18.40			13.71			18.73			6.24	

AP: Adequate precision, CE: Coefficient estimate, CV: Coefficient of variance, GRD: Gamma radiation dose, OAC: Oil absorption capacity, PSI: Protein solubility index, R^2 : Regression coefficient, SC: Swelling capacity, SN: Sieve number, SP: Suspension period, WAC: Water absorption capacity the effect of process

cessing factors and the functional properties of the flour was explained by developing the polynomial regression equations.

$$\text{WAC (\%)} = 399.27 + 41.06X_1 - 13.06X_2 + 1.31X_3 - 24.99X_1^2 - 13.36X_2^2 - 4.36X_3^2 - 8.63X_1X_2 + 4.63X_1X_3 + 9.62X_2X_3$$

$$\text{OAC (\%)} = 184.20 + 5.31X_1 + 12.81X_2 - 0.31X_3 - 23.52X_1^2 - 7.27X_2^2 - 9.15X_3^2 - 10.63X_1X_2 + 6.88X_1X_3 + 16.88X_2X_3$$

$$\text{SC (\%)} = 109.90 + 0.47X_1 - 0.87X_2 - 0.23X_3 - 1.35X_1^2 - 0.07X_2^2 - 1.58X_3^2 - 1.39X_1X_2 - 0.27X_1X_3 - 0.01X_2X_3$$

$$\text{PSI (\%)} = 13.80 - 0.88X_1 + 1.50X_2 + 0.13X_3 - 0.48X_1^2 - 0.60X_2^2 - 1.10X_3^2 + 0.00X_1X_2 - 0.25X_1X_3 - 0.75X_2X_3$$

These regression equations present the main, linear, quadratic, and interaction effects of the selected input variables on the functional

properties of the flour. The sign and number of the main effect determine the trend and extent of variation in the response variable under the influence of the selected input variables. The results of the one-way analysis of variance (ANOVA) of the experimental data are presented in Tab. 3. The measurement of F-value and p-value showed a statistically significant main effect ($F=5.304-36.633$, $p=0.000-0.008$) of the selected input factors on the functional properties of the *N. nucifera* rhizome flour. The PS showed a significant linear positive effect on the WAC and SC, and a linear negative effect on PSI ($F=6.613-107.863$, $p=0.000-0.028$). The GRD showed a significant linear positive effect on OAC and PSI while a linear negative effect on WAC and SC ($F=10.915-31.393$, $p=0.000-0.008$). However, the SP showed no significant linear effect on the studied functional properties of the flour. The response-surface analysis also showed a significant interaction effect of PS and GRD on OAC and SC and that of GRD and SP on OAC. The PS also showed a significant negative quadratic effect on WAC, OAC, and SC while GRD showed a quadratic negative effect on WAC, OAC, and PSI. The SP also showed a significant quadratic effect on OAC, SC, and PSI. The main, linear, quadratic, and interaction effects of various combinations of the PS, GRD, and SP on the studied functional properties are graphically presented in Fig. 1a-l.

The degree of fitness of data points on the regression line was determined by the regression coefficient (R^2). The observed values of R^2 (0.8268-0.9706) and adjusted R^2 (0.6709-0.9441) suggested that 82-97% variability in the studied functional properties in response to the selected input variables could be explained using the suggested statistical model. The precision and reliability of the suggested

model were checked by calculating the values of coefficient of variation (CV) and adequate precision (AP). A relatively lower value of CV and higher AP suggest that the model is more precise and reliable. The observed values of CV (0.58-11.29%) and AP (6.24-1873%) advocated the precision and reliability of the employed statistical model to study the relationship between the selected factors and the studied functional properties of *N. nucifera* rhizome flour.

The polynomial regression equations generated by the employed model were used to calculate the predicted values of the studied functional properties. Fig. 2a-d presents the correlation plots of the predicted values of the studied functional properties against the experimental ones. The plots were drawn to test the applicability of the employed model to study the effect of the selected factors on the functional properties of the flour. The plots showed very good agreements between the experimental and the predicted values with relatively higher values of R^2 (0.8268-0.9706) that suggested that the selected response-surface quadratic model may successfully be employed to study the relationship between the selected process variables and functional properties of the flour.

