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# Microwave Drying for Safe Storage and Improved Nutritional Quality of Green Gram Seed (*Vigna radiata*)

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**ABSTRACT:** The present study describes the effect of the microwave-heating method on disinfestations and physico characteristics, viz., grain size, grain hardness, and nutritional quality, of the stored green gram seed. It has been observed that the use of the microwave-heating method not only prolongs the storage duration of the green gram seed but also enhances its nutritional quality. The effect of independent parameters, viz., microwave power level and time of exposure, on the moisture content, insect mortality, color, and antinutrient factor (phyic acid) was optimized using response surface methodology (RSM), with the optimized value for power of 808 W and time at 80 s. The optimally treated green gram seed has 8.9% moisture, 99.5% insect mortality, 2.22 $\Delta a^*$ (green color of seed), and 591.79 mg/100 g of antinutrient factor (phytic acid). The grain size (geometric mean diameter, $D_m$) of the control (untreated) sample was 3.75 mm, and that of the microwave-treated sample using optimum conditions was 3.99 mm. The grain hardness of the control sample was 3.31 kg, and that of the microwave-treated sample using optimum conditions was 1.305 kg. *In vitro* protein digestibility (IVPD) of the control (untreated) sample was 83 ± 0.289%, and that of the microwave-treated sample using optimum conditions was 85 ± 0.296%. These values are significantly difference ($p < 0.05$). The mineral elements studied were Zn, Fe, Mg, Mn, Cu, K, Ca, and Na. The microwave treatment resulted in a non-significant ($p < 0.05$) effect for Mg, Mn, Cu, K, and Na but a significant ($p < 0.05$) effect for Zn, Ca, and Fe. The results indicate that the microwave heating not only increases the insect mortality but also reduces the moisture content and antinutritional factor (phytic acid), while the natural green color of the seed is not affected much. This study provides a novel and environmentally safe technique and increase in the nutritive quality.

**KEYWORDS:** *Response surface methodology (RSM), insect mortality, phytic acid, green gram (Vigna radiata), mineral, in vitro protein digestibility (IVPD), moisture, microwave drying*

## INTRODUCTION

India is the largest producer and consumer of green gram in the world. Green gram is a good source of carbohydrates, proteins, and minerals, and its protein quality is similar or superior to other legumes, such as chickpea, black gram, peas, pigeon pea, etc.[1] Green gram with 26–28% protein forms a major staple food source to meet the protein requirement of the vegetarian population of the country. It is consumed in the form of whole pulse as well as split dal, which is an essential supplement of a cereal-based diet. Postharvest losses account for 9.5% of the total pulse production.[2] Among postharvest operations, storage is responsible for the maximum loss (7.5%). Processing, threshing, and transport cause 1, 0.5, and 0.5% losses, respectively. Among storage losses, pulses are also most susceptible to damage because of insects (5%) compared to wheat (2.5%), paddy (2%), and maize (3.5%).[3] Pulses are more difficult to store, and they suffer great damage during storage because of the growth of insect pests and microorganisms.[4] Bruchids are a major threat to stored cowpea grains,[5] and infestations by the most prominent species *Callosobruchus maculatus* and *Callosobruchus chinensis* are responsible for grain losses estimated at 20–60%.[6] Stored pulses are severely infested by beetles of the family Bruchidae (Coleoptera), and the most damaging stages are larvae and pupae, which are closely associated with pulses. Many species are important primary pests of stored pulses.

The concept of heating grain to control insect pests is not new. During World War I, stored wheat was heated to 58–60 °C for at least 3 min as an insect control strategy. However, an increasing market preference for residue-free grain, the development of high-level insect resistance to phosphine, and the phasing out of methyl bromide currently used for rapid disinfestations of grain all support the need for research and development in heating technology. Susceptibility of grain to heat damage varies and is influenced by the grain type and moisture content at the time of the heat treatment. The microwave drying results in a high thermal efficiency, shorter drying time, and also potential means of replacing chemical fumigation in pest control. It has also been observed by earlier workers that radiation processing improves the nutritional quality of food legumes to various extents. The major advantage of disinfestations by physical means is that it does not leave any toxic residue on the grain. Microwave drying helps to remove the moisture from the food products without the problem of case hardening.[7] The moisture content is the important factor for storage and controlling the insect pests of grain. Color is an important quality attribute that determines consumer acceptance.[8] Generally, this is a subjective evaluation performed by the human eye. Microwave treatment slightly modified the green color ($\Delta a^*$) of grain, making it a little darker, although these changes were minimal and probably not detectable by the

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Table 1. Central Composite Rotatable Design Arrangement with Actual Level of Factors

| | process variables | | responses | | | |
|---|---|---|---|---|---|---|
| | power (W) | time (s) | moisture content (%, db) | insect mortality (%) | $\Delta a^*$ | phytic acid (mg/100 g) |
| experiment number | $X_1$ | $X_2$ | $Y_1$ | $Y_2$ | $Y_3$ | $Y_4$ |
| 1 | 180.00 | 60.00 | 12.95 | 45 | 1.36 | 1167.78 |
| 2 | 285.44 | 45.86 | 11.5 | 60 | 1.96 | 1100.43 |
| 3 | 285.44 | 74.14 | 11 | 70 | 2.07 | 1000.61 |
| 4 | 540.00 | 40.00 | 10.88 | 92.5 | 1.88 | 1126.08 |
| 5 | 540.00 | 60.00 | 10.32 | 96 | 2.79 | 813.129 |
| 6 | 540.00 | 60.00 | 10.32 | 97.5 | 2.88 | 813.129 |
| 7 | 540.00 | 60.00 | 10.66 | 96 | 2.46 | 875.836 |
| 8 | 540.00 | 60.00 | 10.7 | 95 | 2.42 | 617.131 |
| 9 | 540.00 | 60.00 | 10.21 | 95 | 1.88 | 917.543 |
| 10 | 540.00 | 80.00 | 9.86 | 98 | 2.86 | 709.01 |
| 11 | 794.56 | 45.86 | 10 | 98 | 1.57 | 854.49 |
| 12 | 794.56 | 74.14 | 9.07 | 99 | 2.07 | 800.19 |
| 13 | 900.00 | 60.00 | 9.95 | 99 | 1.33 | 617.131 |

consumer, and microwave drying also improves the nutritional quality of food pulses because of the reduction in antinutrients.[9] Response surface methodology (RSM) consists of a group of mathematical and statistical techniques used in the modeling and analysis of situations in which a response is affected by several variables, alone or in combination.[10] One of the main advantages of RSM is enabling the prediction of the behavior of different parameters under a given set of conditions with a reduced number of experiments.[11]

