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Optimizing Sprouting Conditions for Improved Soybean Quality

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ABSTRACT

Soybean sprouts contain several health-promoting phytochemicals with high antioxidant properties and lower anti-nutritional elements. The purpose of this study was to optimize the sprouting condition variables comprising steeping temperature, germination temperature, germination relative humidity and germination time and their relative effect on the morphological quality parameters such as sprout length and sprout weight and productivity factor (the germination percentage). The response surface methodology (RSM) was utilized in this study to optimize the relationship of sprouting conditions. Sprouting conditions played a decisive role in determining the quality of sprouted soybeans. For the best quality soybean sprouts, the optimized values were 26°C, 24°C, 83% and 3.5 days for steeping temperature, germination temperature, germination relative humidity and germination time, respectively. The results were indicators for better production, handling, management, storage and marketing of soybean sprouts.

Key words: Germination, soybean, optimization, sprouting parameters

INTRODUCTION

Soybean (*Glycine max* L.) has been consumed by people worldwide for centuries due to its nutritional value. Soybean foods are currently attracting attention because studies showed that populations that consumed a considerable amount of soybean suffered a lower risk of some chronic diseases, most notably heart disease and cancer (Swallah *et al.*, 2023). Soybean seeds, on the other hand, contain a variety of anti-nutritional factors, including lectins and enzyme inhibitors (Wang *et al.*, 2022). Therefore, various methods have been explored to enhance the nutritional value of soybean through germination, which has been identified as a low-cost and efficient technology for enhancing the nutritional value. This results in highly palatable sprouts and a good source of protein and minerals.

Germination is a complex biological process which is an initial step of the plant life cycle, that involves several factors such as water imbibition, subcellular structural changes, growth of bud and roots, increase in respiration, formation of enzymes and degradation of some chemical substances (Liu *et al.*, 2022). This process starts when a dry inactive seed absorbs water and softens the seeds outercoat leading to uniform germination under various factors. These factors were dormancy of seed, a sufficient amount of water for absorption, relative humidity, temperature, oxygen, light, chemicals and growth-promoting substrates or other environmental factors (Yan and Chen, 2020). The germination process is an ancient approach for increasing and improving the nutritional and nutraceutical properties of seeds, but many issues arise during the germination or sprouting process such as deterioration of sprout quality, undesirable length of sprout, low yield and putrid during sprouting. Therefore, to overcome this problem, there must be an optimistic condition of sprouts which enhances the quality and quantity of sprouting. Although some factors that play an important role in sprouting are adequate temperature, steeping or soaking time of seed in water, quality of water, ideal moisture and most importantly relative humidity because a dry environment was not appropriate for sprouts, a good growth of sprout occurred in a hot and humid environment (Bishnoi and Ray, 2023).

The current study focused on the effects of temperature and humidity on the percentage of emergence and yield of soybean sprouts. Now-a-days for optimization, a statistical tool named Response Surface Methodology (RSM) is used to design a model and optimize complex processes to generate an appropriately predicted value for a given experiment. It helps in reducing the total number of experimental trials by providing simple and well-arranged experimental runs (Chelladurai *et al.*, 2021).

MATERIALS AND METHODS

The study was conducted in the Department of Food Technology, Guru Jambheshwar University of Science and Technology, Hisar, Haryana, India. The seeds of the soybean (*Glycine max*) variety SL-688 were procured from the Department of Plant Breeding and Genetics, Punjab Agricultural University, Punjab. For further work, the seeds were manually cleaned to remove spoiled, immature, or broken seeds, stone, dust, dirt, sand, stubbles and other extraneous material and stored in a deep freezer (-18°C). All the chemicals and standards used in the present investigation were of high-purity analytical grade purchased from Hi-Media Laboratories Pvt. Ltd., Sigma Aldrich, CHD, Merch Pvt. Ltd. The seeds were initially sterilized in 1% (v/v) sodium hypochlorite for 30 min, then drained and washed three times with distilled water so they reached a neutral pH. The seeds were then placed in distilled water (1:5) and soaked for 2 h at different temperatures. Seeds were germinated in a Seed Germinator (Dewsil Company) on Petri dishes (Diameter 125 mm) lined with absorbent paper at different temperatures, relative humidity (RH) and time combinations. Seedlings were watered daily two times with distilled water.

