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Effect of Microwave Heat on the Nutritional Properties of Infected Red Kidney Beans

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ABSTRACT In this paper, the effect of microwave heat on the nutritional characteristics of affected red kidney beans, i.e., Rajma is studied. Evaluation of crude protein content in microwave heated red kidney beans are carried out using standard Kjeldahl method and Fourier transform infra-red (FTIR) spectroscopy. Red kidney beans affected by Pulse Beetle (Callosobruchus chinensis) have been analyzed throughout the experiments. The heat treatment of the samples is undertaken using a domestic microwave oven at 2.45 GHz. The samples are heated at different microwave powers, such as 90, 270, 450, 750, and 900 W, while the exposure time is varied from 30–90 s. The effective microwave power for maximum disinfestation is found to be 270–450 W at 30–60 s. Most of the red kidney beans are damaged at 900 W for 60 s. Using Kjeldahl method, a constant crude protein level of 22.5 % is obtained for untreated and treated samples at 90–450 W. Crude protein level of red kidney beans is changed at 750–900 W. From FTIR analysis, the absorbance spectrum of carbohydrate peaks is found at 900–1250 cm^{-1}, a moisture peak at 1650 cm^{-1}, and a protein peaks at 2800–3000 cm^{-1}. Statistical analysis of the treated and untreated samples represents the lowest error of 0.0016 at 450 W power level for 60 s. Variances are determined to be the lowest with a value of 270 W and 450 W at 90 s and 60 s. These findings indicate the optimum microwave heat treatment for red kidney beans to retain its protein content.

INDEX TERMS Microwave, crude protein, FTIR, heat treatment, disinfestation, and absorbance.

I. INTRODUCTION

Most of the stored grains are being infected due to the adverse environmental effects, hazardous chemicals, contamination with insects, insect fragments, fungi, mycotoxins and lack of suitable storage facilities. The stored grains are affected by the insects both in quantitatively as well as qualitatively. Infestation of grains result serious detrimental effects on humans and animals. Therefore, disinfestation is a major concern of the grain industry in recent years. Apart from disinfestation, it is pertinent to monitor the nutritional property of grains continuously to maintain the grain quality. Nutritional factors also depend on the ambient temperature and moisture content of the grains. Most of the food grains become spoilage and lose their original nutritional values due to environment temperature and moisture. It is reported that insect growth in food grains depends on the moisture level of food grains and moisture level in food grains should be in the range of 11.5-16 % [1], [2]. The chemical fumigation, sun drying, neem leaves (Azadirachta indica) and bio-products are some of the most commonly used methods to remove/kill insects from the food grains and pulses. But these techniques facilitate distinct difficulty for chemical fumigation. During this process chemicals are deposited on the surface of grains which are further consumed by the human beings [3]. Sun drying is the most common method to remove the insects from food grains but it removes the insects from the surface only and cannot kill the eggs/larvae inside the kernel of food grains. Neem leaves (Azadirachta indica) and other bioproducts, i.e. cow dung may remove the insects for a limited time period. In order to overcome these issues, microwave treatment is considered as a suitable alternative by replacing chemical methods of killing insects in grain. Since it does not leave any toxic residues and thus it is promising for controlling insect infestation compared to other available methods [4]. Moreover, microwave disinfestation allows huge amount of products to pass quickly through continuous process. Furthermore, microwave disinfestation is a safe and competitive substitute to fumigation, since it does not pollute the environment. Reports are available in the literature where microwave heat treatment is used to control the moisture and disinfestation of food grains [5]–[7]. The most commonly used power levels for disinfestation of food grains is 250-500 W at 14-56 s [2], [8]. With increasing the power level, the germination level of food grains decreases as a function of time [5]–[7]. In a report, the effect of continuous microwave heat treatment on nutritional parameters in terms of chemical and biological changes was studied [9], [10]. Bhargava *et al.* [11] reported that dielectric properties of food grains affect the quality of food grains. Park *et al.* [12] developed a method of sonication using different buffer solutions to isolate sorghum starch, which extracts starch from the protein matrix rapidly. Bean *et al.* [13] used ultrasound technique to improve the extraction of sorghum proteins. Generally, Kjeldahl method is used to analyze the crude protein values of the food grains [14]. It is a chemical method to find out the total nitrogen in the food grains [15].

