Application of response surface methodology in process optimization of Soytofu and their sensory evaluation

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Abstract: The objective of this investigation was to optimize the levels of four salt and three acid coagulants and coagulating temperature of soy milk so as to prepare quality soytofu by employing Response Surface Methodology (RSM) and to select an ideal one among the seven coagulants. To optimize the level of coagulant and temperature, Three Level Factorial Design was used. This included three levels of salt (0.2, 0.4 and 0.6%, w/v on soy milk basis) and acid (0.15, 0.25 and 0.35%, w/v on soy milk basis) coagulants with three levels of coagulating temperature (75, 82.5 and 90°C). Thus 13 RSM experiments including five central and eight axial points were conducted to produce soytofu samples with each of the coagulant. Soytofu samples were evaluated organoleptically on nine point hedonic rating scale. Results revealed that the optimum concentration of coagulants (% w/v, on soymilk basis) and coagulating temperature (°C) of soymilk for making quality soytofu was 0.6, 86.55, 0.57, 84.9, 0.6, 87.23, 0.46, 86.18, 0.35, 89.33, 0.3, 80.25 and 0.15%, 87.45°C, respectively, for CaCl₂, CaSO₄, MgCl₂, MgSO₄, lactic acid, acetic acid and citric acid coagulants. Preparation of soytofu sample from RSM optimized level and their organoleptic evaluation indicated that among the seven coagulants calcium chloride was best for making quality soytofu and it contained 68.59, 50.3, 12,

5.9 and 15.35% moisture, protein, crude fat, ash and carbohydrates, respectively.

Keywords: Coagulants, Optimization, Soybean, Soytofu, Temperature

Introduction

Tofu is usually considered as salt or acid coagulated water based gel, with soy lipids and proteins trapped in the gel network (Kohyama et al. 1995). It is an inexpensive, nutritious and versatile meat or cheese substitute with bland taste and porous texture. On the moisture free basis, tofu contains about 50% protein and 27% oil, and the remaining constituents are carbohydrates and minerals (Tripathi and Mishra, 2005). Yield and quality of tofu are important parameters which should be kept in mind during the production of tofu. It is influenced by environmental as well as processing factors such as cultivar (Sun and Breene, 1991, Tanwar et. 1998, Krishna et al. 2002), soaking time (Choi and Kim 1983), type of coagulant (Sutar et al. 2009, Shokunbi et al. 2011), concentration of coagulant (Sun and Breene, 1991), temperature of coagulant (Shih et al. 1997, Agrahar-Murugkar 2015), pressure applied (Gandhi and Bourne 1988) and processing methods (Agrahar-Murugkar 2015).

Till now many coagulants have been used in soytofu preparation like calcium chloride, calcium sulphate, magnesium chloride, lemon juice, Epsom salt and top water of fermented maize (Sun and Breene 1991, Obtalu 2008, Sutar et al. 2009, Shokunbi et al. 2011). There is dearth of information regarding usage of acid coagulants (citric acid, acetic acid and lactic acid). So, in present investigation response surface methodology (RSM) was applied to optimize the level of coagulants and coagulating temperature of seven different alt and acid coagulants. As, RSM is a valuable statistical tool that has been widely used in process optimization as well as in recipe formulation and processing conditions (Sharma et al. 2005, Deshpande et al. 2008, Karuppayya et al. 2009 and Granato et al. 2010).

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Materials and Methods

The present investigation was carried out in the Department of Food Science and Technology, G.B. Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar (Uttarakhand). Mature variety of soybean PS-1347 was procured from Crop Research Centre of G.B. Pant University of Agriculture and Technology, Pantnagar. Soybean seeds were cleaned manually and stored in a cool and dry place until required. Seven coagulants (calcium chloride, calcium sulphate, magnesium chloride, magnesium sulphate, lactic acid, acetic acid and citric acid), and other chemicals employed in the present study were of AR grade. The physiochemical properties of soybean seeds were also analyzed.