The current study focused on the effects of temperature and humidity on the percentage of emergence and yield of soybean sprouts. The germination conditions like soaking temperature (20-60°C) and seed germinator parameters like germination temperature (20-30°C), relative humidity (80-90%) and time (2-4 days) were varied at selected levels based on previous studies. The study was designed on the hypothesis that sprouts length, weight increase percentage and germination percentage for sprouts are related to germination factors like soaking and germination temperature, RH and time of germination.

Tools like Response Surface Methodology (RSM) with Design Expert Software provide more accurate conclusions than one-factor-at-a-time (OFAT) reduce the number of experimental attempts and generate more informative data. Moreover, the Design Expert aids in the analysis of the interaction between several factors and in the optimization of the different sprouting conditions, which ultimately improve process performance (Chelladurai *et al.*, 2021). Hence, the present investigation utilized them to get optimized sprouting conditions.

The levels of factors were chosen within the range based on previous research and on some preliminary studies. The design and levels of factors are shown in Table 1. Response surface methodology was chosen to analyze the effect of variables including steeping temperature (A), germination temperature (B), germination relative humidity (C) and germination time (D) on quality properties (sprout length, sprout weight increase and germination percentage) of sprouted soybean.

The experiments were designed and processed using Design Expert 10.0.7 software (Stat Ease Inc.). Experiments were conducted for four variables using a central composite design with face-centered alpha involving 30 combinations with six center points (Table 2). The runs were randomized to minimize the effects of unexplained variability in the observed responses due to extraneous factors.

RESULTS AND DISCUSSION

In this research, germination conditions showed a significant effect on the sprout length of soybean (Table 2). Further, the three-dimensional response surface plots were constructed from the response surface methodology model representing regression equations that demonstrated the interaction

Table 1. Levels of examined germination variables according to central composite RSM design

Symbol	Experimental factor	Coded value			Actual value		
A	Steeping temperature (°C)	-1	0	1	20	40	60
B	Germination temperature (°C)	-1	0	1	20	25	30
C	Germination relative humidity (%)	-1	0	1	80	85	90
D	Germination time (days)	-1	0	1	2	3	4

Table 2. Experimental design for varying germination conditions and responses obtained

Runs	Factors				Soybean		
	A	B	C	D	SL (mm)	SWI%	G%
1	40	30	85	3	45.88	172	16
2	40	25	85	3	113.24	259	70
3	40	25	85	3	102.41	230	70
4	20	30	90	2	2.16	142	14
5	60	30	80	4	6.29	84	14
6	20	30	80	4	46.33	186	72
7	20	20	90	4	20.06	238	80
8	20	20	80	2	32.68	178	68
9	20	30	80	2	38.55	170	60
10	60	20	90	2	4.33	143	12
11	60	20	80	4	9.68	157	10
12	40	25	85	3	55.44	260	70
13	20	20	80	4	48.41	252	84
14	60	25	85	3	55.67	159	36
15	20	25	85	3	81.92	228	98
16	60	30	90	4	54.44	147	12
17	20	20	90	2	12.38	145	68
18	60	30	90	2	21.1	165	18
19	40	25	85	3	98.54	245	72
20	20	30	90	4	6.43	158	16
21	60	30	80	2	2.29	127	12
22	40	20	85	3	65.85	195	26
23	40	25	85	4	75.41	275	84
24	40	25	85	3	102.51	254	76
25	40	25	90	3	53.35	202	48
26	60	30	90	4	16.81	159	14
27	40	25	85	3	98.02	249	82
28	40	25	85	2	36.54	208	70
29	40	25	80	3	72.81	228	52
30	60	20	80	2	2.58	147	2

A: Steeping temperature, B: Germination temperature, C: Germination relative humidity, D: Germination time, SL: Sprout length (mm), SWI%: Sprout weight increase % with respect to dry seed weight, G%: Germination%.

between independent variables such as steeping temperature, germination time, relative humidity and germination temperature on the response factors which were sprout length, weight and germination percentage. These graphs represented one constant factor with other variables to represent the interactive effect on the dependent variables so that it can be easy to understand the effect of germination conditions on length.