Near infra-red (NIR) spectroscopy is used to measure the bioactive compounds such as fatty acids, glucosinolates and polyphenols in cereals, herbs, spices, vegetables and fruits, etc. [16]. It also determines the moisture content, physical properties, quality and safety attributes of grains and seeds [17]. It may be mentioned here that nutritional parameters (protein, carbohydrates, fats, moisture, etc.) of the food grains are located in the range of 200-4000 cm^{-1} [18]. In a report, NIR technology was used to calculate the moisture, protein, ash, starch damage, particle size, etc., in wheat, barley flour milling, dough and cereals [17]. In a study, NIR spectroscopy was carried out to determine the composition and functionality of pulses and seeds [19]. In addition, 300 sunflower seeds are analyzed during NIR analysis to evaluate the crude protein contents and moisture level [20]. However, the effect of microwave treatment on nutritional properties of red kidney beans have not yet been well studied in terms of disinfestations related to the analysis of various nutritional parameters as explained through FTIR spectroscopy and Principal Component Analysis.

Keeping in view of the above perspectives, efforts have been made to use microwave heat treatment for preventing and controlling the insect infestation and fungal growth during storage. In this study, we thus, report the effect of microwave heat on the nutritional properties of infected red kidney beans, i.e. rajma. The compositions such as crude protein, fats, carbohydrates, moisture, etc. are determined using FTIR spectroscopy and Principal Component Analysis.

II. MATERIALS AND METHODS

Red kidney beans are purchased from a local market. Domestic microwave oven (Electrolux 26L Convection EJ26CSL4, 2.45 GHz) is used to heat the red kidney beans. Samples are stored in desiccators after the treatment. A grinder (Philips JMG model) is used to grind the samples into powder. Crude protein analysis is undertaken using a Kjeloplus (Pelican Inc. Instrument) and Scrubber (Pelican Inc.) Standard Kjeldahl method is used to analyze the data further. FTIR spectroscopy was carried out using a spectrometer (ABB Bomem MB 3000) equipped with ZnSe optics and DTGS detectors. The data was verified using Principal component analysis (PCA).

A. INSECT AND CULTURE PREPARATION

Pulse Beetle (Callosobruchus chinensis) and its eggs are collected from the stored Red Kidney beans. The egg is small and oval in shape. White colored glue is observed on the surface of the beans. The insects are grown in a favorable environment. Hundreds of eggs with different relative humidity are grown at 17-35 °C. Larvae and pupa are directly grown into the food grains throughout the growth cycle. Life cycle of the eggs is 5-20 days. 700 g of red kidney beans is filled in each plastic jar. 50 insects are placed inside the jar covered with an air holed plastic for proper aeration. The jar is placed at 30 ± 5 °C with relative humidity of 50-75 % in a controlled atmosphere for 6 weeks. After 6 weeks, insects are fully grown up through the entire life cycle such as eggs, larvae and pupae. 75 % pulses are damaged by the insects throughout its life cycle.

B. MORTALITY RATE OF PULSE BEETLE

The mortality rate is defined as the ratio of dead insects after microwave exposure to the total no. of insects present in the pulses before drying. The value of mortality rate is calculated during microwave treatment. Insects mortality rate is described in the following expression [21].

$$\text{Insect Mortality}(\%) = (A/B) \times 100 \tag{1}$$

where, A = no. of insects after microwave exposure B = total no. of insects before microwave drying

C. TREATMENT OF INFECTED RED KIDNEY BEANS

Red kidney beans affected by Cowpea or Pulse Beetle (Callosobruchus chinensis) are taken for the microwave treatment. The infected pulses have large number of eggs, larvae, pupae and adults. Three batches of infected red kidney beans are prepared for treatment. Each batch consists of 10 samples having a weight of 20 g per sample. Initially, 3 samples are placed in the desiccators as reference samples, whereas another 27 samples are treated in microwave energy (2.45 GHz) at different power and time. After that it is stored in the desiccators to protect from moisture. 27 samples are treated at different power levels, i.e. 900, 750, 450, 270 and 90 W for 30-90 s and then placed in the desiccators. Finally, all the samples are kept for 90 days to determine the mortality rate of the insects and their life cycle.

