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Optimization of Sprouting and Infrared Radiation Combination Treatment for Production of Ready-to-eat Sprouted Soybean

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Antinutritional factors (ANFs) in the soybean limit its consumption in raw form. Although sprouting reduces ANFs to a certain extent, they are still beyond the safe limit of human consumption, limiting soybean consumption in sprouted form. The inactivation of soybean ANFs necessitates adequate heat treatment. Therefore, in the present study, post-sprouting infrared (IR) treatment was given to reduce ANFs, particularly trypsin inhibitors. The effects of IR power density (4250–4750 W/m^2), exposure time (4–8 min), and stage of germination (5–11 mm length of sprouts) on color, firmness, and Trypsin Inhibitor Activity (TIA) were investigated. The response surface methodology was used to optimize the responses. The optimum conditions were 4497.5 W/m^2 IR power, 4 min exposure time, and 5.54 mm germination stage (average sprout length). The color difference, firmness, and TIA values obtained at optimal conditions were 2.43, 24.66 N, and 2.458 mg/g, respectively. Sprouting and IR combination treatment efficiently lowered the TIA to a safe level (~77% reduction from raw soybean) while retaining the quality of the sprouted grains. The study suggests that combination treatment can be effectively used to produce ready-to-eat soybean sprouts.

Keywords: Antinutrients, Infrared heating, Soybean, Sprouting, Trypsin inhibitor activity

Introduction

According to the USDA Nutrient Database, soybeans contain 36.5% protein, 30% carbohydrates, 19.9% lipids, and 9.3% dietary fiber. Although soybean is nutritionally significant, it has many biologically active antinutrients like trypsin inhibitors, oligosaccharides, phytates, lectins, saponins, and glucosinates.[1] Before consumption, the activity or content of these antinutrients needs to be reduced to a level that will not be detrimental. As these antinutrients are heat labile, traditional thermal treatment such as boiling, autoclaving, steaming, roasting, etc.[2,3] or novel thermal treatment such as ohmic heating, microwave heating, radiofrequency or dielectric heating, etc.[4–6] is proven successful for reducing antinutrients in raw soybeans. Apart from heat treatment, sprouting, a non-thermal and non-chemical technology, is also beneficial in reducing antinutritional factors and enhancing nutrients and mineral availability.[7,8] Reduction in antinutrients "from sprouting is a function of sprout length or duration of germination. Dikshit & Ghadle[9] reported 27.95–51.68% reduction in TIA from raw soybean based on the sprout length or duration of germination. However, sprouting alone is insufficient to reduce antinutrients present in soybean to an acceptable limit (50–60% of inactivation from raw soybean).[10–12] The majority of commercially available soy products for human consumption, including soy milk, tofu, textured meat analogs, soy protein isolates, soy protein concentrates, and soy-based infant formulas, are sufficiently heat treated to inactivate at least 80% of the TIA found in raw soybeans.[10,13] Therefore, to make soybean sprouts ready-to-eat, post-sprouting, suitable treatment is required (as any treatment given to seed before sprouting may reduce or hinder its germination) to bring down antinutrients to a safe limit.

Therefore, in the present study, post-sprouting infrared (IR) heat treatment is used to reduce antinutrients, particularly trypsin inhibitors. The IR radiations have a wide range of applications in the food industry, such as drying, roasting, pasteurization, blanching, peeling, etc.[14] It is a faster and energy-efficient heating method. IR heating has been applied to cereal grains and pulses to reduce antinutrients and improve their digestibility.[15] Peas

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and red kidney beans micronized to 90°C showed significant reductions in antinutrients. The reduction was dependent on pulse type and variety.[16] Yalcin & Basman[17] reported that infrared treatment caused a considerable reduction in TIA (95–97%), and the treatment effect was more pronounced on soaked soybeans than unsoaked.

Till now, the applications of individual heat treatment and sprouting in reducing ANFs have been reported for preparing different value-added products of soybean. Efforts have not been made to make soybean ready-to-eat in sprouted form. Therefore, the objective of the present investigation was to assess the effectiveness of post- sprouting IR treatment on reducing TIA, optimization of the sprouting stage, and IR treatment parameters to make soybean consumable in its sprouted form.