Physico-chemical analysis of soybean

Soybean used in research was test for various physical parameters such as thousand grain weight, hull content, hardness, bulk density, true density and porosity as per the following formula:

$$\text{Bulk density (g/cc)} = \frac{\text{Weight of soybean in gram}}{\text{Volume of soybean in cubic centimeter}}$$

$$\text{True density (g/cc)} = \frac{\text{Weight of grain in gram}}{\text{Volume of toluene displaced in cubic centimeter}}$$

$$\text{Per cent porosity} = \frac{\text{True density} - \text{Bulk density}}{\text{True density}} \times 100$$

Hardness of 10 randomly taken seeds of soybean was determined with a Hardness Tester (Kiya Seisakusho Ltd., Japan). Hardness represented the force required to break the seed vertically and horizontally and average value was expressed in kg. While moisture, fat, protein, total ash, calcium, phosphorus and iron was estimated by Ranganna (1996).

Design of experiment

The investigation comprised of two experiments. In the first experiment, seven coagulants were employed individually for preparation of soytofu. Soymilk was coagulated at three different concentration of each coagulant. Thirteen soytofu samples were prepared with each coagulant by using RSM and level of each coagulant and coagulating temperature were optimized so as to produce soytofu having maximum yield and ideal organoleptic characteristics. In the second experiment, seven soytofu samples were prepared as per the RSM optimized levels for each of the coagulant and corresponding coagulating temperature. These soytofu samples were subjected to organoleptic evaluation so as to find out the best coagulant among the seven and proximate

composition of the best soytofu sample was also determined. The experiments were planned using RSM with Three Level Factorial Design. A full second order model was fitted into each response. The adequacy of the model was tested using coefficient of determination (R^2) and Fisher's F-test.

Preparation protocol

Whole soybeans were immersed in water (14-16 hours) and were washed thoroughly by rubbing them manually between the palms, the hulls were removed completely. Then dehulled soybeans were then ground at a Mixer-Grinder (Philips, HL 1632) in hot water (95°C) for 3 min. During extraction the ratio (bean to water) was 1:8 (w/v). The resultant suspension was passed through a double-layered fabric of muslin. The soymilk thus obtained was boiled to inactivate trypsin inhibitor and other anti-nutritional factors for five minutes (Sutar et al. 2009).

The volume of coagulant needed as per the RSM was dissolved in 20 ml of distilled water and transferred to a 1 liter size stainless steel tank. Freshly prepared 500 ml of soymilk was brought to the coagulating temperature required and then gently poured into the vessel containing coagulant for soytofu preparation. Then for about 30 minutes the contents were left undisturbed and allowed to cool to room temperature. The content was passed through a double layered cheese cloth to separate curd and whey. The curd was then transferred into a wooden box lined with a double layered cheese cloth. Cloth corners were folded over the contents and a constant pressure of 28 g/cm² was exerted for 15 minutes on the contents. The box had holes to drain off the whey at its sides and the edges. The prepared soytofu was then removed from the wooden box and weighed and stored in chilled water at refrigeration temperature until organoleptic analysis. Freshly prepared samples of soytofu were tested organoleptically within 24 hours (Table 1).

Screening of ideal coagulant

For the screening of best coagulants, seven soytofu samples were prepared from each coagulant separately as per the RSM optimized coagulant concentration and corresponding coagulating temperature. An ideal coagulant was selected on the basis of yield and sensory parameters. A soytofu sample was prepared as per the ideal selected coagulant and it was analyzed for proximate composition (moisture, crude protein, crude fat, ash and carbohydrate) as per AOAC methods.

Organoleptic evaluation

The acceptability of soytofu samples prepared from soybean variety PS 1347 was determined organoleptically. The taste panel was comprised of 10 members drawn from staff and PG-students of Department of Food Science and Technology. The panelists evaluated soytofu samples on 9-point hedonic scale for sensory

attributes (appearance, colour, odour, taste, texture and overall acceptability).