Therefore, the aim of the present work was to study the effect of microwave treatment on the moisture content, insect mortality, color, and antinutrient content, which are the important parameters for safe storage of green gram, with the help of a multifactor optimization study, i.e., RSM. The microwave treatment was further studied at optimum conditions for nutritional composition, nutritive value, and microstructure properties of green gram seeds.

## ■ MATERIALS AND METHODS

**Sample Collection.** Green gram (Malviya-12) seeds were procured from the experimental farm of Banaras Hindu University, Varanasi, India. Green gram samples were cleaned, and any foreign matter was manually removed before its use in the experiment.

**Microwave Specification.** The microwave oven used in this study was LG-Intello cook, model number 808 WAR, with a total frequency of 2450 MHz, an input frequency of 50 Hz, a microwave power of 0−900 W, a timer at 0−10 min, and a voltage of 230 V alternating current.

**Insect Cultures.** Experimental cultures (*Callosobruchus* sp.) were taken from stored grain stocks of the Entomology Laboratory of the Department of Agricultural and Food Engineering, Indian Institute of Technology, and maintained at 32 ± 1 °C and 60 ± 5% relative humidity to obtain larvae and adults, which were separated from rearing medium and placed in Petri dishes for studies.

**Sample Preparation.** Cleaned green gram seed samples were artificially infested with pulse beetles (irrespective of sex) in triplicate at room temperature and covered with lids, and the containers were stored at room temperature (32 ± 1 °C and 60 ± 5% relative humidity). Infested grains were sampled at the intervals for microwave treatment and compared to non-irradiated grain. The level of infestation was determined on the basis of the number of holes present in grains.

**Optimization Study for Microwave Treatment.** A five-level, two-factor central composite rotary design (CCRD) obtained using the commercial statistical package, Design Expert, version 7.1.1 (Stat-Ease, Minneapolis, MN), was employed to find out the interactive effects of the power ($X_1$) and exposure time ($X_2$) on the moisture content, insect mortality, color, and phytic acid of microwave-treated green gram seeds in 13 runs. The experimental data obtained from CCRD were analyzed by RSM and were employed to fit a second-order polynomial equation. To optimize the level of each factor for maximum response, a "point optimization" technique was employed. The microwave power level was varied from 180 to 900 W, and the duration of treatment was varied from 40 to 80 s. The maximum and minimum values of independent variables were based on some preliminary experiments. The central composite design at the given range of the above parameters in terms of actual values is presented in Table 1. To calculate the error sum of squares and the lack of fit of the developed regression equation between the responses and independent variables, five replicated experiments were conducted at the central points.[12]

**Moisture Content.** The moisture content was described by Karaca et al.[11]

**Insect Mortality Assessment.** The green gram seeds were taken out of the microwave treatment chambers, cooled, and counted for the number of insects. Samples were also counted for total insects before each treatment. The mortality percent was calculated using[13] the formula in eq 1

$$\text{mortality percent (\%)} = \frac{N_0 - N}{N_0} \times 100 \qquad (1)$$

where $N_0$ is the total number of live insects before treatment and $N$ is the total number of live insects after treatment.

**Color Measurement.** Color measurements (CIE $L^*$, $a^*$, and $b^*$ color space) were performed on whole grain using a chroma meter (CR-400/410, Konica Minlota),[14] where $L^*$ stands for brightness, $+a^*$ stands for redness, $-a^*$ stands for greenness, $+b^*$ stands for yellowness, and $-b^*$ stands for blueness. The green color deference between the harvest sample and heat-treated sample was calculated using eq 2.

$$\Delta a^* = \Delta a^*_{\text{sample}} - \Delta a^*_{\text{standard}} \qquad (2)$$

**Phytic Acid Determination.** Phytic acid was extracted from 3% trichloroacetic acid and assayed spectrophotometrically at 480 nm as described by Wheeler and Ferrel.[15]

**Microwave-Treated Green Gram Seeds Using Optimum Conditions.** *Proximate Analysis.* Proximate analysis (moisture, crude protein using $\%N \times 6.25$, crude fiber, ash, and fat) of the raw and microwave-treated samples was described by Karaca et al.[11] The carbohydrate content was determined on the basis of the percent differential from 100%.[11] Student's unpaired $t$ test was applied to determine the statistical significance of differences in the proximate composition of green gram seed using SPSS software (version 17, SPSS, Inc., Chicago, IL).

*Grain Size.* The average length, width, and thickness of 30 randomly selected grains were determined using a grain shape tester (Japan MK-100). The geometric mean diameter, $D_m$, was calculated using the relationship presented in eq 3,[16] where $L$ stands for length, $W$ stands for width, and $T$ stands for thickness.

$$D = \sqrt[3]{LWT} \tag{3}$$

*Grain Hardness.* The hardness of the single green gram grain was determined by a manually operated Kiya hardness tester (Kiya Seisakusho Ltd., Japan). Bar-type probe (0.5 cm in diameter) with a maximum permissible load of 20 kg was used for the determination of hardness of a single grain. The 20 randomly selected grains were analyzed, and then, the average value is reported.[17]

*In Vitro Protein Digestibility (IVPD).* IVPD was assessed by employing a multi-enzyme mixture using trypsin, chymotrypsin, and peptidase.[18,19] IVPD of the sample was then calculated using the following formula: percent digestibility = 234.84 − 22.56$X$, where $X$ is the pH recorded after a total digestion period of 20 min.[9] Student's unpaired $t$ test was applied to determine the statistical significance of differences in the *in vitro* digestibility of protein using SPSS software (version 17, SPSS, Inc., Chicago, IL).