Material and methods

Sample Preparation

Soybean grains of cultivar *JS-9305* were procured from ICAR-Central Institute of Agricultural Engineering, Bhopal (India) research farm. The grains were cleaned through air screen cleaner to remove foreign material and further graded using a spiral grader to ensure that only bold grains having good vigor and viability were selected for the study. Cleaned grains were further stored at 25 ± 1°C, 50% RH with 8 ± 0.2% of MC (db). Furthermore, seeds were surface sterilized with potassium permanganate solution (0.1% w/v) and soaked in distilled water (30 ± 1°C) for 4 hours. Following soaking (4 h), the seeds were placed on moist filter paper to sprout. Water was sprinkled at regular time intervals to maintain moisture.[18] About 50 g of sprouted soybean with average sprout length observed as 5 ± 1.0 mm, 8.0 ± 1.0, and 11.0 ±1.0 mm at 24, 36, and 48 h, respectively, were collected for infrared radiation treatment.

Experimental Setup for Infrared Treatment

A schematic illustration of the experimental setup for controlled infrared treatments on sprouted soybeans is shown in Fig 1. The treatment system consists of an electrically operated 250 W quartz bulb (Make: Philips India Limited) as an IR source, emitting radiation in the near-infrared region of the electromagnetic spectrum. The IR source was mounted on a horizontal lamp holding bar. The lamp holding bar was attached to a vertical screw which facilitated adjusting the distance between the IR source and the treatment tray. The intensity of infrared radiation was varied by regulating the input electrical power to the lamp with the help of a voltage variac (Graff Electro, Delhi, India) appropriately connected to the circuit. The output radiation intensity was measured with a pyranometer connected to a millivoltmeter. A specially designed inside concave type of mirror polish reflector was fitted over the IR lamp keeping in view that the focal point of radiations comes exactly over the surface of the treatment tray. Based on the preliminary trials, during experiments, the distance between the lower surface of the IR lamp and surface of sample holder was fixed at 150 mm.

Infrared Treatment of Sprouted Soybean

The IR source was switched on 5 minutes before treatment to reach the set power density for the individual experiment. The sample was placed on a circular treatment tray of 145 mm diameter and agitated manually at one minute time interval for uniform exposure of all the surfaces of the sample. After treatment, the samples were immediately immersed in distilled water (30 ± 1°C) for 2 minutes, which enabled the removal of the hull from the sprouted soybean without damaging the sprout. Hull removed samples were kept on tissue paper to remove excess water and further taken for analysis.

Experimental Plan

In the study, three parameters, i.e., infrared power density, exposure time, and stage of germination were selected as independent variables with 5 levels each (Table 1). The ranges of the levels of the independent variables were selected based on preliminary trials. The possible combinations of the experimental conditions were obtained through Central Composite Rotatable Design (CCRD), as shown in Table 2. Total color difference, firmness, and TIA were selected as dependent variables to analyze the effect of different

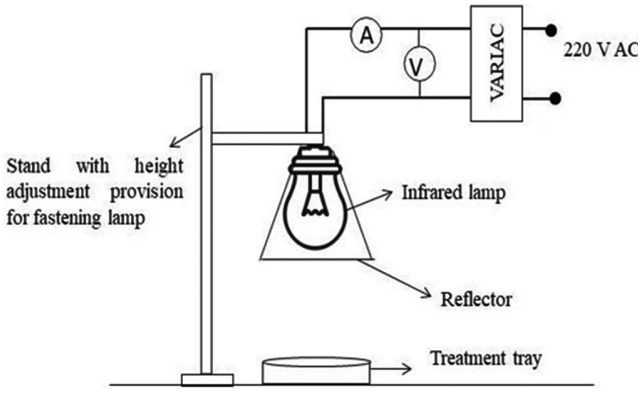

Fig. 1 — Experimental setup for IR treatment of sprouted soybean

Table 1 — Levels of process variables

Variable	Name (units)	Level				
		−1.68	−1	0	1	1.68
X_1	Infrared power density (W/m^2)	4079.55	4250	4500	4750	4920.45
X_2	Exposure time (min)	2.64	4	6	8	9.36
X_3	Stage of germination (mm)	2.95	5	8	11	13.05