The levels of the input variables to achieve the optimal values of the studied functional properties were determined by the numerical optimization method. The calculated values of optimum levels of PS, GRD, and SP to achieve maximum values of WAC, OAC, SC, and PSI were PS: 224.65, 164.65, 184.046, and 136.85 μm , GRD: 8.63, 18.07, 10.15, and 23.37 kGy, and SP: 18, 21, 17, and 14 h respectively. The graphical expression of the numerical optimization of the PS, GRD, and SP to achieve maximum values of WAC, OAC, SC, and PSI is presented as contour graphs in Fig. 3a-l.

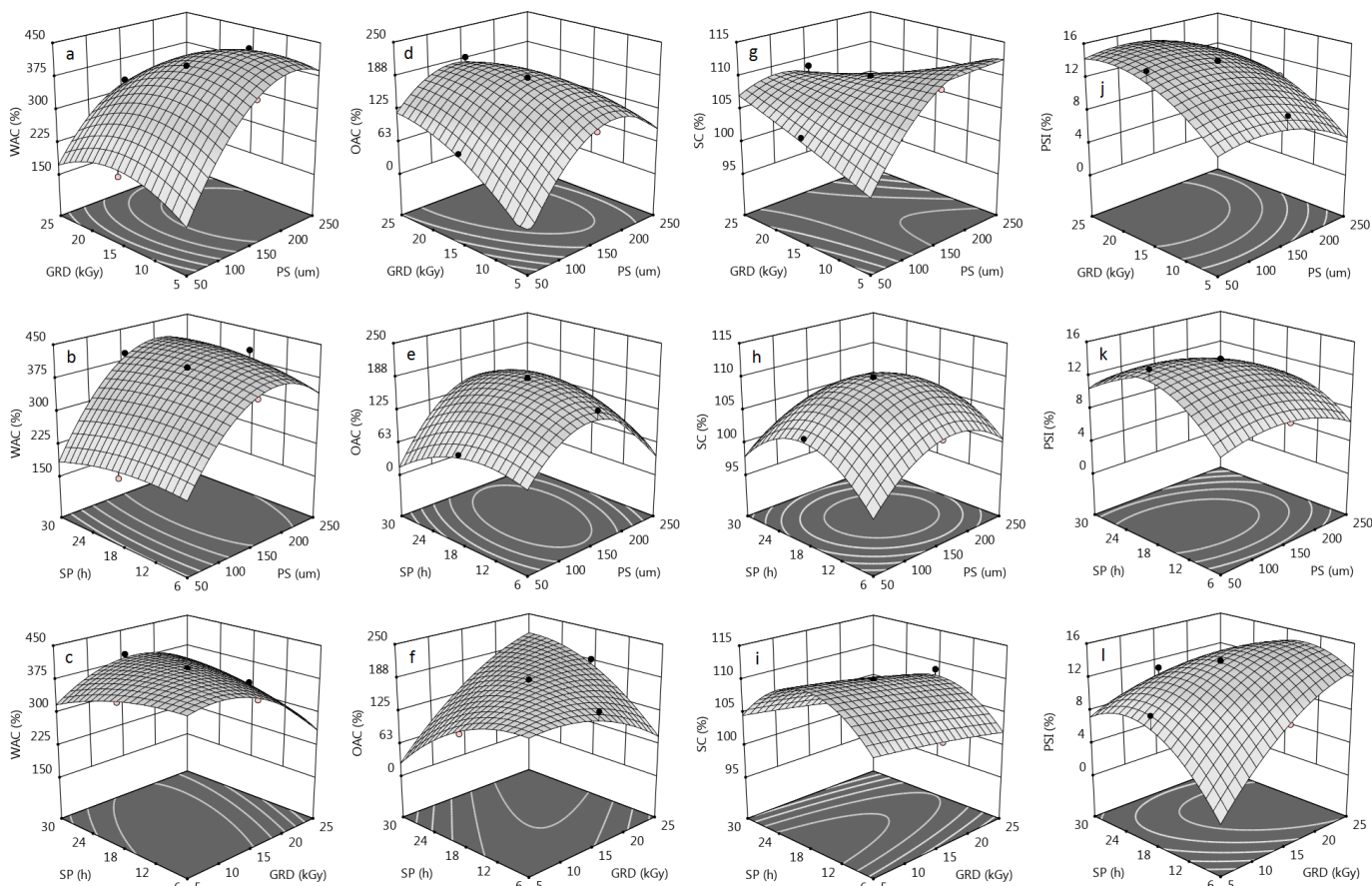


Fig. 1: Response-surface plots of functional properties of gamma-treated *N. nucifera* rhizome flour at selected levels of PS, GRD, and SP.