Statistical analysis

Optimization of levels of each coagulant and coagulating temperature was done using the RSM (Design-Expert 8.0.7.1). Data recorded in the experiments were analyzed and the response functions were developed using the multiple regression. ANOVA was used to analyze the models as described by Snedecor and Cochran (1986).

Results and Discussion

The physico-chemical characteristics of soybean variety PS-1347 are shown in table 2. Similar values with regard to fat content in soybean were also observed by Krishna et al. (2003). The value of ash content in whole soybean was in accordance with those reported by Sutar et al. (2009). The carbohydrate content is slightly lower than reported by Sutar et al. (2010). Jain and Mittal (1992) and Krishna et al. (2003) also estimated calcium in different Indian varieties of soybeans which ranged from 62.38 to 298.47, 252.91 to 304.17 mg/100 g, respectively.

Effects of different levels of salt coagulants and temperature on yield and sensory profile of soytofu

Soytofu prepared by 0.6% CaCl_2 was added to soymilk at 82.5°C had maximum product yield of 27.9% while addition of 0.2% CaCl_2 in soymilk at 90°C resulted in minimum soytofu yield of 22.4%. Soytofu yield was maximum (29%) when CaSO_4 was used at a level of 0.4% while the coagulating temperature of soymilk was 82.5°C. Results indicate that yield of soytofu was maximum when 0.6% MgCl_2 was used at 75°C. An observation of yield by coagulation through lactic acid (0.6%, 82.5°C), acetic acid (0.35%, 75°C) and citric acid (0.35%, 90°C) was noted as 27.5%, 25.7% and 22%, respectively. The sensory score of the prepared soytofu

was done on the basis of nine-point of hedonic scale. Maximum overall acceptability score of soytofu at different coagulant and temperature levels were CaCl_2 (8.75) CaSO_4 (8.25) MgCl_2 (9) MgSO_4 (9) lactic acid (9), acetic acid (8.87) and citric acid (9). Yield was calculated by following formula

$$\text{Yield (\%)} = \frac{\text{Soytofu obtained}}{\text{Soymilk taken for coagulation}} \times 100$$

Calculated F values of soytofu yield based on ANOVA depicted that yield is significantly affected by the level of coagulant and temperature at 1% level of significance when coagulated by CaCl_2 and MgCl_2 , while in case of citric acid and lactic acid yield is only affected by coagulant concentration at 1% level of significance. Product yield was affected by temperature at 1% and 5% level of significance when coagulant was MgSO_4 and CaSO_4 , respectively but at 5% level of significance soytofu yield was affected by using only acetic acid.

When CaCl_2 was used at 0.6% concentration with temperature of soymilk as 82.5°C, the sensory parameters (odour, taste and texture) was maximum as compare to other combination of processing variables, while appearance of soytofu had highest score at 0.4% level of CaCl_2 and 82.5°C. The F value suggests that the effect on appearance and taste are significant at 5% level with values of 5.63 and 5.74, respectively, odour texture and overall acceptability are significant at 1% level with values 12.05, 20.35 and 27.44, respectively. However effect on the color of soytofu is nonsignificant with F value of 2.44.

It can be seen that soytofu produced by 0.6% CaSO_4 with coagulating temperature of 82.5°C of milk was rated superior (overall acceptability score 8.25). The ratings of this soytofu sample with regard to odour (8.5), taste (8.5) and texture (8) were also appreciably higher. The effect on colour of soytofu due to

Table 1 Response Surface experiments to prepare soytofu by employing salt and acid coagulants

Experiment	Salt coagulant		Acid coagulant		Coagulating temperature	
	%	coded value	%	coded value	°C	coded value
1	0.2	-1	0.15	-1	75	-1
2	0.6	+1	0.35	+1	82.5	0
3	0.4	0	0.25	0	82.5	0
4	0.2	-1	0.15	-1	90	+1
5	0.6	+1	0.35	+1	75	-1
6	0.4	0	0.25	0	75	-1
7	0.6	+1	0.35	+1	90	+1
8	0.4	0	0.25	0	82.5	0
9	0.2	-1	0.15	-1	82.5	0
10	0.4	0	0.25	0	82.5	0
11	0.4	0	0.25	0	82.5	0
12	0.4	0	0.25	0	90	+1
13	0.4	0	0.25	0	82.5	0