Table 2 — Response surface design (CCRD) with experimental results

Std	Run	IR power density (W/m^2)	Exposure time (min)	Stage of germination (mm)	Color difference	Firmness (N)	Trypsin inhibitor activity (mg/g)
1	3	4250	4	5	1.953	25.421	2.918
2	19	4750	4	5	2.686	23.674	2.72
3	12	4250	8	5	4.102	22.431	2.685
4	1	4750	8	5	6.967	18.778	1.995
5	5	4250	4	11	3.947	24.928	2.86
6	11	4750	4	11	6.039	23.189	2.67
7	2	4250	8	11	3.473	22.239	2.274
8	16	4750	8	11	7.716	18.681	1.071
9	8	4079.55	6	8	2.678	25.947	3.424
10	15	4920.45	6	8	6.431	19.402	1.561
11	9	4500	2.64	8	2.528	25.902	2.828
12	17	4500	9.36	8	5.647	12.907	0.982
13	4	4500	6	2.95	4.092	21.891	2.204
14	18	4500	6	13.05	5.014	20.343	1.898
15	7	4500	6	8	6.441	22.485	1.906
16	10	4500	6	8	3.85	24.527	1.92
17	14	4500	6	8	3.85	23.905	2.18
18	13	4500	6	8	3.85	21.214	1.78
19	6	4500	6	8	3.85	21.947	1.68
20	20	4500	6	8	3.85	22.395	1.86

operating parameters with their varying combinations. Further, multiple response optimization was carried out using Response Surface Methodology (RSM) with Design-Expert® Software Version 7.0. Table 1 shows the actual and coded levels of independent parameters. Second-order polynomial models were fitted to experimental data to generate prediction equations (Eq. 1). Analysis of variance (ANOVA) was conducted to evaluate the significance of the independent variables, their interactions, and higher degrees on the selected dependent variable.

$$Y = \beta_0 + \sum_{i=1}^{n} \beta_i X_i + \sum_{i=1}^{n} \beta_{ii} X_i^2 + \sum_{i=1}^{2} \sum_{j=i+1}^{3} \beta_{ij} X_i X_j \ldots (1)$$

where,

β_o, β_i, β_{ii}, β_{ij} = regression coefficient

X_i and X_j = independent variables (where, I = 1, 2, … And j = 1, 2,…) and Y is response

Dependent Variables

Color Difference

The surface color of the IR-treated and fresh-sprouted soybean samples was measured in terms of the CIELAB using a colorimeter (LabscanXE, Hunterlab, USA). Color difference (ΔE) of the IR-treated sprouted soybean (L, a, b) in comparison to fresh soybean sprouts (L*, a*, b*) was calculated using Eq. (2)[19].

$$\Delta E = \sqrt{(L - L^*)^2 + (a - a^*)^2 + (b - b^*)^2} \quad \ldots (2)$$

Firmness

Texture Analyzers (Stable micro system, UK) equipped with 50 kg load cells was used to measure the firmness of fresh and treated soy sprouts. The test was conducted with a flat plate (P/75) probe operated in compression mode with 1 mm/s of test speed and 10 mm/s of pre and post-test speed. During the test, Splits of fresh and treated soybean sprouts were placed horizontally on the sample holding platform and compressed twice to 20 of strain with a trigger force of 5 g. The test was performed in 5 replications. The maximum force was recorded as the firmness value.[19]

Trypsin Inhibitor Activity

IR-treated soybean sprouts were dried in an oven for 24 h at 40 ± 2°C. After drying, samples were ground

and sieved. Further, the samples were extracted with 50 mL NaOH (0.01 N) for 2 h under constant stirring on a magnetic stirrer and then filtered. The extracted sample was diluted such that 2 mL of the extract inhibited 40–60% of the trypsin used as a standard in the analysis. TIA was determined by the standard procedure of Hamerstrand *et al.*[20]

Results and Discussion

Prior to IR treatment, the color values (L*, a*, b*), firmness, and TIA of fresh soybean sprouts were measured. The L*, a*, and b* values of the fresh soybean sprouts were 69.08, 1.64, and 29.89, respectively. The firmness of the fresh soybean sprouts was 26.74 ± 0.8 N. The TIA of raw soybean was 11.38 ± 0.15 mg/g of sample. After sprouting the TIA of the soybean reduced significantly ($p < 0.05$). The TIA of sprouted seeds without IR treatment with an average sprout length of 5 ± 1 mm (24 h), 8 ± 1 mm (36 h), and 11 ± 1 mm (48 h) were 8.28 ± 0.09 mg/g (27% reduction from raw soybean), 7.64 ± 0.11 mg/g (33.83% reduction from raw soybean), and 7.55 ± 0.08 mg/g (33.67% reduction from raw soybean), respectively (Fig. 2). The values of the dependent variables under individual experimental conditions are listed in Table 2. Regression analysis and ANOVA of the experimental data were performed for prediction of response and to understand the effect and significance of independent variables on response.