a-c) WAC, d-f) OAC, g-i) SC, and j-l) PSI

PS: Particle size, GRD: Gamma radiation dose, SP: particle size, WAC: water absorption capacity, OAC: Oil absorption capacity, SC: Swelling capacity, PSI: protein solubility index

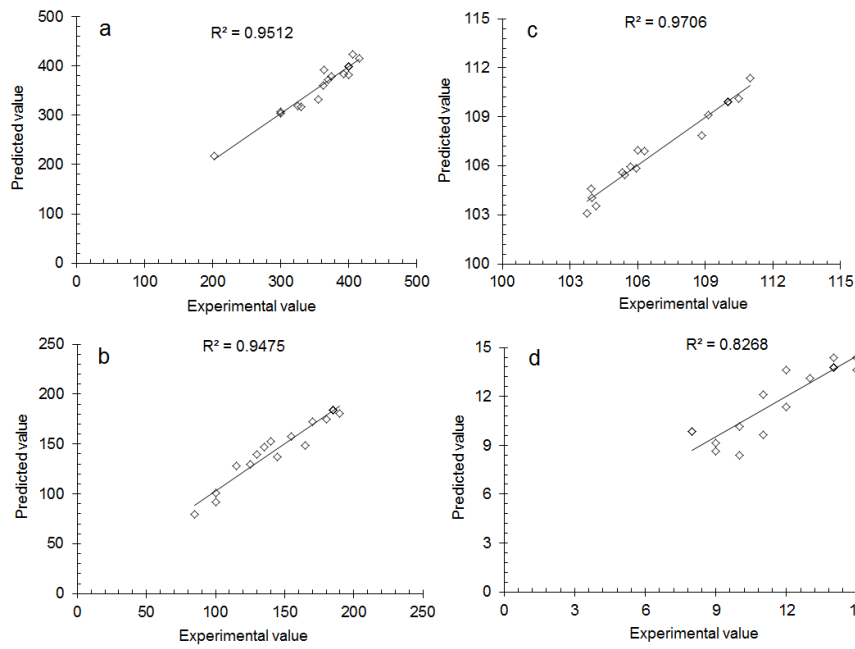


Fig. 2: Correlation plots of the predicted values of functional properties against experimental ones at the selected combinations of the input variables a) WAC, b) OAC, c) SC, and d) PSI

