

## Research Article

# Influence of Processing Factors on Beany Flavor, Trypsin Inhibitor and Colour of Soymilk

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**Abstract**

Research work were planned by Box-Behnken method with three independent variables such as temperature treatment (-20, 30, 80 °C), soaking time (4, 6, 8 h) and grinding time effects (3, 6, 9 min) of three level of each variable on quality attributes such as TI activation, color and beany flavor was observed the details of raw materials. Beany flavor, trypsin inhibition activity and color difference varied from 2-7.5, 67.3-85.4% and 23.34 – 29.54 respectively. Trypsin inactivation increased with increase in grinding time. Color was compared with cow's milk and reported in terms of difference, which decreased with increase in soaking time whereas with increase in temperature treatment, there was an increase in color difference till a temperature of 40°C beyond which, decreased slightly. The independent variables had significant effect on TIA and color. Optimum level of variables was 2.01, 76.5% and 22.6 for beany flavor, trypsin inhibition activity and color respectively. Optimum conditions for production of soymilk were soaking time (8hours), temperature-(20<sup>0</sup>C) and grinding time (8.614 min).

**Keywords:** soymilk, beany flavour, trypsin inhibition activity, colour

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**Introduction**

Soybean is termed as magic crop due to its high oil (20%) and protein (40%) content. Soybean protein is a high quality protein and it is obtained at a cheaper cost compared to other protein. Number of products has been developed based on soybean like tofu, soymilk, soy flour and soybean oil. Soymilk is the rich creamy liquid extract obtained from soybean and is one of easiest method to add quality protein in daily diet and can also act as replacement for cow's milk. Along with its beneficial health effects, soymilk is also known to contain some anti-nutritional properties and off flavor compounds which must be reduced and kept under control with properly designed processing methods that would also benefit small entrepreneurs. Soybean is normally harvested at the end of rainy season, and at the moisture content of around 25-35% (w.b.). It must be dried to 8-10% (w.b.) for safe storage [1]. Soybean comprises approximately 8% seed coat or hull, 90% cotyledons and 2% hypocotyls axis or germ. 100 gm of soybean comprises of 1866 KJ (446 kcal) energy, 7.33 gm sugar, 9.3gm dietary fiber and zero gm cholesterol. Protein and fat content of soybean account for 56% of dry soybean by weight (36% protein and 20% fat). The remainder consists of 30% carbohydrates, 9% water and 5% ash.

About 85% of the world's soybeans are processed or crushed annually into soybean meal and oil. Approximately 98% of the soybean meal that is crushed is further processed into animal feed with the balance used to make soy flour and proteins. Of the oil fraction, 95% is consumed as edible oil; the rest is used for industrial products such as fatty acids, soaps and biodiesel. In fact soybean meal which is used to feed to cattle is currently largest of source of protein feed in world. In present scenario large number of soy and soy based products like tofu, soymilk, biscuits, flour, yoghurt, ice-cream etc. are being manufactured and are available in market. The use of soy flour to substitute wheat flour in suitable proportion (max up to 25 %) for the production of biscuit is already in trend [2]. The traditional technique of manufacturing soymilk includes soaking the soybeans, followed by wet grinding, filtering and cooking. While soymilk is highly nutritional sources and its acceptance throughout the world is still very low due to deleterious result of antinutritional components such as soybean trypsin inhibitors, haemagglutinins, saponins and antivitamin and undesirable beany flavor related with it. Soymilk was produced by traditional technique, does not have the bland flavour or smooth texture for example cow's milk, beany flavour and odour, although acceptable to some, population especially in Asia, it is quite objectionable to others [7]. The off-flavour and odour characterized as 'beany' or 'painty' are due to volatile compounds formed by lipoxygenase catalysed reactions which develop almost

instantaneously upon wet grinding [12]. It is necessary to remove these off-flavouring constituents developed during soymilk processing. Heat treatment of soymilk is necessary for enhancing flavor and nutritional value by denaturing trypsin inhibitors, haemagglutinins, saponins and other antinutritional compounds [6] and [7].

Soy protein has been used and accepted as food ingredients to enhance the value of finished foods. Soybean and soy-foods have been identified with their protein content from a nutritional perspective and as such there is much interest among clinicians and researchers on their potential role in preventing and treating chronic diseases. One of the products of soybean is soymilk. Soymilk is a water extract of soybeans which is known to have significant health-promoting components and are an important source of inexpensive protein and calories for human consumption. It is an excellent dietary supplement with high quality proteins while containing no cholesterol, gluten, or lactose. Along with its beneficial health effects, soymilk is also known to contain beany flavor and trypsin inhibitor activity (TIA), which can have ill health effects if present at a high level. Thermal processing can inactivate TIA, reduce undesirable flavor and odor and helps in improvement of organoleptic acceptability and nutritive value of soymilk. Thus, keeping in view the above facts, this work was taken to study the effect of process parameters on the beany flavor, trypsin inhibitor and colour of soya milk.