D. SAMPLE PREPARATION FOR CRUDE PROTEIN ANALYSIS AND FTIR SPECTROSCOPY

Red kidney beans are grinded to fine powder for crude protein and FTIR analysis. 0.2 g of powder is added to the catalyst mixture containing potassium sulphate and cupric sulphate (in the ratio of 5:1). Then 10 mL of H_2SO_4 (98% purity) is added into the solution. Same process is repeated to analyze the crude protein content.

E. DIGESTION, DISTILLATION AND TITRATION PROCESSES (KJELDAHL METHOD)

Liquid samples are heated at 350 °C for 30 min during digestion process. The samples are further heated at 420 °C for 1 hr. At the same time, the scrubber is used to remove the hazardous gases. After some time, liquid samples are turned into milky green. The samples are subjected to cool at room temperature. Distilled water (10 mL) is added to dilute the sample. Water also prevents the process of crystallization. Process is repeated for the remaining samples of red kidney beans. Subsequently, the digested samples are exposed for distillation. H_2SO_4(98 %), NaOH (40 %) and boric acid (4 %) are used during distillation. Consequently, the by-products are mixed to the ammonia gas. By-product is further mixed with the Methyl Red & Bromo-cresol Green indicator in the ratio of 5:2. Titration of the by-product is undertaken using 0.1N HCl and it turned into a pink colored solution. Finally, the nitrogen and total protein (crude protein) content is determined using the following expressions [14], [15].

$$\%\,\text{Nitrogen} = \frac{(14 \times 0.1 \times (STV - BTV))}{(SW \times 1000)} \tag{2}$$

$$\%\,\text{Protein} = 6.24 \times \%\,\text{Nitrogen} \tag{3}$$

where,

$$STV = \text{Sample Titer Value}$$
$$BTV = \text{Blank Titer Value}$$
$$SW = \text{Sample Weight}$$

Total absorbance (A) is calculated using the following expression.

$$A = 2 - \log_{10}(\%\,\text{Transmittance}) \tag{4}$$

III. RESULTS AND DISCUSSION

Insects of the red kidney beans are found to die during irradiation at 900 W for 60 s and damaged the red kidney beans to some extent. Microwave power of 90-900 W is used throughout disinfestation process. After the heat treatment, the dead insects are removed from the treated samples using a fan. No significant effect on the moisture content of beans is observed. Then the samples are placed into an airtight glass container for 3 months and mortality rate of insects is monitored. No significant effect of insects on the treated samples is observed.

TABLE 1 summarizes the statistical analysis of treated and untreated red kidney beans (rajma). Microwave power is found to be convenient at 270-450 W for 30-60 s. Treated sample shows the lowest error of 0.0016 at 450 W power level with a time interval of 60 s, whereas in case of untreated sample, highest error is found at 750 W for 30 s. The variances are found to be lowest such as 270 W at 90 s, 450W at 60 s and 450 W at 90 s for treated samples. The variance is observed to be the highest, at 750 W at 30 s.

TABLE 1. Statistical analysis of treated and untreated red kidney beans (rajma).

Sample type	SD	Variance	SE
Fresh Sample	0.03996	0.046165	0.0967
270 W 30 s	0.09146	0.091463	0.055771
270 W 60 s	0.04617	0.03996	0.040562
270 W 90 s	0.00165	0.001466	0.002131
450 W 30 s	0.00311	0.009987	0.008366
450 W 60 s	0.00935	0.001551	0.001597
450 W 90 s	0.04218	0.001779	0.038282
750 W 30 s	0.03939	0.0967	0.099935
750 W 60 s	0.09994	0.055771	0.039385
750 W 90 s	0.03828	0.040562	0.04218