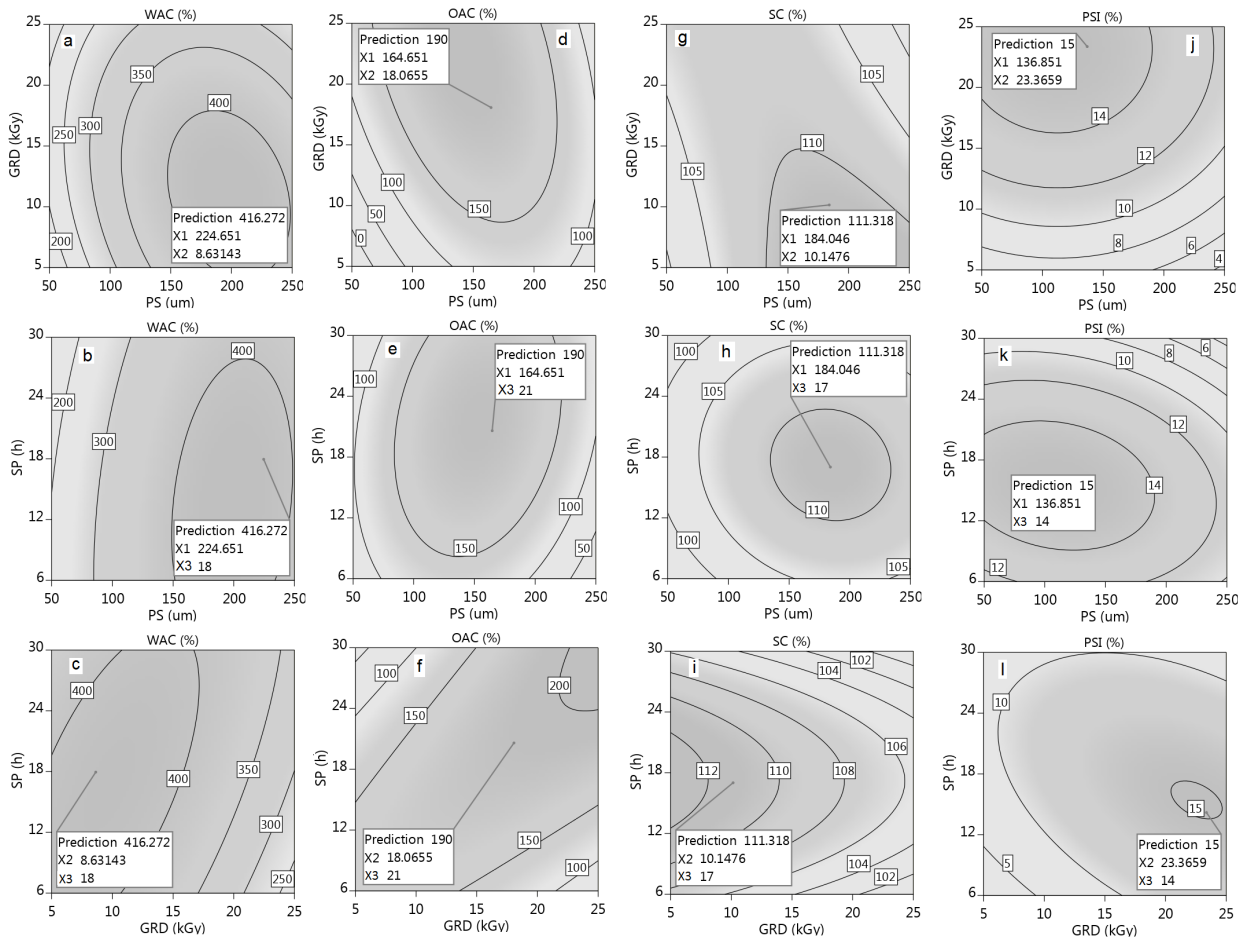


Fig. 3: Optimum levels of process variables for the optimal response of functional properties of *N. nucifera* rhizome flour
a-c) WAC, d-f) OAC, g-i) SC, and j-l) PSI
X1: PS, X2: GRD, X3: SP
PS: Particle size, GRD: Gamma radiation dose, SP: particle size, WAC: water absorption capacity, OAC: Oil absorption capacity, SC: Swelling capacity, PSI: protein solubility index

Discussion

Gamma irradiation is a frequently used technique for the sterilization of pharmaceutical and food products on an industrial scale. The gamma rays being high energy radiations have been also reported to affect the structure and properties of the food materials. The gamma irradiation may either improve or reduce the functional quality of the food products. The present study reports the optimization of the effect of gamma radiation on the functional properties of *N. nucifera* rhizome flour using response surface methodology (RSM). RSM is a set of mathematical and statistical tools effectively being applied for designing a model and developing, improving, and optimization of the experimental process (OLIVEIRA et al., 2015). It has been extensively applied in food processing technologies to develop response-surface models for multivariate analysis (MIRHOSSEINI and TAN, 2009; MONTGOMERY, 2017). Previously, it has also been used to optimize the effect of preparation variables on the characteristics of food products (ASARE et al., 2004).

The WAC and PSI of the non-irradiated flour at different PS were found to be statistically different ($p < 0.05$) while no significant ($p > 0.05$) variation was observed in OAC and SC. The WAC was found to be initially increased with an increase in the PS up to 200 μm but decreased at 250 μm . However, PSI was slightly increased with an increase in particle size. The results of numerical optimization showed that a particle size of 164-224 μm is more favorable for optimal WAC, OAC, and SC of the flour. However, the PSI of the flour was found to be a linear negative function of PS that showed optimum results at 136.85 μm . The observed increase in WAC and PSI as a function of PS may be attributed to the relatively higher number of pores and complex fibrous structure of the flour at a relatively higher particle size of the flour (HEMDANE et al., 2016). The results are in agreement with those reported earlier for wheat bran (MAJZOUBI et al., 2013; ONIPE et al., 2017) and chestnut flour (AHMED et al., 2016).