Effect of Independent Variables on Color Difference

The experimental values of color change in sprouted soybeans with respect to IR treatment varied from 1.953 to 7.716. The minimum value of color change (i.e., 1.953) obtained at 4250 W/m^2 IR power density, 4 min exposure time, and 5 mm stage of germination. A second-order regression Eq. (1) was fitted into color difference data from various experimental conditions. Regression analysis (Table 3) showed that the regression equation for color difference had a coefficient of determination (R^2) of 0.86, indicating that the model could account for 86% of the data. The model was highly significant ($p < 0.01$) with a non-significant lack of fit. The equation representing the change in color difference with respect to experimental conditions as follows:

$$Y_1 = 4.28 + 1.19X_1 + 0.94X_2 + 0.51X_3 + 0.54X_1X_2 + 0.34X_1X_3 - 0.65X_2X_3 + 0.14X_1^2 - 0.025X_2^2 + 0.14X_3^2 \quad \ldots (3)$$

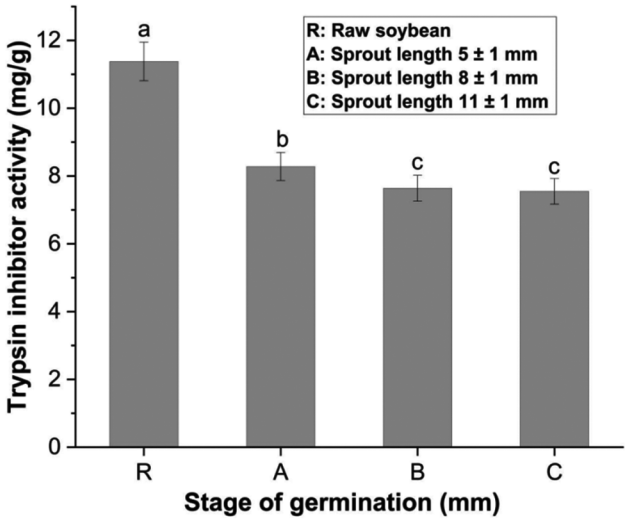

Fig. 2 — Variation in TIA of fresh soybean sprouts with stage of germination

Table 3 — Results of regression analysis

Source	Color difference		Firmness (N)		Trypsin inhibitor (mg/g)	
	Coeff.	p-Value	Coeff.	p-Value	Coeff.	p-Value
Model	4.28	0.002***	22.70	0.006***	1.88	0.001***
X_1	1.19	0.001***	−1.59	0.006***	−0.40	0.001***
X_2	0.94	0.001***	−2.70	0.001***	−0.46	< 0.001***
X_3	0.51	0.038**	−0.28	0.552	−0.14	0.073*
X_1X_2	0.54	0.086*	−0.47	0.457	−0.19	0.072*
X_1X_3	0.34	0.252	0.013	0.983	−0.063	0.514
X_2X_3	−0.65	0.043**	0.086	0.889	−0.15	0.1314
X_1^2	0.14	0.521	0.28	0.544	0.26	0.004***
X_2^2	−0.025	0.906	−0.87	0.08*	0.055	0.442
X_3^2	0.14	0.522	−0.27	0.562	0.11	0.155
R^2	0.8696		0.8391		0.9081	
F value	7.41		5.80		10.98	
LOF	NS		NS		NS	