Table 2 Physico-chemical characteristics of soybean seeds

Physical characters	Values
Hull content (%)	7.98±0.289*
Grain hardness (kg)	8.75±0.238
	19.1±0.185
Bulk density (g/cc)	0.71±0.004
True density (g/cc)	1.21±0.079
% Porosity	43.63±0.312
Colour	Light yellow
1000 grain weight (g)	132.03±0.026
Moisture (%)	10.3±0.395
Protein (%)	39.98±0.036
Crude fat (%)	19.55±0.195
Ash (%)	6.23±0.193
Carbohydrate (by difference) (%)	23.94
Calcium (mg)	257±1.155
Phosphorus (mg)	421.72±1.938
Iron (mg)	11.18±0.068

*Average value ± Standard Deviation

processing variables was negligible. The ratings for odour, taste, texture and overall acceptability of soytofu increased due to increasing level of CaSO_4 from 0.2 to 0.6% irrespective of temperature level while appearance of the product is improved when soymilk was coagulated at 75 and 82.5°C temperature.

Soytofu prepared using 0.4% MgCl_2 and 90°C temperature of soymilk recorded highest ratings for its appearance (9), odour (8.5), taste (8.75) and texture (9). In general, soytofu sample produced with 0.2% coagulant at 75°C was disliked most as indicated by its lowest organoleptic score with regard to appearance (4), taste (5.25), texture (4.25) and overall acceptability (4.5). The F value suggests that the effect on appearance odour, taste and overall acceptability are significant at 1% level with values of 27.74, 77, 11.3 and 11.64, respectively while texture is significant at 5% level with F value of 6.51. However, color is not affected significantly. The R^2 values were found to be 95.26, 97.88, 88.86, 82.31, 89.09% for appearance, odour, taste, texture and overall acceptability, respectively while it was 58.6% for color which further indicate that color was not influenced significantly due to variations in concentrations of MgCl_2 (0.2-0.6%) and temperature (75-90°C) as employed in making soytofu. Ratings for appearance, taste and texture of the product are improved due to either increasing the concentration of MgCl_2 or temperature and both were independent of each other. Rating of odour is maximum with the use of 0.4% MgCl_2 . The odour of soytofu is improved with increasing the coagulating temperature of soymilk and use of MgCl_2 at the level of 0.4 and 0.6%.

With the use of 0.4% MgSO_4 and temperature of soymilk as 90°C registered maximum organoleptic ratings with regard to appearance (8.5), odour (8.75), taste (8.75) and texture (9). Improvement in odour, texture and overall acceptability of the

Table 3 Optimum values of coagulant concentration and temperature of soymilk to produce ideal soy soytofu

Coagulants	Coagulant concentration (%)	Temperature of soymilk (°C)
CaCl_2	0.6	86.55
CaSO_4	0.57	84.9
MgCl_2	0.6	87.23
MgSO_4	0.46	86.18
Lactic Acid	0.35	89.33
Acetic Acid	0.3	80.25
Citric Acid	0.15	87.45

product due to increasing the coagulating temperature of soymilk at all the levels of MgSO_4 (0.2 to 0.6%) were used in the preparation of soytofu. The appearance score of soytofu is maximum at 82.5°C temperature of soymilk and that is independent of concentration of MgSO_4 . However, variations in temperature with any of the concentration of MgSO_4 were not affecting the taste of the soytofu.