The RSM design of germination showed a significant variation of sprout length values from 2.16 to 113.24 mm for soybean seeds. Table 3 demonstrates the ANOVA results and indicates that the response surface model for sprout length showed a significantly fitted and validates the dependent factors corresponding to independent factors. The linear effects showed a non-significant effect on the sprout length of soybean. Whereas the quadratic effect of steeping temperature and germination relative humidity was significant and all other factors i.e. steeping temperature x germination temperature, steeping temperature x germination time, germination temperature with relative humidity and germination time in quadratic effect representing a non-significant effect on soybean sprout length as shown in Table 4.

The response surface plot represents the quadratic model for sprout length as indicated in Fig. 1. The steeping temperature had less curvature for sprout length but with the increase in germination temperature sprout length also increased. It was noticed that length was increased till 26°C of steeping temperature but beyond this temperature, sprouts became short in length. Along with this relative humidity and germination time indicated a high curvature effect that represented their maximum effect on sprout length (Fig. 1A).

As relative humidity rose to 88%, the length

Table 3. Analysis of variance for different response models for soybean

Source	Sprout length	Sprout weight (%)	Germination (%)
Model fitted	F - value	F - value	F - value
Model	7.22***	14.08***	22.19***
A	2.44^NS	28.37***	125.79***
B	0.021^NS	11.73**	11.50**
C	0.86^NS	0.15^NS	5.76**
D	3.13^NS	9.05**	2.62^NS
AB	1.03^NS	1.04^NS	18.62***
AC	8.25*	7.79*	11.77**
AD	0.094^NS	10.45**	0.99^NS
BC	0.19^NS	1.69^NS	8.93**
BD	8.382E-003^NS	9.40**	0.60^NS
CD	0.11^NS	0.48^NS	0.60^NS
A2	0.68^NS	9.30**	0.74^NS
B2	4.05^NS	15.51**	53.79***
C2	1.82^NS	1.29^NS	4.70*
D2	4.01^NS	1.49^NS	6.97*
Lack of Fit	0.63^NS	3.45^NS	4.72^NS
R^2	0.8708	0.9293	0.9539
Adjusted R^2	0.7502	0.8633	0.9109
Predicted R^2	0.5789	0.6965	0.7619

*,** and ***Significant at P<0.05, P<0.01 and P<0.001, respectively. NS-Non-significant.
A: Steeping temperature, B: Germination temperature, C: Germination relative humidity and D: Germination time.

Table 4. Predicted equation for different responses for soybean

Regression coefficient	Predicted equation for the responses in coded factors	R^2
Sprout length	86.37 - 6.43 A + 0.59 B - 3.81 C + 7.29 D + 4.42 AB + 12.55 AC + 1.34 AD + 1.90 BC + 0.40 BD + 1.45 CD - 8.93 A2 - 21.86 B2 - 14.64 C2 - 21.75 D2	0.8708
Sprout weight increase	238.64 -22.72 A - 4.61 B - 1.67 C + 12.83 D + 4.63 AB + 12.63 AC - 14.62 AD + 5.88 BC - 13.87 BD + 3.13 CD - 34.28 A2 - 44.28 B2 - 12.78 C2 + 13.72 D2	0.9293
Germination percentage	67.75 -23.89 A - 7.22 B - 5.11 C + 3.44 D + 9.75 AB + 7.75 AC -2.25 AD - 6.75 BC - 1.75 BD -1.75 CD + 4.82 A2 - 41.18 B2 - 12.18 C2 + 14.82 D2	0.9539

A: Steeping temperature, B: Germination temperature, C: Germination relative humidity and D: Germination time.

of sprouts grew maximum (Fig. 1B). Similarly, three days of germination time reported maximum length in sprouts. A similar report on pearl millet sprouts was examined by Dahiya *et al.* (2018) who reported that the length of sprouts was highly influenced by the steeping temperature and germination time. The length of pearl millet sprouts was significantly increased when the steeping temperature rose along with germination time. This might have happened due to some suitable enzymes that promoted the outgrowth of rudimentary shoots and roots of seeds at 45°C of steeping temperature and it might have taken about 2-3 days of germination period.