*** Significant at 1% level, ** Significant at 5% level, * Significant at 10% level

The coefficients of the regression model for color change in terms of linear order, second-order, and interaction term are presented in Table 3. At the linear level, the color change of IR-treated sprouted soybeans was significantly affected by all three parameters (X_1, X_2, and X_3, $p < 0.001$ or $p < 0.05$). In addition, the interaction parameters X_1X_2 and X_2X_3 were significant at the 10% and 5% levels of significance, whereas the quadratic parameters (X_1^2, X_2^2, and X_3^2) were non-significant ($p > 0.1$). Positive linear coefficient of infrared power (X_1), exposure time (X_2), and stage of germination (X_3) indicated that the total color difference increased with an increase in all three parameters. The simplified prediction equation (Eq. 4) for color change becomes:

$$Y_1 = 4.28 + 1.19X_1 + 0.94X_2 + 0.51X_3 + 0.54X_1X_2 - 0.65X_2X_3 \quad \ldots (4)$$

The visual representation of the effect of process parameters, viz. infrared power density, exposure time, and stage of germination, on the total color difference of sprouted soybeans is depicted in Fig. 3. Increasing the infrared power and exposure time increased the color difference of sprouted soybeans due to the surface browning of the samples. The maximum color difference was observed for samples exposed to higher levels of infrared power and exposure time. Similar results of deterioration in color parameters with an increase in the infrared power level were reported by Baezegar *et al.*[21] for green beans and Doymaz *et al.*[22] for jujube fruit. It may be due to enzymatic and non-enzymatic browning reactions that occur when the sample is exposed to infrared radiation, resulting in quick browning of products.[23] At higher germination stages, the sprout tip became brown, which led to an increase in color difference. These results revealed that a lower infrared power, exposure time, and stage of germination might be preferable to minimize color changes in treated samples.

Effect of Independent Variables on Firmness

In the present investigation, the firmness values of the sprouted soybeans varied from 12.907 N to 25.947 N. The maximum firmness value was obtained at 4079.55 W/m^2 IR power density, 6 min exposure time, and 8 mm germination stage. Regression analysis was used to correlate the firmness of IR-treated soy sprouts obtained in different experimental runs with three independent variables. ANOVA results showed that the second-order equation was highly significant ($p < 0.01$) for firmness. The regression model's coefficient of determination (R^2) was 0.83. A lack of fit test was performed to assess the adequacy of the model, and it was insignificant for $p > 0.05$. Since the models have a sufficiently higher coefficient of determination with a non-significant lack of fit (Table. 3), it is suitable for firmness prediction (Eq. 5).

$$Y_2 = 22.70 - 1.59X_1 - 2.70X_2 - 0.28X_3 - 0.47X_1X_2 + 0.013X_1X_3 + 0.086X_2X_3 + 0.28X_1^2 - 0.87X_2^2 - 0.27X_3^2 \quad \ldots (5)$$