It is clear from the results that the selected process variables including PS, GRD, and SP showed a significant positive main effect on WAC, OAC, SC, and PSI of *N. nucifera* rhizome flour. The linear positive and quadratic negative effects of PS on WAC, OAC, and SC suggest that these properties are initially increased and then decreased in response to an increase in the PS of the flour. These results suggest that *N. nucifera* rhizome flour has less ability to absorb water or oil at very low or very high particle size due to changes in surface area and pore size.

The GRD also showed linear positive and quadratic negative effects on OAC and PSI while negative linear and quadratic effects on WAC and SC. However, no significant linear effect of SP was observed on the studied functional properties of the flour. The linear positive effect of GRD on OAC and PSI was in agreement with that reported for chickpea flour (BASHIR and AGGARWAL, 2016). The results of gamma radiation induced-decrease in WAC and SC are in agreement with those reported earlier for *Sagittaria sagittifolia* tuber flour (WANI et al., 2015) and wheat flour (BHAT et al., 2016). The polynomial change in PSI under the influence of gamma irradiation was also in agreement with that reported earlier for *Sesamum indicum* seed (HASSAN et al., 2018).

The observed variation in the studied functional properties of *N. nucifera* rhizome flour under the influence of an increase in the gamma radiation dose may be attributed to gamma radiation-induced disruption of the granular structure of flour and exposure of different functional groups. The dose-dependent linear decrease in WAC and SC and increase in OAC of the gamma-irradiated flour may be attributed to the gamma radiation-induced exposure of some hydrophobic functional groups in the starch and protein present in the flour. The polynomial change in PSI under the influence of gamma irradiation may also be attributed to gamma radiation-induced exposure of the soluble protein present in the flour. The WAC and OAC entirely depend

on the ratio of hydrophilic and hydrophobic functional groups on the carbohydrates and protein present in the flours. A relatively higher hydrophilic to a hydrophobic ratio of functional groups in starch or protein results in higher WAC and lower OAC of the flour (NAWAZ et al., 2019). The flours with relatively lower WHC and higher OHC are more applicable for the preparation of moisture-free food products possessing good mouthfeel and taste and longer shelf life (AWUCHI et al., 2019). The flours with relatively higher WAC and lower OAC are suitable for the preparations of fat-free formulations (NAWAZ et al., 2015). However, such types of foods are more susceptible to hydrolytic and microbial degradation. The SC has associated with the crystalline arrangement hydrogen binding ability of the flour. The flours with relatively higher SC are useful in the preparation of gravies, jellies, puddings, and bakery products (AWUCHI et al., 2019). The gamma radiation-induced linear increase in OAC and PSI and decrease in WAC and SC suggests that the gamma irradiation of food material within the suggested range of gamma radiation dose may be beneficial for sterilization as well as improving the functional quality of the carbohydrate and protein-based food products (NAWAZ et al., 2019). The present study would be a valuable contribution to the literature regarding the functional quality and product stability of food materials.

Conclusion

The studied functional properties of *N. nucifera* rhizome flour including WAC, SC and PSI were found to be linear positive and quadratic negative functions of particle size. Gamma radiation dose showed a linear negative effect on WAC and SC and a linear positive effect on OAC and PSI. However, the quadratic effect of GRD was found to be negative on each of the studied functional properties suggesting the decrease in the functional properties in response to further increase in GRD. The results suggest the use of gamma irradiation in the range of 5-25 kGy for improving the functional quality and product stability of carbohydrate and protein-based food materials.

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Conflicts of interest

No potential conflict of interest was reported by the authors.