## Material and Methods

### *Experimental Procedure for making soymilk*

Dry soybean seeds procured from market were prepared by removing dust, dirt, foreign matters, and other unwanted materials. Soaking loosens the tissues and facilitates in grinding of soybean. Soaking was done in tap water at room temperature (30-35 °C) taking 50g of seed in 150 ml of water for 4, 6, 8 h. Proportion of water to soybean was kept three times. At the end of experiment, water was drained. Properly drained soybean seeds were given temperature treatment at three different temperatures of -20 °C, 30 °C and 80 °C to improve quality and yield of soymilk. Three samples of soaked seeds were taken. One was kept in deep freezer at -20 °C for 5 h, second one at ambient temperature (30°C) while third sample was kept in warm water at 80 °C for 7 min. Temperature plays an important role in processing of soymilk which affects its texture, palatability as well as nutritional and sensory quality characteristics [8] showed that using frozen soybean affected the structure and coagulation of soymilk and tofu prepared from it. The rehydrated beans were drained with enough water to give the desired solids content to the final product. The ratio of water to soybean on a weight basis was kept as 9:1 [14] i.e 450 ml of cold water was added to soaked soybean seeds then grinding was done in grinder (model). Grinding time was taken as variable and was carried for three different timings of 3, 6 & 9 min The resulting slurry obtained after grinding was cooked at 94°C in order to improve its inactivating soybean trypsin inhibitor and other are these harmful biologically active components (nutritional value, flavor and to reduce beany flavor). Heating at 94°C was continued for a period of 15 min, followed by the removal of an insoluble residue (soy pulp fiber or okara) by filtration using muslin cloth. The effect of cooking temperature on quality characteristics of soyamilk and found 100 °C as best processing condition in terms of physiological, microbiological and sensory attributes [5]. Excessive heating results in the destruction or inactivation of some essential amino acids thus adversely affecting the nutritional value of soy protein [14]. Also the digestibility of soy protein by proteolytic enzymes therefore, becomes considerably reduced when the protein has been subjected to excessive heat treatment. Cooking soy milk at 110°C for 30 min could cause some damage to the soy protein [11]. Thus, cooking temperature and time were decided giving importance to nutritional value of milk.

### *Storage of soymilk*

The soymilk obtained was packed in low density poly ethylene pouch, after cooling it to room temperature and stored under refrigerated condition (4°C) to avoid any deterioration in quality.

### *Analysis of soymilk*

Analysis was done to determine the effect of independent variables on soymilk obtained in quality characteristics. The procedure adopted to carry out the analysis of each soymilk samples has been discussed in subsections.

### *Trypsin inhibitor*

The sample extract was prepared based on procedures of Hamerstrand *et al.*, 1981 with some modifications [4]. Freeze-dried soy milk (0.5 g) was extracted with 50 mL of 0.01 N NaOH for 5 h, while being shaken constantly at room temperature. The suspension was then diluted to 100 mL with distilled water and allowed to stand for 2 h at

4°C. The supernatant was collected and diluted, so that 2 mL of the extract could produce a trypsin inhibition rate between 40% and 60%. The TIA assay was used with some modifications [4]. Briefly, to two test tubes for the sample and sample blank, 2 mL of diluted sample was added, while 2 mL of distilled water was added to another two tubes for the standard and standard blank. Trypsin standard solution (2 mL) was then added to sample tubes and standard tubes, and then all the tubes were vortexed and incubated in a water bath at 37 °C for 10 min. Then, 5 mL of BAPA solution preheated to 37 °C was added rapidly to each tube; the tubes were vortexed and incubated in a water bath at 37 °C for another 10 min before mixing with 1 mL of 30% acetic acid. After 2 mL of trypsin standard was added to tubes for standard and sample blanks, the mixtures in tubes were centrifuged at 6000 rpm for 10 min, and the supernatants were subjected to vacuum filtration. The absorbances of the standard and sample filtrates were measured at 410 nm against the respective blank. The TIA was expressed as trypsin inhibitor units (TIU) per gram of freeze-dried soy milk (TIU/g), as well as milligrams of trypsin inhibitor equivalent (mg TI/g) by dividing the TIU by 1900.

### Colour

High resolution images of the soymilk samples were captured using an 18.2 MP digital camera at 3.6x zoom. A standard illumination system consisting of two horizontally mounted tubes of 40W power rating was used to provide uniform light intensity over the soymilk sample. The angle between the camera lens axis and light source axis was kept at 45° and the camera was placed vertically 60 cm over the sample. A black background was used for the white coloured samples to provide maximum contrast enabling better colour recognition.

Adobe Photoshop CS 5 software v.12.0 was used to determine L, a, b values. The mean and standard deviation values were determined from the histogram obtained from the software. L, a, b values are not standard colour values and need to be converted to L\*, a\* and b\*. The values for L\*, a\*, b\* were evaluated as per the formulae (Eqn 1-3) given by Yam and Papadakis, 2004 [13].

$$L^* = \frac{L}{255} \times 100 \quad (1)$$

$$a^* = \frac{240a}{255} - 120 \quad (2)$$

$$b^* = \frac{240b}{255} - 120 \quad (3)$$

The color difference ( $\Delta E$ ) between the fresh (subscript 2) and freeze dried sample (subscript 1) was calculated using the formula prescribed by the *International Commission on Illumination* (CIE) as given by Eqn. 4A  $\Delta E^*$  value around 2.3 indicates noticeable difference [10].

$$\Delta E^* = \sqrt{(L_2^* - L_1^*)^2 + (a_2^* - a_1^*)^2 + (b_2^* - b_1^*)^2} \quad (4)$$

### Beany flavour

The undesirable flavor of soybeans is described generally as beany, bitter, grassy, and astringent and is attributed partially to degradation of lipids. Cooking significantly reduces the beany odor compositions [14]. Sensory evaluation may be seen as the scientific discipline which looks at how measurements evoked and interpret the characteristics of food and materials as permitted by the senses. Sensory evaluation was used to identify the beany flavor of the soymilk. A 15 member sensory panel rated for different quality attributes on hedonic rating test scale as given in **Table 1** the panel member were requested to evaluate the milk sample in terms of taste, flavor and overall acceptability on a 9 point hedonic scale ranging from 1= dislike very much to 9= like extremely.