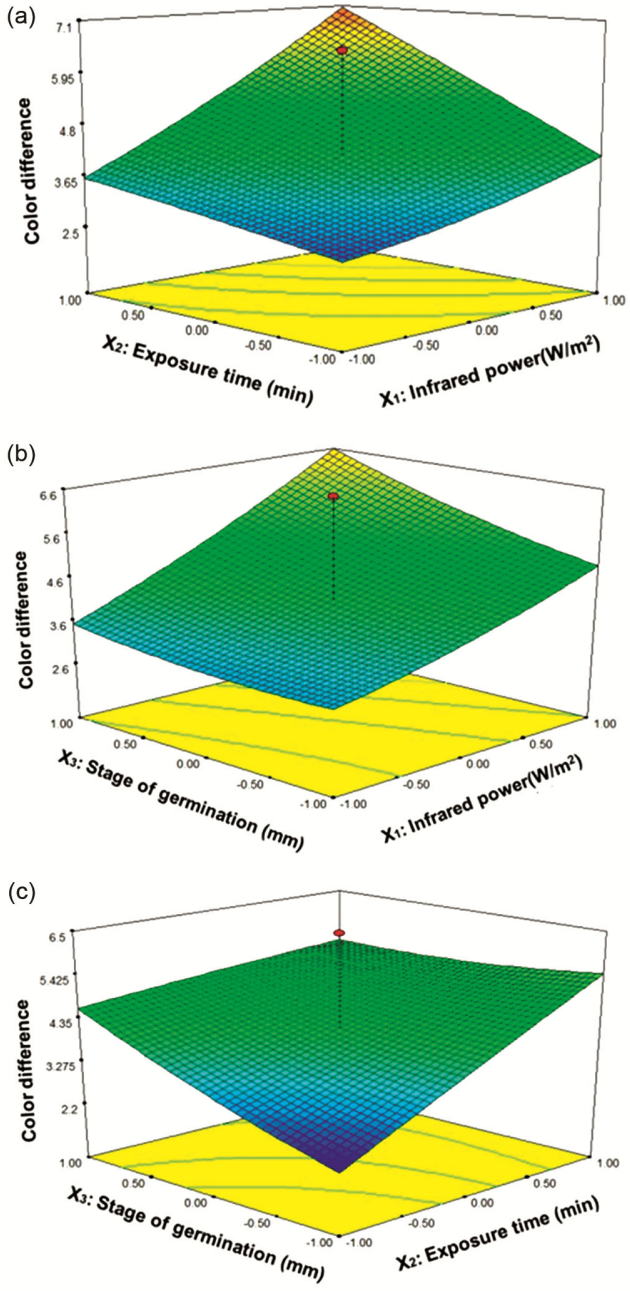

Fig. 3 — Effect of independent variables on color difference

The coefficient of the regression model for firmness in terms of linear order, second order, and interaction term are given in Table 3. It was observed that the firmness of IR-treated sprouts was affected significantly by infrared power and exposure time (X_1 and X_2, $p < 0.01$). The germination stage had a negative effect on firmness, but the effect was not significant ($p > 0.1$). Interaction effect of infrared power, exposure time, and germination stage was not-significant ($p > 0.10$). The non-significance of all the interactive terms might be due to the individual variable's counteracting effect, which resulted in an overall null effect on the response[24]. The quadratic effect of exposure time was significant at 10% significance level. The predictive second-order equation was rearranged by ignoring the terms that were not significant at 10% level of significance, and the corresponding Eq. (6) is given below:

$$Y_2 = 22.70 - 1.59X_1 - 2.70X_2 - 0.87X_2^2 \quad \ldots (6)$$

An increased infrared power and exposure time resulted in decreased firmness of sprouted soybean (Fig. 4). Lowest firmness was observed at the highest infrared power and exposure time within the experimental range. Andrejko *et al.*[25] also reported that the compression resistance of wheat grain depends on the temperature and duration of the IR interaction. When processing temperature and time were increased, the compression resistance of wheat grain decreased. Similar result of a reduction in firmness was also reported by Lara *et al.*[26] for vegetable soybean. The reason behind this lies in the structure of soybean. The primary cell wall of soybeans is made up of polysaccharides, mainly pectin (50–70% on cell wall weight) and protein. Pectin rich layer known as the middle lamella ensures the adhesion between the cells.[27] Due to heat, some pectin substances become soluble.[28] Additionally, heating also disrupts the middle lamella and softens the protein matrix of beans, which might have reduced firmness.[29] 3D surface plots (Fig. 3) also revealed that stage of germination did not affect the firmness of sprouted soybean.

Effect of Independent Variables on TIA

TIA of treated samples ranged between 0.982–3.424 mg/g (69.96–91.37% reduction from raw soybean). In comparison, TIA of sprouted soybean without IR treatment ranged between 7.55–8.28 mg/g sample. A substantial decrease in TIA was observed after IR treatment (~70% from fresh sprouts). This reduction in TIA post-IR treatment was influenced by infrared power and exposure time. The results obtained for TIA during different experimental run (Table 2) was correlated with three independent variables using a second-order regression equation. Regression analysis (Table 3) reveals that the coefficient of determination (R^2) for the regression model for TIA was 0.90, with non-significant lack of fit; the model was highly significant ($p < 0.001$) to represent the change in behavior of TIA during overall experimental conditions. The second-order polynomial equation for TIA is shown in Eq. (7)