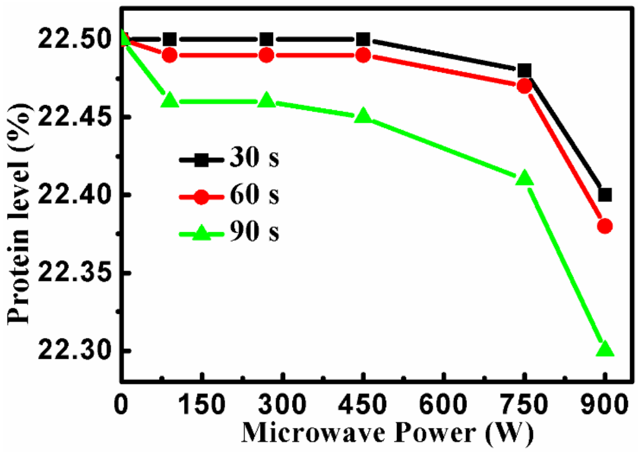

FIGURE 1. Protein levels of red kidney beans at different microwave power and time.

Crude protein content of the processed kidney beans is calculated using standard Kjeldahl method. Crude protein content for treated and untreated red kidney beans at different power and time is shown in Fig. 1. Protein level of treated samples is found to be 22.5 % at 90 W and 450 W. 0.02 % protein is lost for the beans treated at the power level of 450 W and 750 W. These findings well match with the reported results in the literature [22], [23]. The difference in protein content of infected and fresh kidney beans can be attributed to the presence of Pulse Beetle (Callosobruchus chinensis).

The moisture content into the beans is determined using DMM8 standard digital moisture meter. The moisture level is observed to be 10 %. It is noted that insects affect the kernel of red kidney beans, when moisture level is less than the predefined level (11.75 %) [23]. The FTIR spectroscopy is carried out to analyze other nutritional parameters such as carbohydrates, moisture, fats, amides, etc. The pellets are made using powder samples and KBr. It is kept into the furnace for 10 min at 40 °C to remove the moisture. The samples are scanned through transmittance mode of FTIR

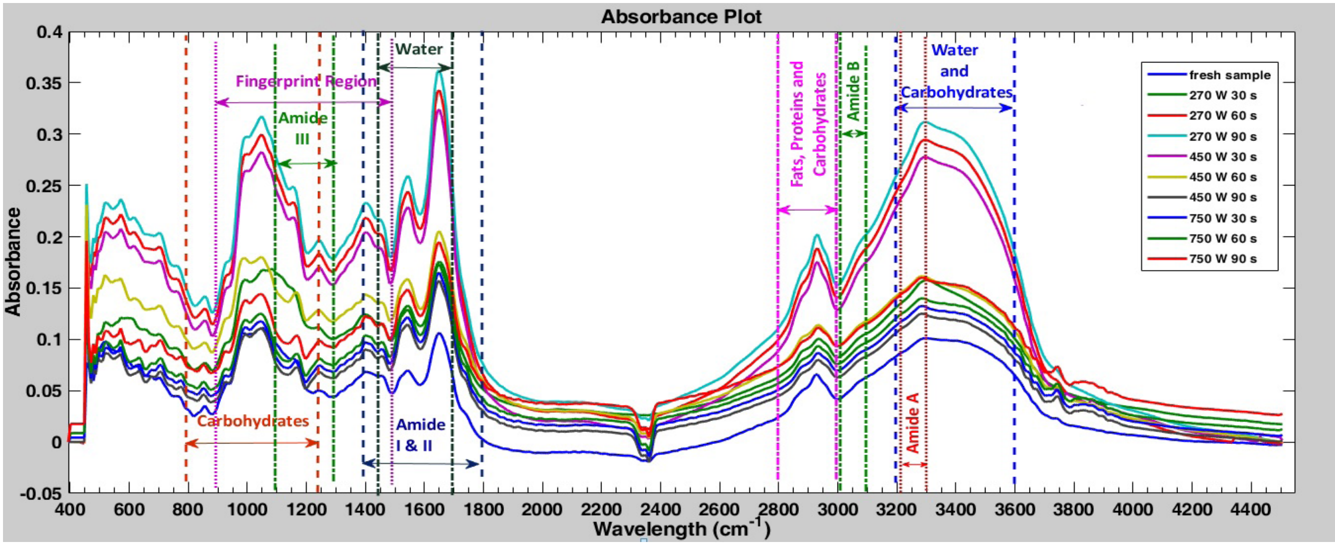

FIGURE 2. FTIR spectroscopy of treated and untreated red kidney beans at different powers and heating time within the range of 400-4500 cm^{-1}.