Data indicate that overall acceptability score of the soytofu sample by lactic acid was maximum (9). Furthermore, ratings for the other sensory attributes i.e. odour, taste and texture were also highest (9, 9 and 8.85, respectively) for this soytofu sample. Soytofu produced with use of 0.25% lactic acid and temperature of soymilk as 82.5°C was of poorest sensory value due to its least overall acceptability score of 3. The model F value suggests that the effect on appearance, odour and taste was significant at 1% level with values of 54.1, 37.7 and 13.33, respectively while texture and overall acceptability, was significant at 5% level with values 6.05, and 6.49, respectively. The R^2 values were found to be 97.51, 96.33, 90.87, 81.19 and 82.22% for appearance, odour, taste, texture and overall acceptability, respectively while it was 43.38% for color which further indicate that color was not influenced significantly due to variations in concentration of lactic acid (0.15-0.35%) and temperature of soymilk (75-90°C) as used in making soytofu. Lack of Fit for the significantly influenced sensory attributes (appearance, odour, taste, texture and overall acceptability) was non significant.

Organoleptic ratings in respect of appearance, odour, taste, texture and overall acceptability were 8.75, 8.88, 9, 9 and 8.75, respectively for soytofu sample wherein acetic acid concentration and soymilk temperature were 0.25% and 82.5°C, respectively. By regression analysis model F value suggests that the effect on appearance, odour, taste, texture and overall acceptability are highly significant at 1% level with values of 80, 153, 19.29, 15 and 15.9, respectively. The R^2 values were found to be 98.35, 99.15, 93.03, 91.52 and 92% for appearance, odour, taste, texture and overall acceptability, respectively while it was 72.11% for color which indicate that color was not influenced significantly due to variations in concentration of acetic acid (0.15-0.35%) and temperature of soymilk (75-90°C) as used in making soytofu. Lack

Fig.1. Response surface plot of appearance score of tofu prepared by calcium chloride

Fig.2. Response surface plot of odour score of tofu prepared by calcium chloride

Fig. 3. Response surface plot of taste score of tofu prepared by calcium chloride

Fig. 4. Response surface plot of texture score of tofu prepared by calcium chloride

Table 4 Yield and organoleptic score of soytofu prepared using seven coagulants at optimized levels of coagulant concentration and temperature of soymilk

Responses	Coagulants						
	CaCl ₂	CaSO ₄	MgCl ₂	MgSO ₄	Lactic Acid	Acetic Acid	Citric Acid
Yield (%)	27	27.02	25.78	26.91	25	21.93	20
Appearance	8.71	7.29	8.29	7.86	7.29	6.14	6.14
Colour	9	7.43	8.43	7.86	8.86	8	6.86
Odour	8.57	7.8	7.29	7.3	6.57	4.86	7.86
Taste	8.72	7.57	7.86	7.11	7.57	5.43	5.43
Texture	8.71	7.1	7.29	8	7.43	5.57	5.79
Overall acceptability	8.73	6.29	7.57	8.01	7.43	5	6.14
F-value	**	**	**	**	**	**	**
S.Em±	-	0.35	0.43	1.64	0.19	0.55	0.48
CD at 1%	-	0.85	0.95	1.85	0.60	1.0	1.0

** Significant at 1% level of significance

of Fit for the significantly influenced sensory attributes (appearance, odour, taste, texture and overall acceptability) was non significant.

Soytofu occupied first position with regard to overall acceptability score (9) at 0.15% citric acid at 82.5°C. The ratings for appearance, taste and texture in respect of this sample was

highest (9). The model F value suggests that the effect on appearance, odour, taste, texture and overall acceptability are highly significant at 1% level with values of 9.42, 7.61, 16.81, 9.91 and 9.58, respectively. The R² values were found to be 87.22, 84.46, 92.29, 87.63 and 87.21% for appearance, odour, taste, texture and overall acceptability, respectively while it was 31.26% for color which further indicate that color was not influenced

significantly due to variations in concentration of citric acid (0.15-0.35%) and temperature of soymilk (75-90°C) as used in making soytofu. Ratings for appearance, taste, texture and overall acceptability declined with increase in concentration of citric acid at any of three coagulating temperatures used in the study. Odour score is unaffected due to variations in the levels of citric acid at all the three levels of temperature. Texture and odour of the soytofu sample are improved with increase in temperature of soy milk and variations in the concentration of coagulant used. Increase in coagulating temperature of soy milk does not affect the score of appearance at all the three concentrations of citric acid.