*Mineral Content.* The samples were wet-acid-digested using a mixture of nitric, perchloric, and sulfuric acids (3:2:1). The amount of minerals in the digested samples was determined by an atomic absorption spectrophotometer (AAnalyst 700 Perkin-Elmer) following the method described by Sreeramaiah et al.[20] Calibration of measurements was performed using standards obtained from the Merck Chemical Company. All measurements were carried out using standard flame operating conditions. The reproducibility values were within 2.0% for all of the minerals. Student's unpaired $t$ test was applied to determine the statistical significance of differences in the mineral content of green gram seed using SPSS software (version 17, SPSS, Inc., Chicago, IL).

*Scanning Electron Microscopy (SEM).* Samples of green gram (microwave-treated and untreated) were taken and cut into small blocks of 3 × 2 × 1 mm with a razor blade and oriented in horizontal and vertical directions in such a way on a panel or stubs so that the longest side comes under the microscope to analyze the microstructural spread properly. All of the samples were fixed on the panel one after another. Thin slices of dehydrated green gram samples were then subsequently coated with gold to provide a reflective surface for the electron beam. Gold coating was carried out in a sputter coater (BIO-RAD E-5200) under a low vacuum environment in the presence of an inert gas, namely, argon. The gold-coated samples were subsequently viewed under the scanning electron microscope.

Observation were taken with low-vacuum SEM using a JSM-5800LV instrument coupled with an energy-dispersive X-ray micro analytical system (OXFORD ISIS-300), operated at an accelerating voltage of 20 kV. Images of representative parts of the sample were taken and observed at magnification of 2000×.

*Determination of Mycoflora.* Green gram seed samples were surface-sterilized in 1% sodium hypochlorite solution to remove unwanted things and then washed with three changes of sterile distilled water. The surface-sterilized seeds (200) were plated on potato dextrose agar plus 60 $\mu$g/mL streptomycin as a bacteriostat using three seeds per plate. Plates were incubated at 28 °C for 5 days. The seeds were then observed under a microscope, and the growth of fungi was recorded.

## RESULTS AND DISCUSSION

Response surface analysis (Design Expert 7)[21,22] was applied to the experimental data (Table 1). The nonlinear second-order polynomial response surface model was fitted to each of the response variables (moisture, insect mortality, and color), except phytic acid, which is a linear equation. Regression analysis and analysis of variance (ANOVA) were conducted for fitting the model and to examine the statistical significance of the model terms. The estimated regression coefficients of the quadratic polynomial models for the response variables, along with the corresponding $R^2$ values and ANOVA data, showed that all of the models were significant ($p < 0.05$) for all of the responses, viz., moisture content, insect mortality, color difference, and phytic acid.

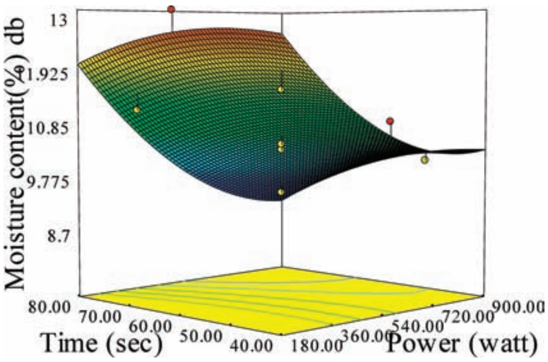

**Figure 1.** Response surface showing the effect of the microwave power level (W) and time (s) of exposure on the moisture content (%, db) of green gram seed.

**Table 2. ANOVA Data Showing the Effect of the Microwave Power Level and Exposure Time on the Moisture Content, Insect Mortality, Green Color Difference, and Phytic Acid**

| | *F* value | | | |
|---|---|---|---|---|
| source | moisture content (%, db) | insect mortality (%) | Δ$a$* | phytic acid (mg/100 g) |
| model | 16.71[a] | 221.12[b] | 5.32[c] | 9.55[c] |
| $X_1$ | 64[b] | 756.40[b] | 0.23[d] | 13.95[c] |
| $X_2$ | 8.85[c] | 13.13[c] | 5.02[d] | 5.14[c] |
| $X_1X_2$ | 0.40[d] | 6.03[c] | 0.37[d] | |
| $X_1^2$ | 8.38[c] | 319.61[b] | 20.89[c] | |
| $X_2^2$ | 1.63[d] | 1.31[d] | 0.094[d] | |
| lack of fit | 4.17[d] | 6.13[d] | 0.22[d] | 1.02[d] |

[b] $p < 0.0001$. [a] $p < 0.001$. [c] $p < 0.05$. [d] Non-significant.

**Moisture Content.** Initially, the moisture content of the control sample was 13.5% dry weight basis (db), and after microwave treatment, it decreased. It is observed from Figure 1 that the moisture content in grains decreases with an increase in the microwave power level up to 720 W and, thereafter, it becomes constant. A similar trend was followed with time. At a fixed power level (900 W), the moisture content decreases with time up to 80 s. At a fixed value of time (60 s), the moisture content decreased with power up to 720 W and becomes constant thereafter up to 900 W. A similar trend was observed for wheat during microwave drying.[23] The change in the moisture content was highly influenced by the microwave power level and time of treatment and more significant at the quadratic level ($p < 0.05$). It is observed from ANOVA data that the model was significant and the lack of fit was non-significant (Table 2). The $F$ values shows that the linear term of power and exposure time influenced the moisture content ($p < 0.0001$ and $p < 0.05$, respectively), as compared to other terms (Table 2). Interaction terms of the two variables did not show a significant effect on the moisture content. The numerical presentation in the variation of the moisture content (%) with different variables $X_1$ (microwave power level) and $X_2$

(exposure time) was fitted well in a polynomial equation (eq 4), with a coefficient of determination ($R^2$) of 0.92.