### Statistical Analysis

The model development was done using response surface methodology through use of Design expert V 9.6.0.2. Multiple regression analysis was used to analyze the experimental data in order to develop response functions and obtain variable parameters optimized corresponding to best outputs. Using a statistical approach called the method of least square (MLS) which is a multiple regression technique used to fit a mathematical model to a set of

experimental data generating the lowest residual possible. The results of regression analysis are obtained in terms of ANOVA, regression coefficient and associated statistics, standard deviation, coefficient of determination ( $R^2$ ), Lack of fit, etc. These determine adequacy of predictive model and effect of independent parameters on the responses. The measured responses were optimized using response surface methodology (RSM).

**Table 1** Rating test of soymilk at 9- Hedonic scale

Degree of preference	Score
Like extremely	9
Like very much	8
Like much	7
Like moderately	6
Neither like nor dislike	5
Slightly dislike	4
Dislike moderately	3
Dislike much	2
Dislike very much	1

## Results and Discussion

### Beany Flavour of Soymilk

Flavour was determined by providing 50 ml soymilk samples to a panel of 21 people. The rating was done on a 9 point hedonic scale. The average values for each sample were noted. The values recorded were deducted from 9.0 to obtain the results as acceptable taste of soymilk. The maximum point obtained for beany flavour was 7.0 with the combination of temperature treatment ( $-20^{\circ}\text{C}$ ), soaking time (4 h) and grinding time (6 min) for experiment no. 1 due to decrease in temperature and increase in grinding time. Minimum value of 1.5 obtained at parameters temperature treatment ( $30^{\circ}\text{C}$ ), soaking time (6 h) and grinding time (6 min) for experiment no. 16. Higher temperatures gave lower beany off-flavour [14]. The results obtained for higher temperatures in the current work gave acceptable flavour; however, lower temperatures gave the best flavour which was in contradiction to that proposed in literature. Analysis of variance (ANOVA) was conducted to check the adequacy of the developed model for beany flavour; the results were presented in **Table 2** which shows the effect of parameters at linear, interactive and quadratic level. The effect of variables on beany flavour of soymilk, at quadratic level was found significant ( $p < 0.01$ ) whereas linear and interactive terms showed no significant effect. It was observed that temperature treatment affected the beany flavour at 1% level of significance. Soaking and grinding time showed no significant effect.

**Table 2** Analysis of variance of beany flavour for soymilk

Source	Df	SS	MS	F-value	F-tab
Model	9	39.22	4.36	5.07**	3.67
Linear	3	4.06	1.35	1.57	
Quadratic	3	33.13	11.04	12.84***	8.45
Interactive	3	1.06	0.35	0.41	
Residual error	7	6.01	0.86		

\*\*\* 1%, \*\*5%, level of significance

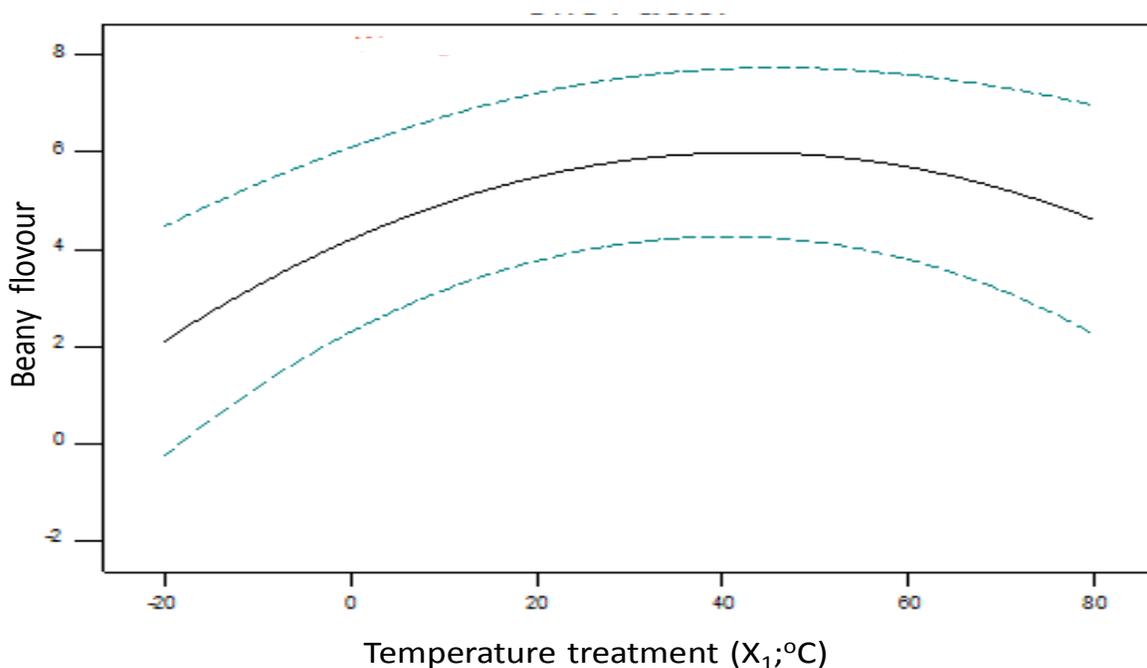
Experimental data fitted into second order mathematical model (Eqn. 5) for beany flavour. The model was checked using a numerical method implying the coefficient of determination ( $R^2$ ) and then calculated and shown in Eqn. 5. The coefficient of determination ( $R^2$ ) for the regression model for beany flavour of soymilk was 0.867 which implies that the model could account for 86.70% similarity in data. The second order predictive equation was used to examine the effect of variables. The model was found to be highly significant ( $p < 0.05$ ). Therefore, it was found that the second order model adequately describes the effect of process parameters for beany flavour for the production of better soymilk. The predictive regression equation is given below