Islam *et al.* (2017) also reported that germination temperature and time highly affected the growth of Lignosus bean sprouts, who found that the interaction of high temperature (near 30°C) and duration (about 4 days) had a significantly positive effect and found that length of bean sprout increased by 8-fold at these conditions. This study also supported the work in the case of soybean sprouts, where it was noticed from Table 2 that at 40°C steeping temperature and 3-4 days of sprouting led to a maximum length of sprout, in experimental run numbers 2, 3, 24 and 27 where the length of the sprouts was 113.24, 102.41, 102.51 and 98.02 mm.

The percentage weight of the sprout significantly increased by the germination conditions as observed in RSM (Table 2). Various combinations of germination conditions implied different effects on weight.

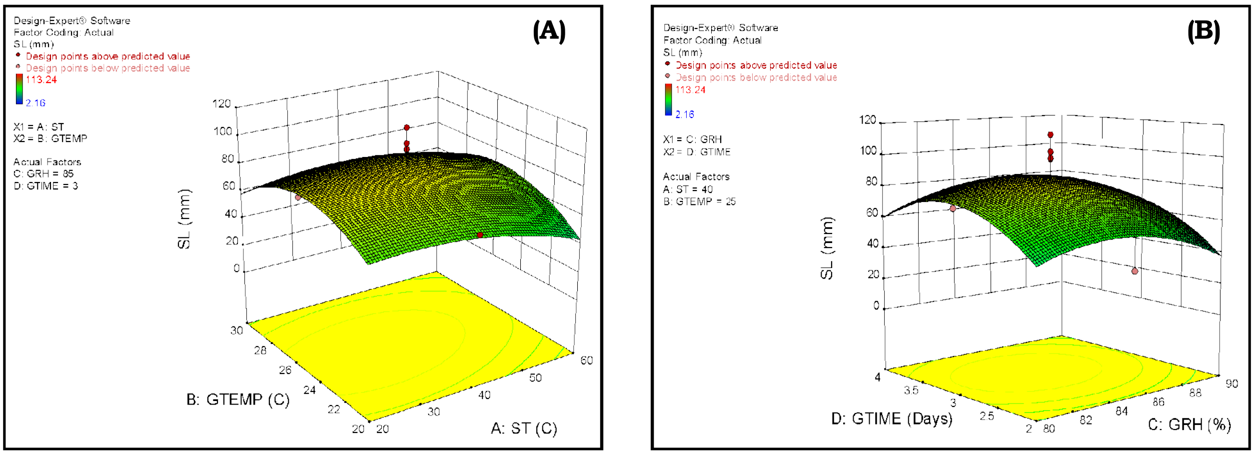

Fig. 1. Response surface plots of sprout length for soybean: (A) Effect of steeping temperature and germination temperature and (B) Effect of relative humidity and germination time.

The range of percentage soybean sprout weight increase was 84 to 275%. The ANOVA in Table 3 indicated that linear factors showed a significantly positive (P<0.01, P<0.001) effect on soybean sprout weight increase except for the relative humidity which individually showed a non-significant effect. The quadratic effect of steeping temperature x relative humidity along with germination time had a significant (P<0.05, P<0.01) effect on sprout weight increase. Germination temperature x germination time also indicated a significant (P<0.01) effect on the sprout weight increment. The response surface plots represented the interaction between germination factors (Fig. 2). It was observed that the interaction of steeping temperature and relative humidity had deep curvature, with an increase in temperature near 80 to 84% relative humidity. The weight consistently increased onward but 40 or 45°C steeping temperature showed decline format meaning weight increase percentage was minimum at 60°C (Fig. 2A). Germination temperature also had a significant effect (near 26°C temperature) sprout weight was found maximum but at lower (20°C) and higher (30°C) germination temperatures sprout weight was minimal. Further, 3.5 to 4 days of germination time indicated maximum weight increase percentage (Fig. 2B).