$$\begin{aligned}\text{moisture content (\%,db)} \\ &= 10.44 - 0.96X_1 - 0.36X_2 - 0.11X_1X_2 + 0.37X_1^2 \\ &\quad - 0.17X_2^2 \qquad (4)\end{aligned}$$

**Insect Mortality.** Green gram samples with 100% infestation were given microwave treatment at varying power levels for different exposure times. When the insect mortality increased with the increase in the microwave power level up to 900 W, a somewhat similar trend of insect mortality was noted with exposure time, as shown in Figure 2. The insect mortality was highly influenced by the microwave power level and exposure time ($p < 0.05$). The same result was found in refs 24 and 25 in stored corn and wheat, respectively; as the power and exposure time increase, the insect mortality also increased. ANOVA showed that the model was significant and the lack of fit was non-significant (Table 2). The linear and quadratic terms of power and the linear term of time influenced the insect mortality more, as compared to other terms. The insect mortality was highly influenced by power ($p < 0.0001$) and time ($p < 0.05$), respectively (Table 2). Interaction terms of the two variables significantly affect the insect mortality. The numerical presentation in the variation of the insect mortality (%) with different variables $X_1$ (microwave power level) and $X_2$ (exposure time) was fitted well in a polynomial equation (eq 5), with a coefficient of determination ($R^2$) of 0.99.

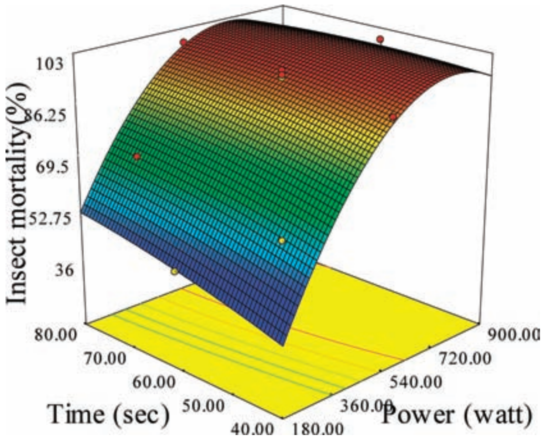

**Figure 2.** Response surface showing the effect of the microwave power level (W) and time (s) of exposure on insect mortality (%) of green gram seed.

$$\begin{aligned}\text{insect mortality (\%)} \\ &= 95.90 + 17.92X_1 + 2.35X_2 - 2.25X_1X_2 - 12.42X_1^2 \\ &\quad - 0.79X_2^2 \qquad (5)\end{aligned}$$

**Color Measurement.** It is observed from Figure 3 that the $\Delta a^*$ difference between the green color of the grain sharply decreases after 720 W up to 900 W and increases with the exposure time. $\Delta a^*$ was highly influenced by the microwave power level and exposure time ($p < 0.05$). Reference 26 also found the effect of microwave heating on the color of Spanish rice varieties. ANOVA showed that the model was significant and the lack of fit was non-significant (Table 2). The qadartic term of power influenced the color difference ($\Delta a^*$) more, as compared to other terms. Interaction terms of the two variables were not a significant effect on the $\Delta a^*$ difference. The numerical presentation in the variation of the color with different variables $X_1$ (microwave power level) and $X_2$ (exposure time) was fitted well in a polynomial equation (eq 6), with a coefficient of determination ($R^2$) of 0.79.

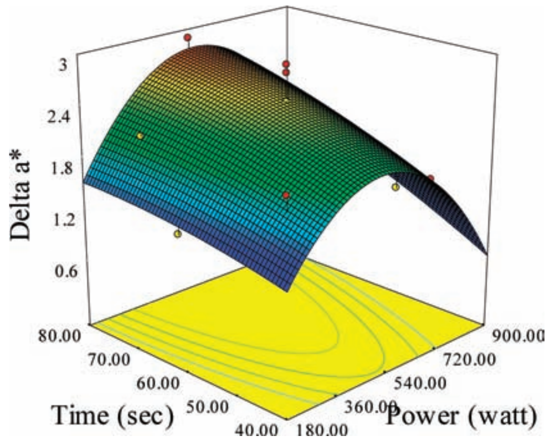

**Figure 3.** Response surface showing the effect of the microwave power level (W) and time (s) of exposure on $\Delta a^*$ seed.

$$\begin{aligned}\Delta a^* &= 2.49 - 0.054X_1 + 0.25X_2 + 0.098X_1X_2 - 0.56X_1^2 \\ &\quad - 0.037X_2^2 \qquad (6)\end{aligned}$$

**Phytic Acid.** The raw green gram sample contained 1251.195 mg/100 g of phytic acid, which, after microwave treatment, decreases linearly at different microwave power levels and exposure times. The observed microwave heating lowered the trypsin inhibitor, which is also an antinutrient factor, without eliminating it completely in raw fava beans, lentils, and green and mature peas.[27] The phytic acid content of green gram decreased with an increase in the microwave power level up to 900 W, and a similar trend was followed with exposure time (Figure 4). The phytic acid content was highly influenced by the microwave power level and exposure time ($p < 0.05$). ANOVA showed that the model was significant and the lack of fit was non-significant (Table 2). The linear term of the microwave power level and exposure time influenced phytic acid, as compared to other terms. The numerical presentation

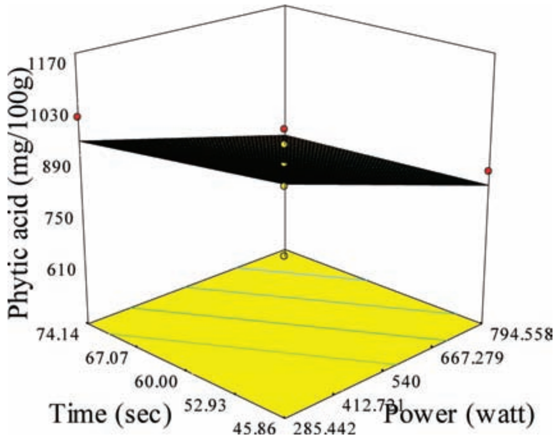

**Figure 4.** Response surface showing the effect of the microwave power level (W) and time (s) of exposure on phytic acid of green gram seed.