References

- AHMED, J., AL-ATTAR, H., ARFAT, Y.A., 2016: Effect of particle size on compositional, functional, pasting, and rheological properties of commercial water chestnut flour. *Food Hydrocoll.* 52, 888-895. DOI: [10.1016/j.foodhyd.2015.08.028](https://doi.org/10.1016/j.foodhyd.2015.08.028)
- ARINOLA, S.O., AKINGBALA, J.O., 2018: Effect of pre-treatments on the chemical, functional and storage properties of breadfruit (*Artocarpus altilis*) flour. *Int. Food Res. J.* 25, 109-11.
- ASARE, E.K., SEFA-DEDEH, S., SAKYI-DAWSON, E., AFOAKWA, E.O., 2004: Application of response surface methodology for studying the product characteristics of extruded rice-cowpea-groundnut blends. *Int. J. Food Sci. Nutr.* 55, 431-439. DOI: [10.1080/09637480400003238](https://doi.org/10.1080/09637480400003238)
- AWUCHI, C.G., IGWE, V.S., ECHETA, C.K., 2019: The functional properties of foods and flours. *Int. J. Adv. Acad. Res.* 5, 139-160.

- BALJEET, S.Y., YADAV, R., YADAV, R., 2010: Studies on functional properties and incorporation of buckwheat flour for biscuit making. *Int. Food Res. J.* 17, 1067-1076.
- BASHIR, K., AGGARWAL, M., 2016: Effects of gamma irradiation on the physicochemical, thermal and functional properties of chickpea flour. *LWT-Food Sci. Technol.* 69, 614-622. DOI: [10.1016/j.lwt.2016.02.022](https://doi.org/10.1016/j.lwt.2016.02.022)
- BHAT, N.A., WANI, I.A., HAMDANI, A.M., GANI, A., MASOODI, F.A., 2016: Physicochemical properties of whole wheat flour as affected by gamma irradiation. *LWT-Food Sci. Technol.* 71, 175-183. DOI: [10.1016/j.lwt.2016.03.024](https://doi.org/10.1016/j.lwt.2016.03.024)
- BISHNOI, S., KHETARPAUL, N., 1993: Variability in physico-chemical properties and nutrient composition of different pea cultivars. *Food Chem.* 47, 371-373. DOI: [10.1016/0308-8146\(93\)90179-J](https://doi.org/10.1016/0308-8146(93)90179-J)
- CHANDRA, S., SAMSEHER, 2013: Assessment of functional properties of different flours. *Afr. J. Agric. Res.* 8, 4849-4852. DOI: [10.5897/AJAR2013.6905](https://doi.org/10.5897/AJAR2013.6905)
- CHEN, G., ZHU, M., GUO, M., 2019: Research advances in traditional and modern use of *Nelumbo nucifera*: phytochemicals, health promoting activities and beyond. *Crit. Rev. Food Sci. Nutr.* 59, 189-209. DOI: [10.1080/10408398.2018.1553846](https://doi.org/10.1080/10408398.2018.1553846)
- CHEN, S.Y., TENGKU, R.T.M., 2020: Effect of cooking methods on nutritional composition and antioxidant properties of lotus (*Nelumbo nucifera*) rhizome. *Food Res.* 4, 1207-1216. DOI: [10.26656/fr.2017.4\(4\).359](https://doi.org/10.26656/fr.2017.4(4).359)
- GUILLARD, V., MAURICIO-IGLESIAS, M., GONTARD, N., 2010: Effect of novel food processing methods on packaging: structure, composition, and migration properties. *Crit. Rev. Food Sci. Nutr.* 50, 969-88. DOI: [10.1080/10408390903001768](https://doi.org/10.1080/10408390903001768)
- HASSAN, A.B., MAHMOUD, N.S., ELMAMOUN, K., ADIAMO, O.Q., AHMED, I.A.M., 2018: Effects of gamma irradiation on the protein characteristics and functional properties of sesame (*Sesamum indicum* L.) seeds. *Radiat. Phys. Chem.* 144, 85-91. DOI: [10.1016/j.radphyschem.2017.11.020](https://doi.org/10.1016/j.radphyschem.2017.11.020)