Adetokunboh *et al.* (2022) demonstrated that a longer time and steeping process of germination led to increase in the water uptakes in legume seed which enhanced the weight of sprouts. In the same way, this study supported the condition that in three days of the germination period 157 to 250% of weight with different combinations of factors but in four days, the weight of the sprout was found to increase up to 238 to 275% at different combinations of germination factors.

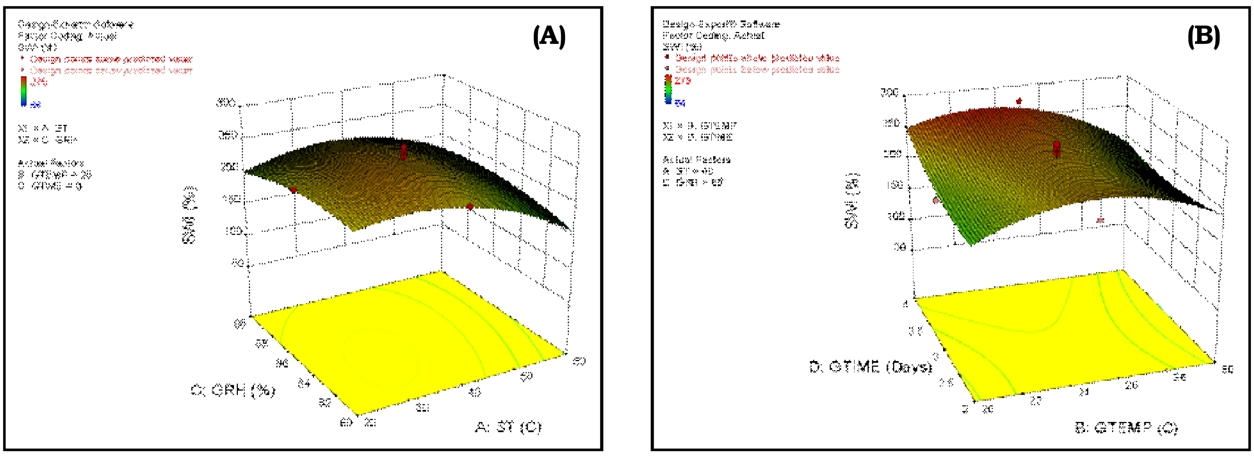

Fig. 2. Response surface plots of sprout weight increase (%) for soybean: (A) Effect of steeping temperature and relative humidity and (B) Effect of germination temperature and germination time.

The range of germination percentage was 2 to 98 at various combinations of germination conditions (Table 2). There was a huge variation in the lower and upper range of germination percentage of soybean sprouts; the experimental run 30 showed minimum germination i.e. 2%, which indicated that at higher steeping temperature (60°C) and lower germination temperature (20°C) and period (2 days) caused minimum growth of sprout percentage. Table 3 also reveals that linear factors such as steeping temperature, germination temperature and relative humidity had highly significant ($P<0.01$, $P<0.001$) effects on the sprout germination percentage. The quadratic factors i.e. steeping temperature × germination temperature, steeping temperature × relative humidity and germination temperature × relative humidity, showed a significant ($P<0.01$, $P<0.001$) effect. The response surface plots for soybean germination percentage, with an increase in steeping temperature (towards 60°C) the germination percentage was gradually decreased, in the case of germination temperature, the maximum value was observed near 24°C, and at towards 30°C it showed progressively decrease (Fig. 3A). The steeping or soaking temperature for soybean at 30°C showed a satisfied germination percentage which might be due to optimal temperature increasing moisture content of soybean enhancing the germination process which significantly influenced the growth-related enzymes. Therefore, steeping temperature and germination temperature were considered a critical factor for the germination process. Furthermore, a higher percentage of germination was obtained at relative humidity near 84% and four days of germination. This optimal condition indicated that near 84 to 88% of humidity was found satisfactory for germination percentage (Fig. 3B). Sofi *et al.* (2020) found that sprouting temperature at 25°C was suitable for desirable sprout length, growth and percentage of sprouted cowpea seeds, but beyond this temperature, there was a mild off flavour and sliminess in sprouts. They also concluded that a prolonged germination period (more than 3 to 4 days) caused dryness in seeds and shrunken sprouts might be due to long exposure to constant temperature which also developed an off flavour, colour and odor in the sprouts. These conditions were found identical in this study of soybean sprouting during a prolonged germination period.