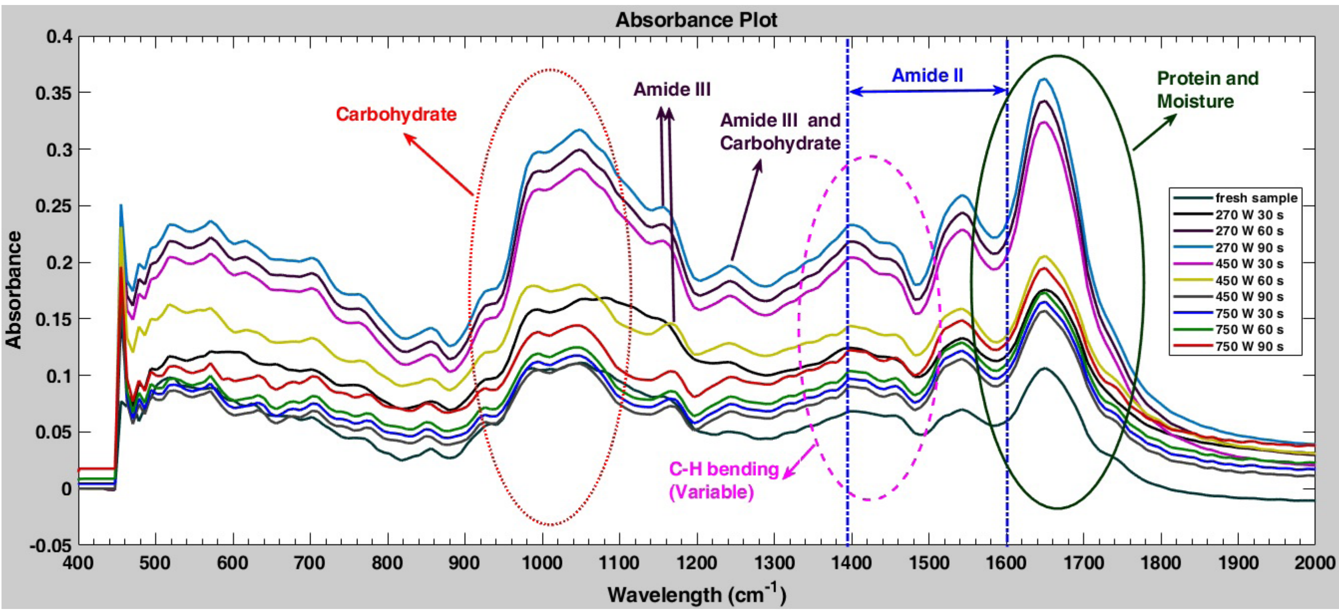

FIGURE 3. FTIR spectroscopy of treated and untreated red kidney beans shows the presence of the nutritional parameters in the range of 400 to 2000 cm^{-1}.

covering the full range of 400-4500 cm^{-1}. FTIR spectra of treated and untreated samples are shown in Fig. 2. The peaks at 800-1250 cm^{-1}, 3200-3600 cm^{-1} and 2800-3000 cm^{-1} are related to carbohydrates [15]. The wavebands located at 1450-1700 cm^{-1} and 3200-3600 cm^{-1} belong to moisture, whereas that at 1100-1300 cm^{-1}, 1400-1800 cm^{-1}, 2800-3000 cm^{-1}, 3050-3100 cm^{-1}, and 3250-3300 cm^{-1} denote the protein and amides in the samples [15].

FTIR spectra pertaining to nutritional factors of treated and untreated red kidney beans are shown in Fig. 3 and Fig. 4. One strong absorbance at 1650 cm^{-1} belongs to O-H bond, while the other at 1600-1700 cm^{-1} corresponds to amide I (C = O strong stretch), as shown in Fig. 3. Peaks for aromatic multiple bands stretching (C = C) including amide II band (N-H bending and C-O stretch) are located at 1390-1600 cm^{-1}, whereas the bands at 1150-1300 cm^{-1} correspond to amide III. The signature of C = C-H, C-O stretching and C-O-H bending vibrations of carbohydrates are appeared at 900-1100 cm^{-1} and 1250 cm^{-1}, as shown in Fig. 3. The peak at 1350-1500 cm^{-1} is related to C-H variable bending. Peptide linkage of proteins such as amides, water and carbohydrates are clearly shown in Fig. 3. The C-H stretching (sharp and strong) and vibrations (strong and medium) correspond to 3300 cm^{-1} and 2850-3100 cm^{-1}, respectively. The appearance of N-H (stretch and vibrations) bond and C = O stretch bonds are observed in the range