- HEMDANE, S., JACOBS, P.J., DORNEZ, E., VERSPREET, J., DELCOUR, J.A., COURTIN, C.M., 2016: Wheat (*Triticum aestivum* L.) bran in bread making: A critical review. *Compr. Rev. Food Sci. Food Saf.* 15, 28-42. DOI: [10.1111/1541-4337.12176](https://doi.org/10.1111/1541-4337.12176)
- HUANG, B., HE, J., BAN, X., ZENG, H., YAO, X., WANG, Y., 2011: Antioxidant activity of bovine and porcine meat treated with extracts from edible lotus (*Nelumbo nucifera*) rhizome knot and leaf. *Meat Sci.* 87, 46-53. DOI: [10.1016/j.meatsci.2010.09.001](https://doi.org/10.1016/j.meatsci.2010.09.001)
- HUSSAIN, S.Z., ALI, F., JABEEN, R., ZARGAR, I., 2017: Twin screw extrusion cooking of lotus rhizome and broken rice flour blends: A response surface analysis. *JSIR.* 76, 485-493.
- IYENAGBE, D.O., MALOMO, S.A., IDOWU, A.O., BADEJO, A.A., FAGBEMI, T.N., 2017: Effects of thermal processing on the nutritional and functional properties of defatted conophor nut (*Tetracarpidium conophorum*) flour and protein isolates. *Food Sci. Nutr.* 5, 1170-1178. DOI: [10.1002/fsn3.508](https://doi.org/10.1002/fsn3.508)
- JANG, Y.-A., PARK, S.-H., KIM, B., PARK, J.-Y., JEOUNG, Y.-O., LEE, J.-T., 2017: Effect of ethanol extract of Lotus Rhizome and node of Lotus Rhizome. *J. Korean Appl. Sci. Technol.* 34, 657-665. DOI: [10.1016/j.nutres.2014.01.003](https://doi.org/10.1016/j.nutres.2014.01.003)
- KANEYASU, M., NAGATA, M., IKEDA, H., OHNUKI, K., SHIMIZU, K., 2019: Anti-allergic activity of lotus root (*Nelumbo nucifera*) powder in TDI-sensitized nasal allergy model mice. *Food Agric. Immunol.* 30, 968-978. DOI: [10.1080/09540105.2019.1651255](https://doi.org/10.1080/09540105.2019.1651255)
- KAUR, P., KAUR, L., KAUR, NAVJEET, SINGH, A., KAUR, J., KAUR, H., KAUR, NAVJOT, KAUR, M., 2019: A brief review on pharmaceutical uses of *Nelumbo nucifera*. *J. Pharmacogn. Phytochem.* 8, 3966-3972.
- MAJZOBI, M., FARAHNAKY, A., NEMATOLAH, Z., MOHAMADI, H.M., TAGHIPOUR, A.M., 2013: Effect of different levels and particle sizes of wheat bran on the quality of flat bread. *J. Agric. Sci. Technol.* 15, 115-123.
- MIRHOSSEINI, H., TAN, C.P., 2009: Response surface methodology and multivariate analysis of equilibrium headspace concentration of orange beverage emulsion as function of emulsion composition and structure. *Food Chem.* 115, 324-333. DOI: [10.1016/j.foodchem.2008.11.090](https://doi.org/10.1016/j.foodchem.2008.11.090)
- MONTGOMERY, D.C., 2017: Design and analysis of experiments. John Wiley & Sons.
- NAWAZ, H., SHAD, M., SAFDAR, S., JABEEN, R., 2019: Process optimization and characterization of gamma irradiation induced variations in functional properties of maize (*Zea mays* L.) flour. *Asian Food Sci. J.* 1-12. DOI: [10.9734/afsj/2019/v12i430089](https://doi.org/10.9734/afsj/2019/v12i430089)
- NAWAZ, H., SHAD, M.A., MEHMOOD, R., REHMAN, T., MUNIR, H., 2015: Comparative evaluation of functional properties of some commonly used cereal and legume flours and their blends. *Int. J. Food Allied Sci.* 1, 67-73. DOI: [10.21620/ijfaas.2015267-73](https://doi.org/10.21620/ijfaas.2015267-73)
- NAWAZ, H., SHAD, M.A., SALEEM, S., KHAN, M.U.A., NISHAN, U., RASHEED, T., BILAL, M., IQBAL, H.M., 2018a: Characteristics of starch isolated from microwave heat treated lotus (*Nelumbo nucifera*) seed flour. *Int. J. Biol. Macromol.* 113, 219-226. DOI: [10.1016/j.ijbiomac.2018.02.125](https://doi.org/10.1016/j.ijbiomac.2018.02.125)