in the variation of phytic acid with different variables $X_1$ (microwave power level) and $X_2$ (exposure time) was fitted in a linear equation (eq 7), with a coefficient of determination ($R^2$) of 0.90.

$$\text{phytic acid (mg/100 g)} = 877.88 - 153.14X_1 - 92.99X_2 \tag{7}$$

**Optimization of the Microwave Treatment.** The numerical values and the graphical optimization (Figure 5 and Table 3) investigated the independent parameters of the microwave treatment to obtain the optimum moisture, insect mortality, difference between green color of green gram seed, and phytic acid. The Design Expert program (version 7.1.1) software was used for simultaneous optimization of the multiple responses. Desired goals (maximized or minimized) for each variable and response were chosen, and different weights (i.e., a number between 0.1 and 1.0 that showed the importance of the desired goal) were assigned to each goal to adjust the shape of its particular desirability function. Software generated six optimum conditions of independent variables, with the predicted values of responses shown in Table 3. The values given in the flagged area in Figure 5 were grouped together, and the optimized values of variables, such as power of 808 W, time at 79 s, moisture content of 8.91%, insect mortality of 99.5%, difference between the green color of green gram of 2.22, and phytic acid of 591.79 mg/100 g, were determined. The values obtained in solution number 1 in Table 3 of the numerical optimization method were found to be closer to the values obtained in the graphical optimization method. On this basis, microwave treatment at a power of 808 W and time at 80 s (used for experimental purpose) for the control of stored insect pest and without a significant loss of physicochemical quality of stored green gram (solution 1 in Table 3) was obtained.

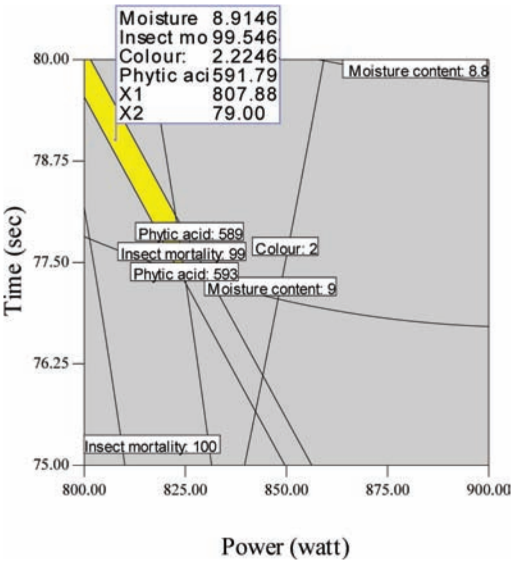

**Figure 5.** Overlay plot showing the optimum process parameters for the moisture content, insect mortality, green color difference, and phytic acid of green gram seed.

**Proximate Analysis of Raw and Microwave-Treated Green Gram Seeds Using Optimum Conditions.** The proximate compositions (moisture, fat, crude fiber, protein, ash, and carbohydrate) of Malviya-12 green gram (*Vigna radiata*) in their raw and microwave-treated green gram seeds are shown in Table 4. The moisture range was 13.39−8.91%; the fat range was 1.46−1.43%; the crude fiber range was 6.45−6%; the protein range was 21.43−21%; the ash range was 3.99−3.77%; and the carbohydrate range was 53.97−59.28%. There was no significant difference (fat, crude fiber, protein, and ash) between control (untreated) and microwave-treated green gram seeds under optimum conditions. Only the moisture content (because of heat treatment) and carbohydrate content show a significant difference. A similar result for the above study was in good agreement with the value reported by several researchers.[28,29] The slight differences may be due to the location and variety effect. The ash content in these pulses indicates that the pulse seed provides essential minerals, and a high fiber content makes green gram a good digestive food.

**Table 4. Proximate Composition of Raw (Untreated) and Microwave-Treated Green Gram Seeds Using Optimum Conditions (Expressed as a Percentage on a Dry Weight Basis)[a]**

| proximate composition | raw (untreated) | microwave treated |
|---|---|---|
| moisture | 13.39 ± 0.3 | 8.91 ± 0.05 |
| fat | 1.43 ± 0.05 | 1.46 ± 0.00 |
| crude fiber | 6.45 ± 0.09 | 6 ± 0.03 |
| protein | 21 ± 0.3 | 21.43 ± 0.1 |
| ash | 3.99 ± 0.16 | 3.77 ± 0.14 |
| carbohydrate | 53.97 ± 0.04 | 59.28 ± 0.09 |

[a] Values are the average of triplicate determinations expressed as a percentage ± standard deviation, for an unpaired $t$ test, with $p < 0.05$.

**Grain Size of Raw and Microwave-Treated Green Gram Seeds Using Optimum Conditions.** The grain size (geometric mean diameter, $D_m$) of the 30 randomly selected control (untreated) samples was 3.75 mm, and that of the optimized sample was 3.99 mm. It may be due to some water changing into vapor, and its causes expansion or swelling of the grain.

**Grain Hardness of Raw and Microwave-Treated Green Gram Seeds Using Optimum Conditions.** The grain hardness of the 20 randomly selected control (untreated) samples was 3.31 kg, and that of the optimized sample was 1.305 kg. Because of the swelling of the grain, the intercellular

**Table 3. Solutions for Numerical Optimization of Process Parameters of Microwave Treatment**

| number | power (W) | time (s) | moisture content (%) | insect mortality (%) | color difference (Δa*) | phytic acid (mg/100 g) | desirability |
|---|---|---|---|---|---|---|---|
| 1 | 807.88 | 79 | 8.91 | 99.54 | 2.22 | 591.798 | 1 |
| 2 | 780.90 | 78.41 | 8.99 | 100.67 | 2.32 | 611.907 | 1 |
| 3 | 788.70 | 76.96 | 9.07 | 100.59 | 2.27 | 616.781 | 1 |
| 4 | 787.76 | 77.52 | 9.03 | 100.55 | 2.28 | 613.618 | 1 |
| 5 | 822.03 | 77.61 | 8.99 | 99.09 | 2.13 | 592.453 | 1 |
| 6 | 772.87 | 78.90 | 8.97 | 100.86 | 2.36 | 613.486 | 1 |