$$\text{Beany flavour (Y)} = 6.60 + 0.69X_1 + 0.19X_2 + 0.13X_1X_2 + 0.50X_1X_3 - 2.49X_1^2 - 1.24X_2^2 + 0.39X_3^2 \quad (5)$$

Where, Y = beany flavour,  $X_1$ ,  $X_2$  and  $X_3$  are coded values of temperature treatment and soaking time respectively. The positive sign in front of the terms indicates a synergistic effect while the negative sign shows the antagonistic effect.

The regression coefficients in the model of beany flavour for production of soymilk are in linear, quadratic and interactive terms. It was inferred from regression analysis that the independent variables affected beany flavour, at a quadratic level. At quadratic level, temperature treatment had significant ( $p < 0.1$ ) negative effect on the beany flavour of soymilk. Negative coefficient suggests that the quadratic value of one parameter can be increased while other decreased for constant value of response.

At linear level, **Figure 1** represents the effect of temperature treatment on beany flavour at optimum value of soaking time (8h) and grinding time (8.6 min). It was concluded that the beany flavour slightly increases with increasing of temperature treatment for instant from (2,  $-20^{\circ}\text{C}$ ) to (6,  $40^{\circ}\text{C}$ ) and after that beany flavour slightly decreases with increase of treatment temperature from (6,  $40^{\circ}\text{C}$ ) to (4,  $80^{\circ}\text{C}$ ).



**Figure 1** Effect of temperature treatment on beany flavour at optimum value of soaking time (8h) and grinding time (8.6 min)

**Table 3** Effect on beany flavour of soymilk

Source	Df	SS	MS	F-value	F-tab
Model	39.22	9	4.36	5.07**	3.67
Temperature treatment ( $X_1$ )	30.89	4	7.72	8.98***	7.84
Soaking time ( $X_2$ )	6.79	4	1.69	1.97	
Grinding time ( $X_3$ )	1.63	4	0.41	0.47	
Residual error	6.01	7	0.86		

\*\*\* 1%, \*\*5%, level of significance

### **Trypsin Inhibitor (TI) Inactivation of Soymilk**

The TI inactivation was obtained in percentage using standard chemical procedure. The inactivation values recorded a maximum value of 85.4% with the combination of temperature treatment ( $80^{\circ}\text{C}$ ), soaking time (6 h) and grinding time (9 min) for experiment no. 8 due to increase in temperature and grinding time. Minimum value of 67.3% was obtained with the combination of parameters as temperature treatment ( $-20^{\circ}\text{C}$ ), soaking time (4 h) and grinding time (6 min) for experiment no. 1 due to decrease in temperature and grinding time. Similar results were obtained by Ellenrieder *et al.*, 1980 on treating soy beans at a high temperature of  $96^{\circ}\text{C}$  [3]. The maximum value obtained by them was slightly higher (86.4%). Even though, the temperature treatment difference is higher, the results obtained in this work may be closer to that cited in literature due to partial inactivation of trypsin inhibitors during boiling of soymilk. Analysis of variance (ANOVA) results were presented in **Table 4** which shows the effect of parameters at linear, interactive and quadratic level. The effect of variables on TI Inactivation for soymilk, at linear and quadratic levels were found significant ( $p < 0.01$ ) while no significant effect was observed at interactive level.

Total effect of individual parameter on TI inactivation for the production of soymilk was calculated (**Table 5**). It was observed that temperature treatment affected TI inactivation at 5% level of significance) while grinding time highly affected TI inactivation at 1 % level of significance.

Experimental data of TI inactivation were fitted into a second order mathematical model (Eqn. 6) for soymilk. The model was checked using a numerical method implying the coefficient of determination ( $R^2$ ) and then calculated as shown in Eqn. 6. The coefficient of determination ( $R^2$ ) for the regression model of TI inactivation for soymilk was 0.907 which implies that the model could account for 90.70% similarity in data.