- NAWAZ, H., SHAH, M., NISHAN, U., IQBAL, J., MANNAN, R., 2021: Microwave-induced modification of physical and functional characteristics and antioxidant potential of alkali-soluble cell wall polysaccharides of *Nelumbo nucifera* rhizome. *J. Polym. Environ.* 1-13. DOI: [10.1007/s10924-021-02131-4](https://doi.org/10.1007/s10924-021-02131-4)
- OLIVEIRA, T., LEAL, C., LIMA, R., 2015: Experimental design and data analysis: methods for the response optimization and applications using R., in: Embrapa Amazônia Ocidental-Resumo Em Anais de Congresso (ALICE). In: Conferência Internacional Da Amazônia Em Estatística Experimental de Risco.
- ONEH ABU, J., GYEBI DUODU, K., MINNAAR, A., 2006: Effect of γ -irradiation on some physicochemical and thermal properties of cowpea (*Vigna unguiculata* L. Walp) starch. *Food Chem.* 95, 386-393. DOI: [10.1016/j.foodchem.2005.01.008](https://doi.org/10.1016/j.foodchem.2005.01.008)
- ONIFE, O.O., BESWA, D., JIDEANI, A.I.O., 2017: Effect of size reduction on colour, hydration and rheological properties of wheat bran. *Food Sci. Technol.* 37, 389-396. DOI: [10.1590/1678-457X.12216](https://doi.org/10.1590/1678-457X.12216)
- PÉREZ, I.C., MU, T.-H., ZHANG, M., JI, L.-L., 2017: Effect of heat treatment to sweet potato flour on dough properties and characteristics of sweet potato-wheat bread. *Food Sci. Technol. Int.* 23, 708-715. DOI: [10.1177/1082013217719006](https://doi.org/10.1177/1082013217719006)
- SHAD, M.A., NAWAZ, H., HUSSAIN, M., YOUSUF, B., 2011: Proximate composition and functional properties of rhizomes of lotus (*Nelumbo nucifera*) from Punjab, Pakistan. *Pak. J. Bot.* 43, 895-904.
- SHOWKAT, Q.A., RATHER, J.A., JABEEN, A., DAR, B.N., MAKROO, H.A., MAJID, D., 2021: Bioactive components, physicochemical and starch characteristics of different parts of lotus (*Nelumbo nucifera* Gaertn.) plant: a review. *Int. J. Food Sci. Technol.* 56, 2205-2214. DOI: [10.1111/ijfs.14863](https://doi.org/10.1111/ijfs.14863)
- SUN, S., ZHANG, G., MA, C., 2016: Preparation, physicochemical characterization and application of acetylated lotus rhizome starches. *Carbohydr. Polym.* 135, 10-17. DOI: [10.1016/j.carbpol.2015.07.090](https://doi.org/10.1016/j.carbpol.2015.07.090)
- SUN, X., OHANENYE, I.C., AHMED, T., UDENIGWE, C.C., 2020: Microwave treatment increased protein digestibility of pigeon pea (*Cajanus cajan*) flour: Elucidation of underlying mechanisms. *Food Chem.* 329, 127196. DOI: [10.1016/j.foodchem.2020.127196](https://doi.org/10.1016/j.foodchem.2020.127196)
- THANUSHREE, M.P., SUDHA, M.L., CRASSINA, K., 2017: Lotus (*Nelumbo nucifera*) rhizome powder as a novel ingredient in bread sticks: rheological characteristics and nutrient composition. *J. Food Meas. Charact.* 11, 1795-1803. DOI: [10.1007/s11694-017-9561-y](https://doi.org/10.1007/s11694-017-9561-y)
- TORBICA, A., BELOVIĆ, M., POPOVIĆ, L., ČAKAREVIĆ, J., 2021: Heat and hydrothermal treatments of non-wheat flours. *Food Chem.* 334, 127523. DOI: [10.1016/j.foodchem.2020.127523](https://doi.org/10.1016/j.foodchem.2020.127523)
- WANI, I.A., WANI, A.A., GANI, A., MUZZAFFAR, S., GUL, M.K., MASOODI, F.A., WANI, T.A., 2015: Effect of gamma-irradiation on physico-chemical and functional properties of arrowhead (*Sagittaria sagittifolia* L.) tuber flour. *Food Biosci.* 11, 23-32. DOI: [10.1016/j.fbio.2015.04.003](https://doi.org/10.1016/j.fbio.2015.04.003)

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