**Table 5. Mineral Content of Raw (Untreated) and Microwave-Treated Green Gram Seeds Using Optimum Conditions (Expressed as mg/100 g)[a]**

| sample | Zn | Fe | Mg | Mn | Cu | K | Ca | Na |
|---|---|---|---|---|---|---|---|---|
| raw (untreated) | 7.4 ± 0.002 | 13 ± 0.005 | 230.1 ± 0.010 | 3.5 ± 0.02 | 0.7 ± 0.003 | 917 ± 0.028 | 99.8 ± 0.001 | 212.9 ± 0.006 |
| treated | 7.9 ± 0.001 | 16.35 ± 0.007 | 231.1 ± 0.014 | 3.6 ± 0.001 | 0.4 ± 0.001 | 913.2 ± 0.052 | 97.9 ± 0.003 | 211.7 ± 0.003 |

[a] Values are the average of triplicate determinations expressed as mg/100 g ± standard deviation, for an unpaired *t* test, with $p < 0.05$.

bond force becomes week and the grain easily cracks because the drying temperature reduces the breakage.

**IVPD of Raw and Microwave-Treated Green Gram Seeds Using Optimum Conditions.** Experimental data for IVPD of raw (untreated) green gram seeds were 83 ± 0.289%, and those of the microwave-treated sample using optimum conditions were 85 ± 0.296%. These values are significantly different ($p < 0.05$). The heat treatment significantly improves the protein quality in pulses by destruction or inactivation of heat-labile antinutritional factors. Microwave heating results in significant reductions in phytic acid and tannins in pulses.[30] Evidence from *in vitro* studies indicates that digestion of native legume seed storage protein is limited because of the structure and conformation of the protein.[31] Also, *in vitro* studies have shown that phytate−protein complexes are insoluble and less subject to attack by proteolytic enzymes than the same protein alone[32] and, subsequently, affect the functional properties of protein. It is well-known that microwave irradiation could induce and or stimulate other factors. Molecular rearrangement and changes in peptide linkages between the amino groups of amino acids could affect the nutritive availability and the biological use of the irradiated proteins. Such changes could interfere with the protein digestibility and or its biological value. Thus, protein digestibility may be decreased and/or increased without incurring amino acid destruction.[33] Therefore, it could be concluded that the irradiation process offers a good treatment for green gram to reduce or eliminate their antinutritional factor(s) with a consequent increase in their digestibility and, thereby, increase the use of their proteins.[9]

**Mineral Content of Raw and Microwave-Treated Green Gram Seeds Using Optimum Conditions.** The data presented in Table 5 show that the mineral contents (Zn, Fe, Mg, Mn, Cu, K, Na, and Ca) of untreated and microwave-treated green gram seeds. The data presented in Table 5 show that the mineral contents of Mg, Mn, Cu, K, and Na are not statistically significant ($p < 0.05$), because there is no additional process, such as leaching, etc., at the time of sample preparation. A similar result was found by ref 34. They also studied the microwave heating on the increased power level effect on mineral contents. Zn, Fe, Na, and Ca are statistically significant ($p < 0.05$). The heat processing decreases the antinutrient factor, i.e., phytic acid, and may cause an increase of the bioavailability of the mineral content, mainly Fe, Zn, and Ca. The complexing of phytic acid with nutritionally essential elements and the possibility of interference with proteolytic digestion have been suggested as being responsible for the antinutritional activity.

**Scanning Electron Microscope of Raw and Microwave-Treated Green Gram Seeds Using Optimum Conditions.** Scanning electron micrographs at a magnification of 2000× of raw (untreated) and microwave-treated green gram seeds using optimum conditions in both horizontal and vertical sections are presented in panels a and b of Figures 6 and 7 respectively. Control (untreated) cotyledons have starch granules and the cytoplasmic matrix, exposing inner cell wall surfaces. Small pits, arranged in a semi-organized pattern, were present in the membranes that lined the interior walls of cells. In contrast to the control (untreated) sample, in the microwave-treated sample at optimum conditions, cell walls ruptured when the cotyledon was fractured and little dispersions of intact cells were obtained.

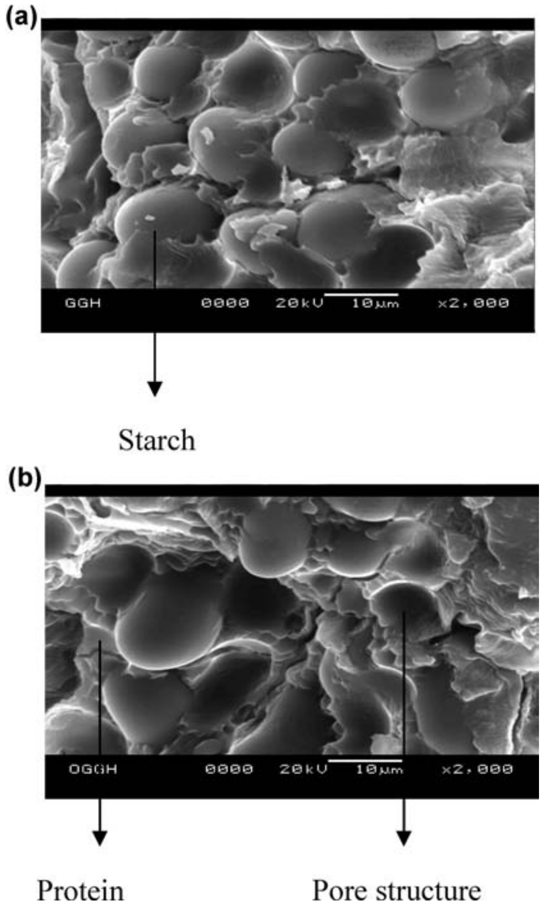

**Figure 6.** Horizontal microscopy image at (2000× magnification) of (a) raw (untreated) and (b) microwave-treated green gram seeds using optimum conditions.