**Table 4** Analysis of variance for TI inactivation in soymilk

Source	Df	SS	MS	F-value	F-tab
Model	9	518.41	57.60	7.64***	6.718
Linear	3	269.24	89.75	11.90***	8.45
Quadratic	3	248.59	82.86	10.99***	8.45
Interactive	3	2.04	0.68	0.09	
Residual error	7	52.78	7.54		

\*\*\* 1%, \*\*5%, level of significance

**Table 5** Effect of individual parameters on TI inactivation of soymilk

Source	Df	SS	MS	F-value	F-tab
Model	9	518.41	57.60	7.64***	6.718
Temperature treatment ( $X_1$ )	4	177.77	44.44	5.89**	4.12
Soaking time ( $X_2$ )	4	83.83	20.96	2.78	
Grinding time ( $X_3$ )	4	260.32	65.08	8.63***	7.84
Residual error	7	52.78	7.54		

\*\*\* 1%, \*\*5%, level of significance

The second order predictive equation was used to examine the effect of variables. The model was found to be highly significant ( $p < 0.01$ ). Therefore, it was found that the second order model adequately describes the effect of process parameters on TI inactivation for making soymilk. The predictive regression equation is given below

$$\text{TI inactivation (Y)} = 80.30 + 1.07X_1 + 2.10X_2 + 5.30X_3 + 0.10X_1X_2 - 0.10X_1X_3 - 0.70X_2X_3 - 6.32X_1^2 - 3.32X_2^2 + 2.82X_3^2 \quad (6)$$

Where, Y = TI inactivation (%),  $X_1$ ,  $X_2$ , and  $X_3$  are coded values of temperature treatment, soaking time, and grinding time respectively. In Eqn 6 the coefficient with one factor i.e. the one in front of  $X_1$ ,  $X_2$  and  $X_3$  represent the effect of that particular factor, while those with second order terms i.e.  $X_1^2$ ,  $X_2^2$  and  $X_3^2$  represent the quadratic effect. The positive sign in front of the terms indicates a synergistic effect while the negative sign shows the antagonistic effect.

The regression coefficients in the model of TI inactivation for making soymilk are in terms of linear, quadratic and interactive. It was inferred from regression analysis that independent variables affected TI inactivation, both at linear and quadratic level. There was no significant effect of variables at interactive level. At linear level, grinding time had highly significant effect at 1% level while no effect was found, but effect of temperature was at 5% level of significance. Negative coefficients of quadratic terms showed the maximum response at the centre value of the parameter and it decreased with increase or decrease in parameter level from centre point.

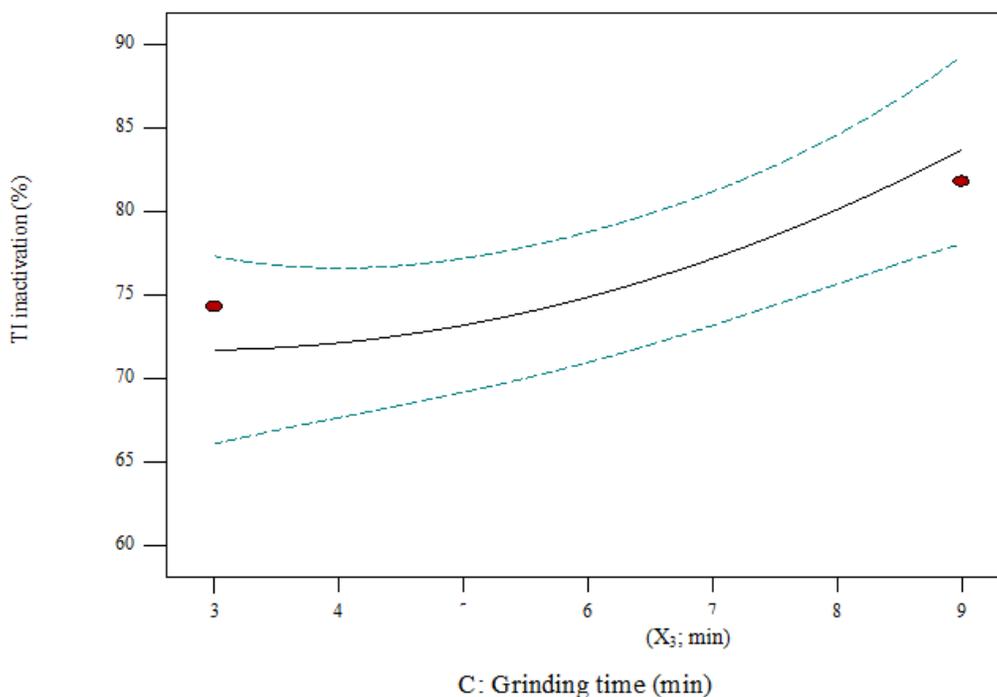
At linear level, **Figure 2** shows effect of grinding time on soymilk TI inactivation at optimum value of temperature treatment ( $-20^\circ\text{C}$ ) and soaking time (8 min). It was concluded that the TI inactivation was increasing continuously with increasing of grinding time from (70%, 3min) to (79%, 9min).

At linear level, **Figure 3** shows effect of soaking time on soymilk TI inactivation at optimum value of temperature treatment ( $-20^\circ\text{C}$ ) and soaking time (8 min). It was concluded that the TI inactivation was slightly increasing with increase of soaking time from (4h, 75%) to (6.5h, 80%) and after that TI inactivation slightly decreased with increasing of soaking time from (6.5h, 80%) to (8h, 77.5%).