To obtain the optimal level for germination responses for soybean, numerical optimization was carried out. The selection criteria for responses sprout length and sprout weight increase % was set to maximum and germination % was set in range. After applying these constraints, it was found that optimal levels for steeping temperature, germination temperature, germination relative humidity and germination time were 26°C, 24°C, 83% and 3.5 days, respectively. The adequacy of the model for predicting optimum response value was verified by carrying out the analysis of sprouts prepared using the optimum levels of the germination conditions (Table 5). The results showed that the experimental values were close to the predicted values, thus, validating the optimal levels of factors for optimum germination conditions for soybean.

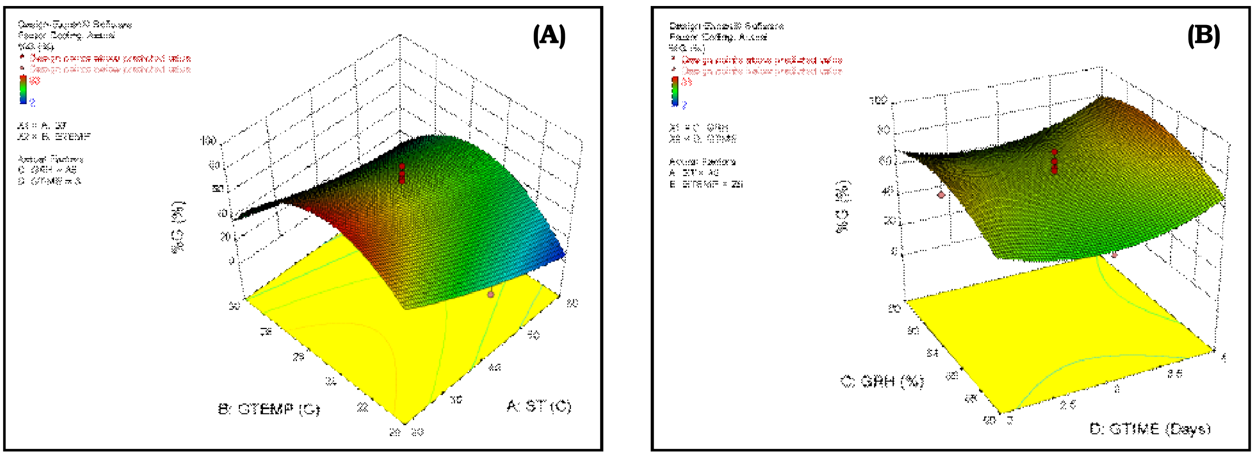

Fig. 3. Response surface plots of germination percentage for soybean: (A) Effect of steeping temperature and germination temperature and (B) Effect of relative humidity and germination time.

Table 5. Predicted and actual response values at optimum levels of germination conditions for soybean

Response	Predicted value	Actual value
Sprout length (mm)	84	89.34
Sprout weight increase (%)	263	227.00
Germination (%)	98	86.00

CONCLUSION

Germination conditions had an important role to play in the quality of final sprouts. The optimal levels for steeping temperature, germination temperature, germination relative humidity and germination time for soybean were found to be 26°C, 24°C, 83% and 3.5 days, respectively. Further, sprouting conditions had a crucial influence on the quality of soybean sprouts as indicated by the wide variation among response values obtained for various combinations of sprouting conditions obtained through RSM. The optimized values were selected to achieve good quality soybean sprouts in terms of sprout length, sprout weight increase and germination percentage of sprouts for better availability of products throughout the year without diminishing the product quality which certainly improves human health through natural means of availability of quality nutrition using for soybean as functional food.

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