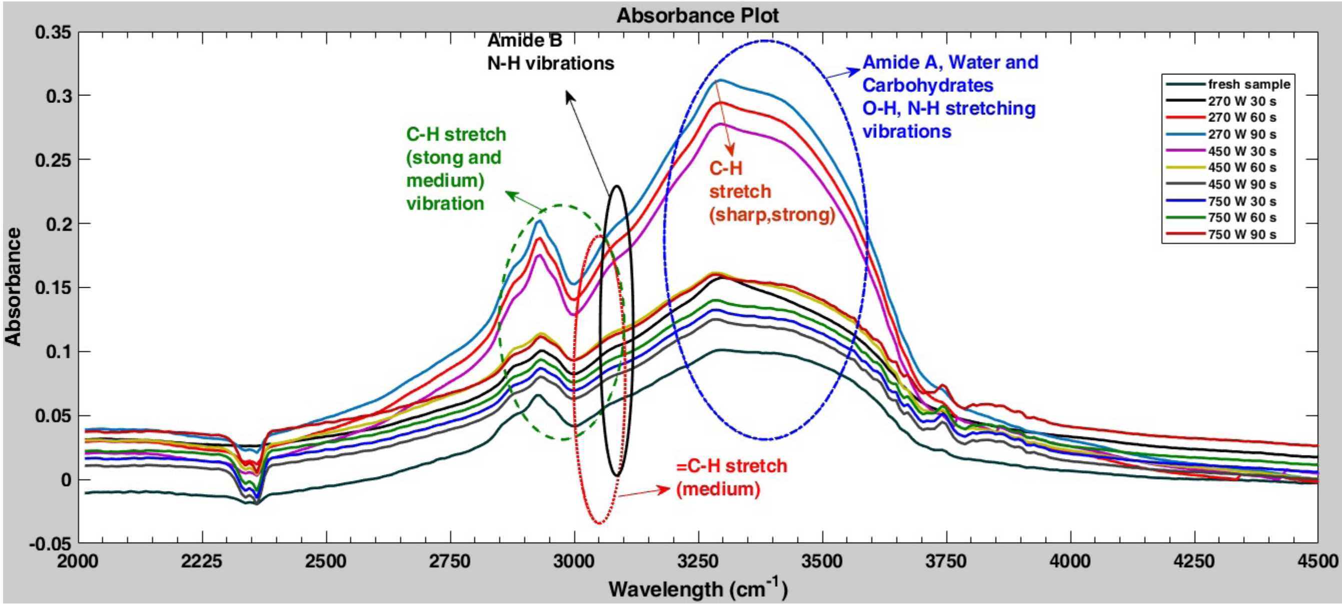

FIGURE 4. FTIR spectroscopy of treated and untreated red kidney beans. Chemical bindings such as C-H, N-H, O-H for nutritional parameters are present in the range of 2000-4500 cm^{-1}.