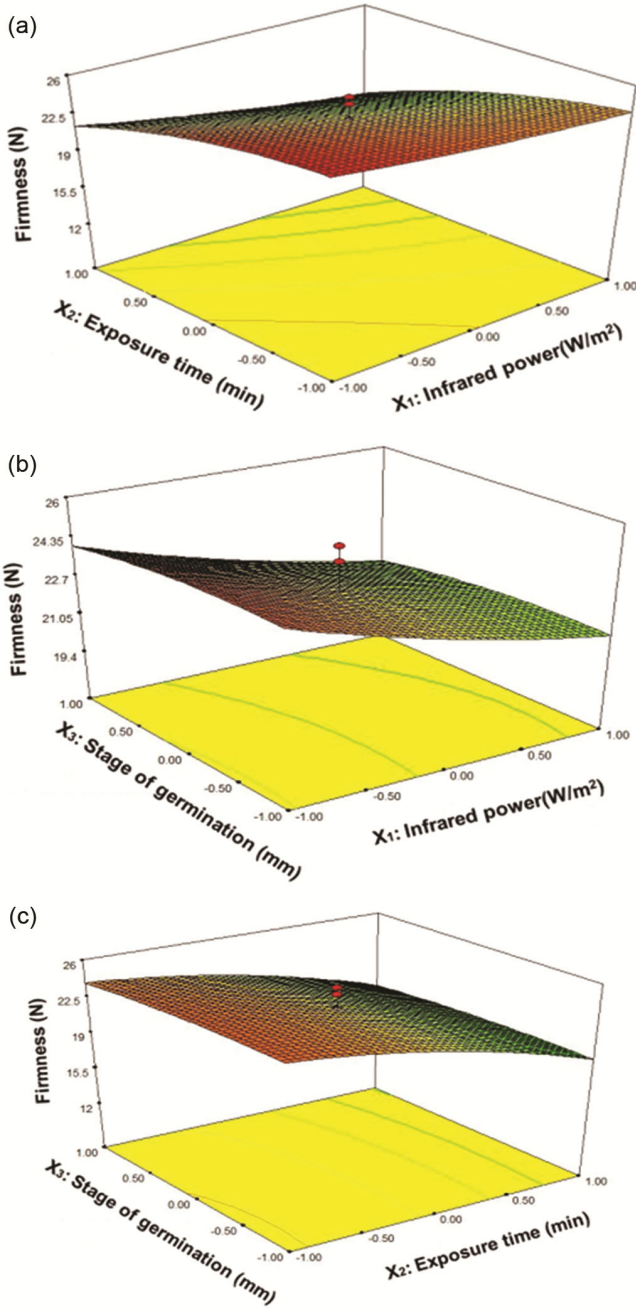

Fig. 4 — Effect of independent variables on firmness

$$Y_3 = 1.88 - 0.40X_1 - 0.46X_2 - 0.14X_3 - 0.19X_1X_2 - 0.063X_1X_3 - 0.15X_2X_3 + 0.26X_1^2 + 0.055X_2^2 + 0.11X_3^2 \quad \ldots (7)$$

The partial coefficients of the regression model for TIA are expressed in Table 3. The sign and magnitude of the coefficients indicate the effect of the variable on TIA. The negative linear coefficient of X_1 (infrared power), X_2 (exposure time), and X_3 (stage of germination) indicated that TIA decreased with increase in all three independent variables. Infrared power and exposure time had significant effects at the 1% significance level, while germination stage was significant at the 10% level of significance. The interaction effect of infrared power and exposure time and the quadratic effect of infrared power were significant at 10% and 1% significance, respectively. A predictive regression equation having only significant terms is given as follows

$$Y_3 = 1.88 - 0.40\,X_1 - 0.46X_2 - 0.14X_3 - 0.19X_1X_2 + 0.26X_1^2 \quad \ldots (8)$$