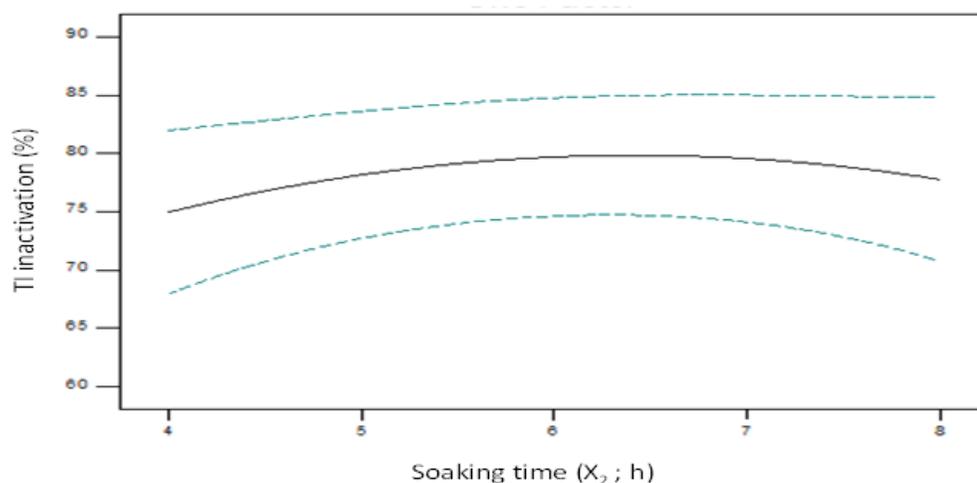
### Colour of Soymilk

Colour was measured for the soymilk samples by analysis of digital images in Adobe Photoshop CS5 software. The images were taken by a 18.2 MP camera at 3.2x zoom under uniform lighting conditions. The digital images used for colour analysis are shown in **Figure 4**. The colour difference ( $\Delta E$ ) was calculated with cow milk as reference. The

maximum value of  $\Delta E$  was obtained as 29.54 with combination of parameters as temperature treatment ( $-20^{\circ}\text{C}$ ), soaking time (6 h) and grinding time (9 min) for experiment no. 7 and a minimum  $\Delta E$  was obtained as 23.34 with combination of parameters as temperature treatment ( $80^{\circ}\text{C}$ ), soaking time (6 h) and grinding time (3 min) for experiment no. 6. The results obtained were in contradiction to that obtained by Oloye, 2014 [9]. The colour difference may be due to different varieties of soy bean.

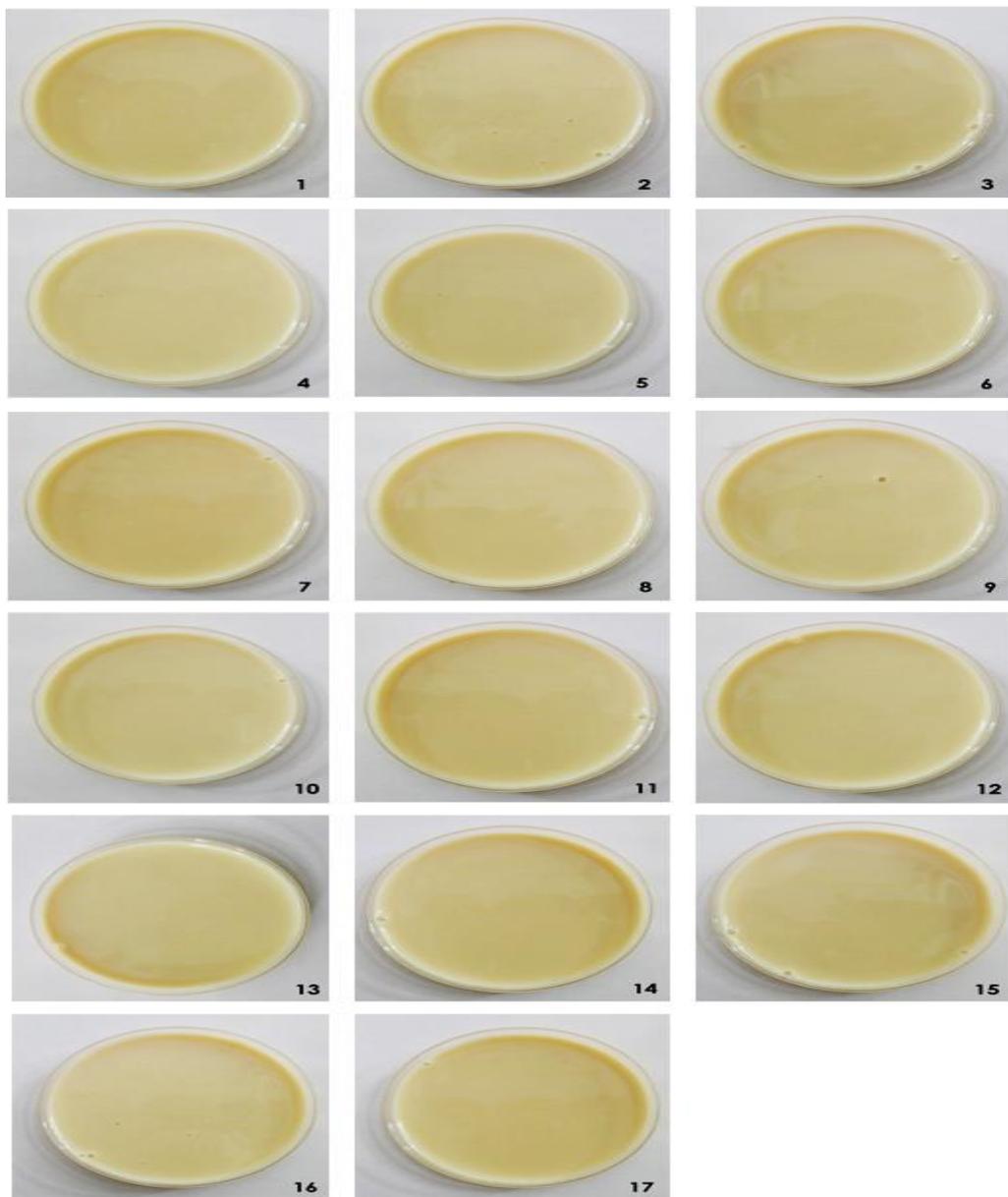


**Figure 2** Effect of grinding time on soymilk TI inactivation at optimum value of temperature treatment ( $-20^{\circ}\text{C}$ ) and soaking time (8 min)