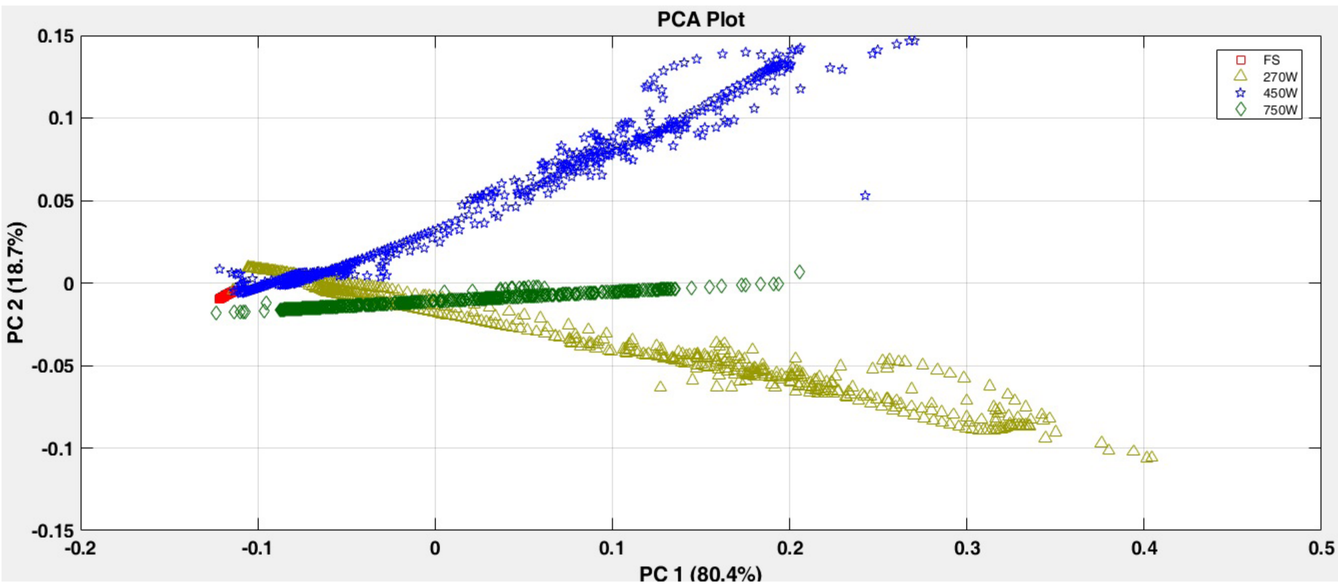

FIGURE 5. Principal component analysis of the most effective components (PC1 and PC2).

of 3200-3600 cm^{-1} and 3050-3100 cm^{-1}, respectively. From Fig. 4, it is seen that peaks of = C-H stretch (medium), C-H aromatic stretch (medium) and O-H strong are located at 3000-3100 cm^{-1}, whereas broad stretch with carbohydrates peaks appears in the range of 3200-3600 cm^{-1}. Untreated and treated samples exhibit almost identical characteristics at the specific chemical bond. The nutritional values of treated samples are also found to be same to the untreated samples. This result gives a clear notion that the nutritional parameters are not affected after the exposure of microwave heat. In addition, the life span of disinfested red kidney beans is increased.

Generally, principal component analysis (PCA) is used to understand the nutritional behavior of samples. PCA is done on the absorbance data to derive the PCA components, loadings and scores. The coefficient of determination, standard deviation (SD), variance and standard error (SE) are derived from the spectrum. The PCA is used to reduce the dimensionality of the absorbance using orthogonal principal components (PCs) and latent variables. The data is analyzed by score factors for microwave heated samples at different power levels as depicted in Fig. 5. The scores denote the projection of spectra for each PC and loading. Fig. 6(a) exhibits the characteristics of PC1 and PC2 for untreated and treated samples at different power and time. Region 'A' indicates the z-score of the sample at zero. In Fig. 6(b), region A is defined broadly, and it is observed that the data are in close proximity.