**Determination of Mycoflora.** After the microwave treatment, there is no growth observed on potato dextrose agar (Figure 8). This might be due to the decrease of the moisture content after the microwave treatment, and the required moisture content was not available for the fungal growth on the surface of the seed.

In conclusion, RSM was effectively used to identify two important parameters, i.e., power level and exposure time of microwave, which have the potential to kill the stored grain insects of green gram, i.e., *Callosobruchus* sp., reduce the moisture content, provide 100% insect mortality, reduce the green color of the grain, and lower phytic acid obtained at 808 W and 80 s exposure time without any adverse effect on the nutritive value and microstructure properties of green gram

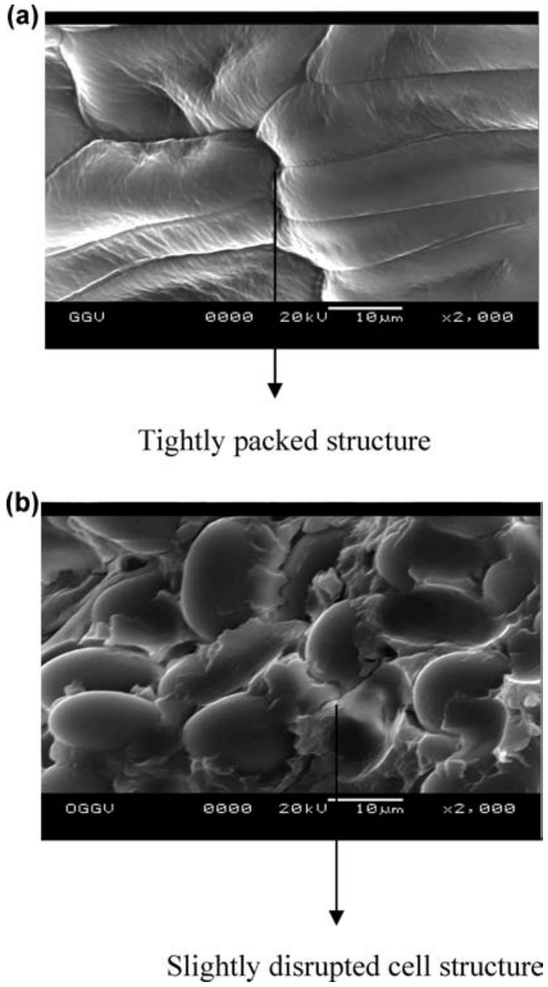

**Figure 7.** Vertical microscopy images at (2000× magnification) of (a) raw (untreated) and (b) microwave-treated green gram seeds using optimum conditions.

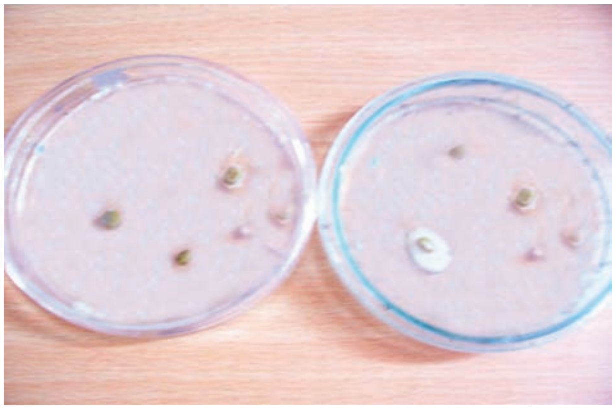

**Figure 8.** No fungal growth on potato dextrose agar after microwave heating using optimum conditions of green gram seed.

seeds of raw (untreated) and microwave-treated samples using the optimized conditions.

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## ABBREVIATIONS USED

IVPD, *in vitro* protein digestibility; RSM, response surface methodology

## REFERENCES

(1) Chandrasekher, C. P.; Ghosh, J. Food economy in disarray. *Indian Food Ind.* **2002**, *21*, 10−14.

(2) Reddy, A. A. Pulse production technology: Status and way forward. *Rev. Agric.* **2009**, *52*, 73−80.

(3) Sumit, K.; Bourai, V. K.; Hitendra, K. Post harvest losses in pulses of Uttarakhand: A specific study of sample villages of Assan valley. *Econ. Aff.* **2011**, *56*, 243−247.

(4) Akhtari, N; Sardar, M. A.; Rahman, M. H.; Abedin, S. M. Z.; Rahman, A. K. M. M. Feeding and oviposition preference of pulse beetle, *C. chinensis* on different genotypes of bean and its control. *Ann. Bangladesh Agric.* **1993**, *3*, 63−69.

(5) Solleti, S. K.; Bakshi, S.; Purkayastha, J.; Panda, S. K.; Sahoo, L. Transgenic cowpea (*Vigna unguiculata*) seeds expressing a bean a amylase inhibitor 1 confer resistance to storage pests, bruchid beetles. *Plant Cell Rep.* **2008**, *27*, 1841−1850.

(6) Tarver, M. R.; Shade, R. E.; Shukle, R. H.; Moar, W. J.; Muir, W. M.; Murdock, L. M.; Pittendrigh, B. R. Pyramiding of insecticidal compounds for control of the cowpea bruchid (*Callosobruchus maculatus* F.). *Pest Manage. Sci.* **2007**, *63*, 440−446.

(7) Prabhajan, D. J.; Ramaswamy, H. S.; Raghavan, G. S. V. Microwave assisted convective air drying of thin layer carrots. *J. Food Eng.* **1995**, *25*, 283−293.

(8) Benedito De Barber, C.; Barber, S.; Martinez, J. Quality of temperate rice in Europe. *Proceedings of the Temperate Rice Conference*; Yanco, New South Wales, Australia, Feb 21−24, 1994; Vol. *1*; pp 187−191.

(9) El-Niely, H. F. G. Effect of radiation processing on anti nutrients, in-vitro protein digestibility and protein efficiency ratio bioassay of legume seeds. *Radiat. Phys. Chem.* **2007**, *76*, 1050−1057.