**Figure 3** Effect of soaking time on soymilk TI inactivation at optimum value of temperature treatment ( $-20^{\circ}\text{C}$ ) and soaking time (8 min)

Analysis of variance (ANOVA) was conducted to check the adequacy of the developed model of colour for making soymilk; results were presented in **Table 6** which shows the effect of parameters at linear, interactive and quadratic level. The effect of variables on colour for soymilk, at linear, quadratic and interactive levels were found significant ( $p < 0.05$ ). Total effect of individual parameter on colour for the production of soymilk was calculated (**Table 7**). It was observed that temperature treatment highly affected the colour at 1% level of significance. Soaking time affected the colour at 5% level of significance. Grinding time showed no significant effects. Experimental data fitted into a second order mathematical model (Eqn. 7). The coefficient of determination ( $R^2$ ) for the regression model of colour for soymilk was 0.8917 which implies that the model could account for 89.17% variability in data. The  $F_{\text{cal}}$  6.42 was found to be greater than  $F_{\text{tab}}$  3.67, suggesting model was significant at 5% level of significance.



**Figure 4** soymilk samples for colour analysis

**Table 6** Analysis of variance for colour of soymilk

Source	Df	SS	MS	F-value	F-tab
Model	9	41.63	4.62	6.42**	3.67
Linear	3	9.53	3.17	4.41**	4.34
Quadratic	3	14.38	4.79	6.66**	4.34
Interactive	3	16.59	5.53	7.68**	4.34
Residual error	7	5.06	0.73		

\*\*\* 1%, \*\*5%, level of significance

**Table 7** Effect of individual parameters on beany flavour of soymilk

Source	Df	SS	MS	F-value	F-tab
Model	9	41.62	4.62	6.42**	3.67
Temperature treatment ( $X_1$ )	4	28.35	7.09	9.84***	7.84
Soaking time ( $X_2$ )	4	22.16	5.54	7.69**	4.12
Grinding time ( $X_3$ )	4	6.58	1.65	2.29	
Residual error	7	5.06	0.72		

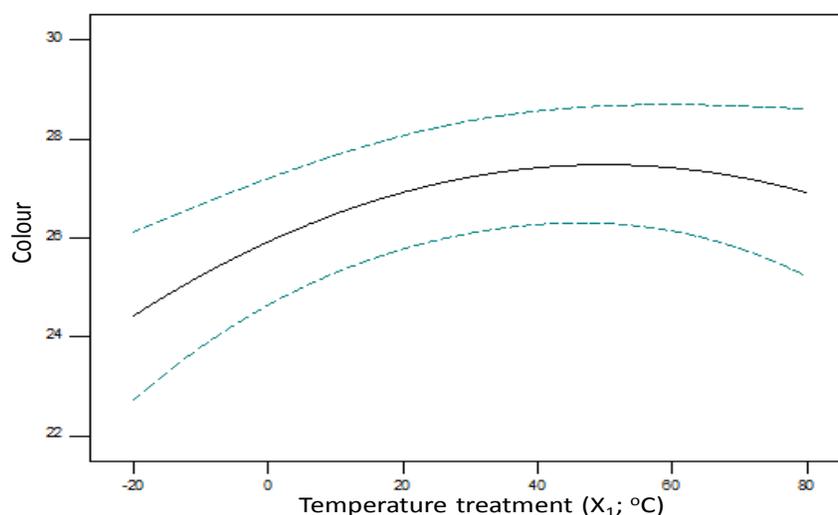
\*\*\* 1%, \*\*5%, level of significance

The second order predictive equation was used to examine the effect of variables. The model was found to be significant ( $p < 0.05$ ). Lack of fit was non-significant. Therefore, it was found that the second order model adequately describes the effect of process parameters on the colour of soymilk. The predictive equation is given below