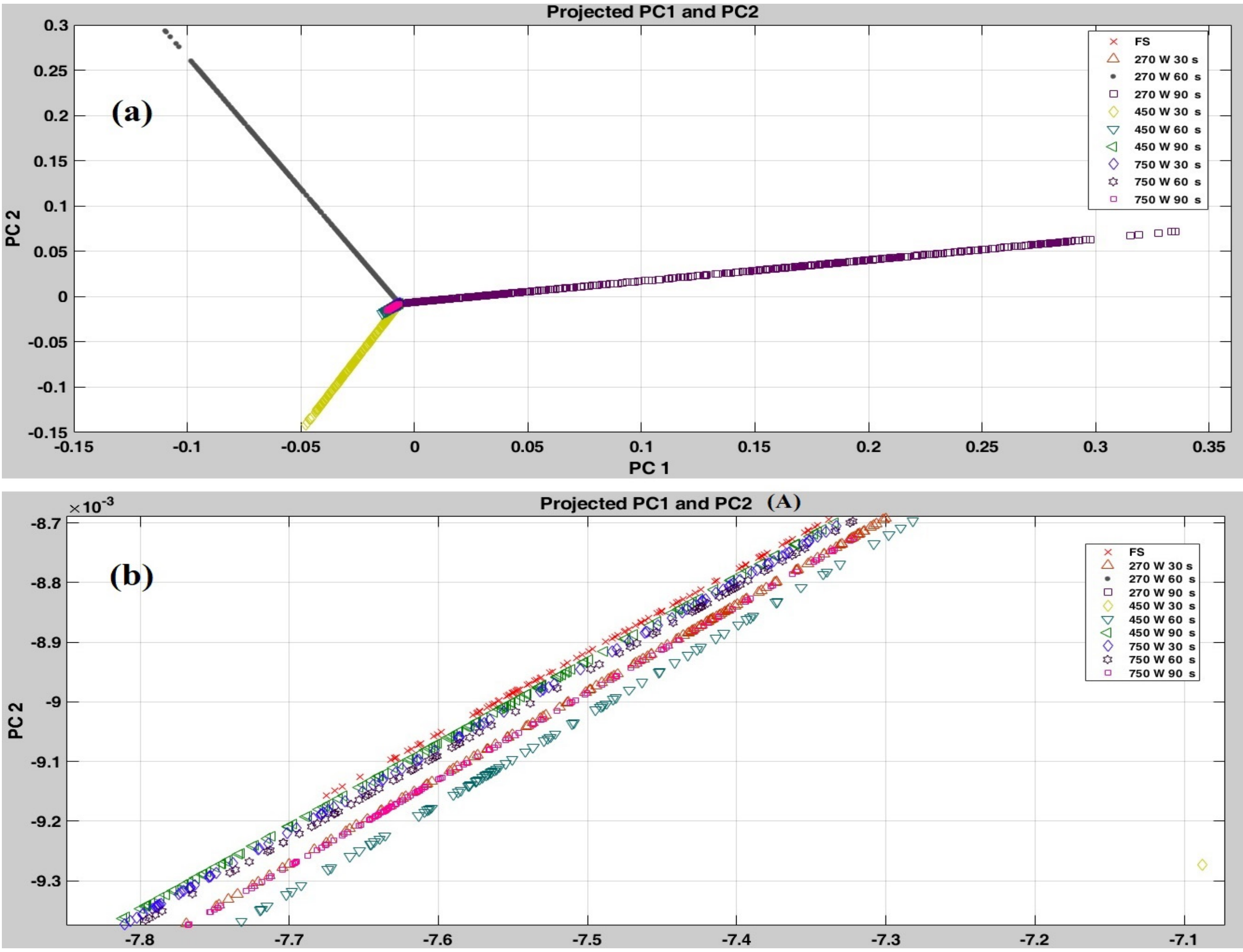

FIGURE 6. **(a) Scores of most effective components such as PC1 and PC2 for treated and untreated samples, and (b) principal component analysis of the selected parameters for red kidney beans.**

Hence the scores cannot be identified separately, as shown in Fig. 6(b).

IV. CONCLUSION

In conclusion, the effect of microwave heat on the nutritional properties of affected red kidney beans (rajma) is studied systematically. Pulse Beetle (Callosobruchus chinensis) shows the mortality rate of 95 % at 270 W and 450 W for 30 s and 60 s, respectively. FTIR spectroscopy also confirms the presence of nutritional parameters such as carbohydrates, protein (amide), moisture or water molecules and aromatic substances in kidney beans. No significant change in crude protein contents are observed at 90-450 W for 30-60 s. Microwave heat treatment is found to be safe in the range of 270-450 W, which does not affect the nutritional properties of red kidney beans. Error is determined to be the lowest with a value of 0.001597. Therefore, microwaves not only can be lethal for insects but it could also prevent the growth of new generation. All these results indicate that high power microwave treatments are a very promising and eco-compatible solution for disinfestations. Thus, microwave heat treatment has great potential for disinfestations of food grains without affecting the nutritional values of red kidney beans.

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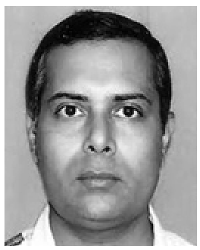

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