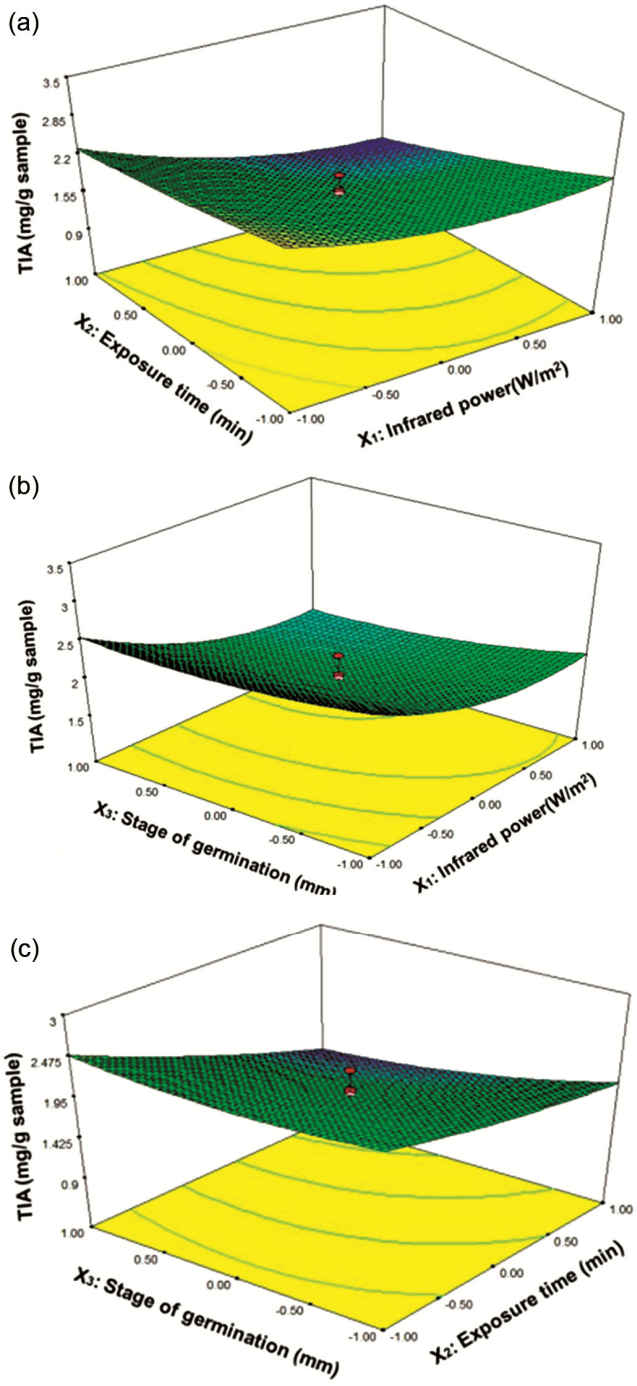

Fig. 5 — Effect of independent variables on trypsin inhibitor activity

The visual representation of the behavior of TIA with change in independent variables is represented in Fig 5. The figure revealed that TIA decreased with an increase in the level of infrared power and exposure time. Kouzeh-Kanani *et al.*[30] and Yalcin & Basman[17] also reported a similar result for soybean. This is attributed to the heat-sensitivity of trypsin inhibitors.[31,32] Infrared energy might have broken the chemical structure of trypsin inhibitors, leading to reduced TIA.[33] TIA was found to reduce with an increase in the stage of germination. A decrease in TIA with an increase in germination time or stage of germination was also reported by Murugkar & Jha[8] and Dikshit & Ghadle[9] for soybean. The reduction in TIA may be attributed to the synthesis of amino acids due to the proteolytic activity of enzymes, which are activated during germination to support the growth of sprouts.[34,35]

Optimization of Process Parameters

The multiple response based numerical optimization of independent variable was carried out using Design-Expert 7.0 statistical software. The goals were fixed for all independent and dependent variables as per the objective of the study. Equal importance (+++) was given to all the responses, and independent variables. The goal was fixed to in range for infrared power and stage of germination, while the minimum was for exposure time. Among the responses, the goal was set to minimize for color and TIA, whereas firmness was fixed to the maximum. Based on the criteria mentioned above, the optimization was carried out. During optimization, 8 possible solutions were obtained in all the cases, out of which the one possible solution that suited the criteria and had higher desirability (0.756 was selected. The optimum conditions for producing ready-to-eat sprouted soybean were 4497.5 W/m^2 IR power, 4 min exposure time, and 5.54 mm stage of

germination (average sprout length). At these conditions, the values of responses obtained as 2.43 of color difference, 24.66 N firmness, and 2.458 mg/g of TIA.

Conclusions

This study aimed to optimize sprouting and IR radiation combination treatment to make sprouts ready to consume. The combination treatment effectively reduced TIA by ~77% (from raw soybean) without affecting the quality of the sprouts. The color and hardness of sprouted soybeans were negatively influenced by higher IR power density and exposure time, whereas these parameters positively affected TIA. The second-order polynomial model was well-fitted to predict the experimental data for all responses. The results of the study suggest the potential usefulness of the combination treatment in reducing the TIA to a safe level while maintaining color and firmness. Consumption of soybean in the sprouted form will help achieve nutritional security and prevent various diseases, as reported for soybean. However, the effect of combination treatment on other ANFs and *in-vitro* protein digestibility of the sprouted soybean needs to be studied further.

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