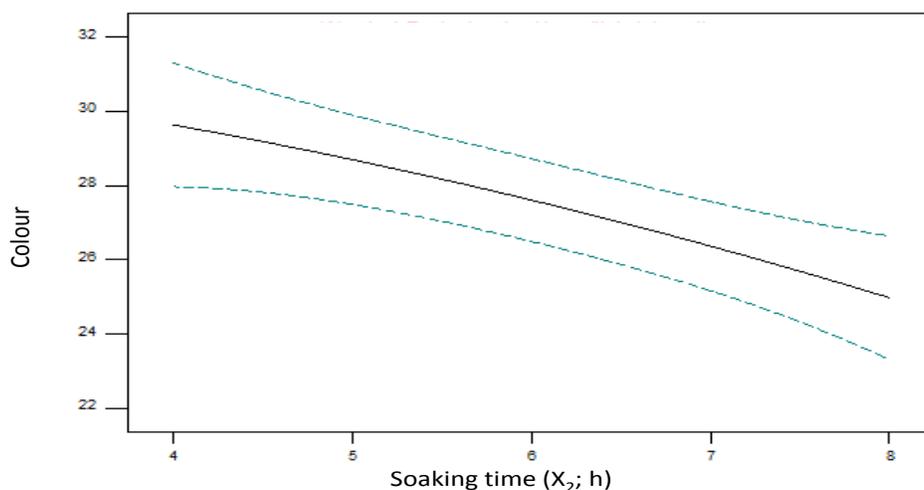
$$\text{Colour (Y)} = 28.89 + 0.66X_1 - 0.86X_2 - 0.12X_3 + 1.86X_1X_2 - 0.43X_1X_3 - 0.71X_2X_3 - 1.56X_1^2 - 0.30X_2^2 - 0.94X_3^2 \quad (7)$$

Where,  $Y$  = colour,  $X_1$ ,  $X_2$  and  $X_3$  are coded values of temperature treatment, soaking time and grinding time respectively.

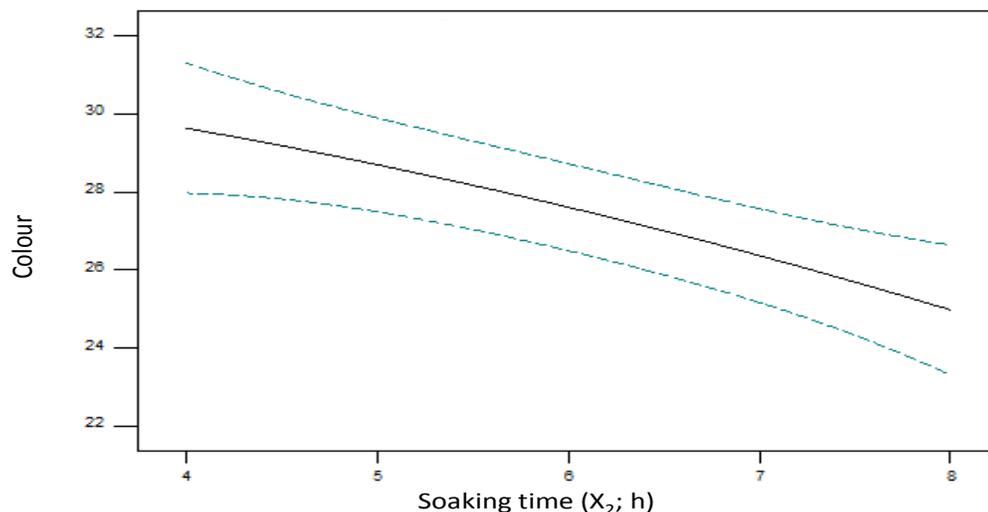
At linear level, soaking time ( $p < 0.05$ ) had a significant effect while temperature treatment had highly significant effect on colour of soymilk but no effect of grinding time observed. At quadratic level, temperature treatment had significant ( $p < 0.01$ ) negative effect. Negative coefficient suggests that the quadratic value of one parameter can be increased while other decreased for constant value of response. At linear level, **Figure 5** shows the effect of temperature treatment on soymilk colour at optimum value of grinding time (8.6min) and soaking time (8 min). It was concluded that the colour was increased with increasing of treatment temperature from (-20°C, 24.3) to (50°C, 27.8) and after that colour slightly decreased with increasing of treatment temperature from (50°C, 27.8) to (80°C, 26.9). At linear level, **Figure 6** shows effect of soaking time on soymilk colour at optimum value of temperature treatment (-20°C) and grinding time (8.6min). It was concluded that the colour was gradually decreasing with increasing of soaking time from (4h, 29.9) to (8h, 24.9). At interactive level, **Figure 7** shows effect of temperature treatment and soaking time on colour of soymilk at optimum grinding time (8.6min). It was observed that colour was increasing with decreasing soaking time from 6h to 4h while also increasing with increasing temperature treatment from -20°C to 59°C after that no effect on colour was observed.



**Figure 5** Effect of temperature treatment on soymilk colour at optimum value of grinding time (8.6min) and soaking time (8 min)



**Figure 6** Effect of soaking time on soymilk colour at optimum value of temperature treatment (-20°C)



**Figure 7** Effect of temperature treatment and soaking time on soymilk colour at optimum grinding time (8.6min)

## Conclusion

Beany flavour score was maximum at treated temperature of 30°C, soaking time 6 h and grinding time 6 min whereas minimum value at temperature of -20°C, soaking time 4 h and grinding time 6 min. Trypsin inhibition activity showed higher value of inactivation and maximum inactivation value was obtained under the treatment temperature of 80°C, soaking time 6 h and grinding time 9 min but minimum value under operating conditions of treatment temperature -20°C, soaking time 4 h and grinding time 6 min. It was observed that Trypsin inactivation increased with increase in grinding time. The highest color difference ( $\Delta E$ ) was obtained at treatment temperature of -20°C, soaking time of 6 h and grinding time of 9 min whereas minimum value was observed at treatment temperature of 80°C, soaking time of 6 h and grinding time of 3 min. It was observed that colour difference decreased with increase in soaking time whereas with increase in temperature treatment, there is an increase in color difference till a temperature of 40°C beyond which it decreases slightly.

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