(10) Fomuso, L. B.; Corredig, M.; Akoh, C. C. Metal-catalyzed oxidation of a structured lipid model emulsion. *J. Agric. Food Chem.* **2011**, *50*, 7114−7119.

(11) Karaca, A. C.; Nickerson, M. T.; Low, N. H. Lentil and chickpea protein-stabilized emulsions: Optimization of emulsion formulation. *J. Agric. Food Chem.* **2011**, *59*, 13203−13211.

(12) Das, H. Food processing operations analysis. In *Empirical Model Development*, 1st ed.; Das, H., Ed.; Asian Books Private Limited: New Delhi, India, 2005; pp 355−366.

(13) Abott, W. S. A method of computing the effectiveness of an insecticide. *J. Econ. Entomol.* **1925**, *18*, 265−267.

(14) Anton, A. A.; Fulcher, R. G; Arntfield, S. D. Physical and nutritional impact of fortification of corn starch-based extruded snacks with common bean (*Phaseolus vulgaris* L.) flour: Effects of bean addition and extrusion cooking. *Food Chem.* **2009**, *113*, 989−986.

(15) Wheeler, E. L.; Ferrel, R. E. A method for phytic acid determination in wheat and wheat fraction. *Cereal Chem.* **1971**, *48*, 312−316.

(16) Mollazade, K.; Ahmadi, H.; Khorshidi, J.; Rajabipour, A.; Mohtasebi, S. S. Moisture-dependent physical and mechanical properties of cumin (*Cuminum cyminum* L.) seed. *Int. J. Agric. Biol. Eng.* **2009**, *2*, 49−56.

(17) Jambunathan, R.; Kherdekar, S. M.; Stenhouse, W. J. Sorghum grain hardness and its relationship to mold susceptibility and mold resistance. *J. Agric. Food Chem.* **1992**, *40*, 1403−1408.

(18) Hsu, H. W.; Vavak, D. L.; Satterlee, L. D.; Miller, G. A. A multienzyme technique for estimating protein digestibility. *J. Food Sci.* **1977**, *38*, 126−130.

(19) Satterlee, L. D.; Marshall, H. F.; Tennyson, J. M. Measuring protein quality. *J. Am. Oil Chem.* **1979**, *56*, 103−108.

(20) Sreeramaiah, H.; Kalpana, P.; Krishnapura, S. Influence of heat processing on the bioaccessibility of zinc and iron from cereals and pulses consumed in India. *J. Trace Elements Med. Biol.* **2007**, *21*, 1−7.

(21) Singh, K. P.; Pardeshi, I. L.; Kumar, M.; Srinivas, K.; Srivastva, A. K. Optimization of machine parameters of a pedal-operated paddy thresher using RSM. *Biosyst. Eng.* **2008**, *100*, 591−600.

(22) Mangaraj, S.; Singh, K. P. Optimization of machine parameters for milling of pigeon pea using RSM. *Food Bioprocess Technol.* **2011**, *4*, 762−769.

(23) Walde, S. G.; Balaswamy, K.; Velu, V.; Rao, D. G. Microwave drying and grinding characteristics of wheat (*Triticum aestivum*). *J. Food Eng.* **2002**, *55*, 271−276.

(24) Vadivambal, R.; Jayas, D. S.; White, N. D. G. Wheat disinfestation using microwave energy. *J. Stored Prod Res.* **2007**, *43*, 508−514.

(25) Vadivambal, R.; Deji, O. F.; Jayas, D. S.; White, N. D. G. Disinfestation of stored corn using microwave energy. *Agric. Biol. J. North Am.* **2010**, *1* (1), 18−26.

(26) Marzal, A.; Osca, J. M.; Castell, V.; Martinez, J.; Benetido, C.; Balbastre, J. V.; Sanchez-Hernandez, D. Effect of microwave energy on grain quality of four Spanish rice varieties. *Spanish J Agric. Res.* **2005**, *3*, 310−318.

(27) Hernandez, I. H.; Sousa, V.; Montalvo, I.; Tena, E. Impact of microwave heating on hemagglutinins, trypsin inhibitors and protein quality of selected legume seeds. *Plant Foods Hum Nutr.* **1998**, *52*, 199−208.

(28) Agugo, U. A.; Onimawo, I. A. Heat treatment on the nutritional value of mungbean. *Electron. J. Environ., Agric. Food Chem.* **2009**, *8*, 924−930.

(29) Paul, T.; Mozumder, N. H. M. R.; Sayed, M. A.; Akhtaruzzaman, M. Proximate compositions, minerals contents and determination of protease activity from green gram (*Vigna radiata* I. Wilczek). *Bangladesh Res. J.* **2011**, *5*, 207−213.

(30) Wang, N.; Hatcher, D. W.; Gawalko, E. J. Effect of variety and processing on nutrients and certain anti-nutrients in field peas (*Pisum sativum*). *Food Chem.* **2008**, *111*, 132−138.

(31) Carbonaro, M.; Grant, G.; Cappelloni, M.; Pusztai, A. Perspectives into factors limiting in vivo digestion of legume proteins: Antinutritional compounds or storage protein. *J. Agric. Food Chem.* **2000**, *48*, 742−749.

(32) Ravindran, V.; Bryden, W. L.; Kornegay, E. T. Phyates: Occurrence, bioavailability and implications in poultry nutrition. *Poult. Avian Biol. Rev.* **1995**, *6*, 125−143.

(33) El-Hakeim, N. F.; Yousri, R. M.; Roushdy, H.; Farag, M. D. El-D. H. Nutritional evaluation of irradiated animal protein by-products. *Isotopenpraxis* **1991**, *27*, 104−108.

(34) Erdogan, S.; Erdemoglu, S. B.; Kaya, S. Optimization of microwave digestion for determination of Fe, Zn. Mn and Cu in various legumes by flame atomic absorption spectrometry. *J. Sci. Food Agric.* **2006**, *86*, 226−232.