Variations in yield and sensory attributes of soytofu prepared by coagulating soymilk at different temperature with different types of coagulants might be ascribed to nature of coagulating agent and their variable mode of formation of soy gel. Two types of coagulants as employed in this investigation for formation of soytofu were salt type and acid type. Formation of protein gel due to addition of salt in soymilk depend upon ionic strength, water affinity, molecular weight, molecular size, ionization ability of forming cross linkage etc. of coagulants. Similarly, formation of protein gel by incorporation of different acid coagulants is governed by type of acid and their pH lowering effect. Gel strength is influenced by coagulating temperature, type of coagulants and its concentration. Softness or compactness of gel is affected by coagulants water binding or water trapping property of gel which inturn control the yield as well as sensory properties of gel. Several previous researches have also recognized different factors which affect the yield and sensory quality of soy soytofu. Gandhi (2009), Sun and Breene (1991), Shih et al. (1997), Choi et al. (1983), Lim et al. (1990), Sun and Breene (1991), Yuwono (2013), Shih et al. (1997), Gandhi and Bourne (1988), reported that yield of soy soytofu depends upon soybean variety, processing method, soaking time, type of and concentration of coagulants, temperature of soymilk for coagulation, stirring speeds and time of coagulation and pressing of curd, respectively. Similarly, Narayan et al. (1997), Tanwar et al. (1998), Yuwono (2013), Li et al. (2014), Smith et al. (1960) and Wang (1967) also bring into being that soybean variety, storage condition of soybean, method of production, type and concentration of coagulant and pressing of soytofu affect the sensory profile of soytofu.

Yield and sensory evaluation of soytofu prepared by optimized levels of coagulants and temperature of soymilk

Yield and organoleptic score of soytofu prepared using selected coagulants at optimized levels shows that use of calcium sulphate and calcium chloride as coagulant gave maximum yield 27.02 and 27%, respectively as compared to other coagulants. Soymilk coagulated with citric acid produced minimum yield (20%) of soytofu. Krishna et al. (2002) also observed similar results of calcium chloride in preparation of soytofu (Table 4).

Soytofu was evaluated by the panelists to determine its acceptability. It was evaluated for appearance, colour, odour, taste, texture and overall acceptability on nine-point hedonic scale. Soytofu prepared from calcium chloride at concentration of 0.6% and 86.55°C temperature of soymilk gives highest scores for appearance, colour, odour, taste, texture and overall acceptability with values 8.71, 9, 8.57, 8.72, 8.71 and 8.73, respectively. Statistical analysis of the data showed significant difference at 1% level among the soytofu samples prepared from different coagulants. Soytofu prepared from calcium chloride was found to be most satisfactory as it had utmost overall acceptability (Fig. 1-4). This was followed by magnesium sulphate, magnesium chloride and lactic acid. Previous researches reported that soytofu prepared with calcium salts had very superior general acceptability in terms of colour, texture, taste and overall acceptability (Table 4).

Soytofu prepared by using CaCl_2 as a coagulant was found to be finest for making soytofu. The moisture, protein, crude fat, ash and carbohydrate content of soytofu were recorded as 68.59 ± 0.02 , 50.3 ± 0.302 , 12 ± 0.120 , 5.9 ± 0.911 and $15.35 \pm 0.03\%$, respectively. Shokunbi et al. (2011) also determined proximate composition of soytofu which were slightly higher than present investigation. The ash content reported in this work (5.9%) is slightly higher than reported by Shih et al. (1997). Such variations can be attributed to the variation in preparation methods as well as the types of soybean used to produce soytofu. However, Obiegbuna et al. (2014) recorded lower values for proximate composition of soytofu.

Conclusions

On the basis of above observations it can be concluded that the concentration of coagulants and levels of coagulating temperature of soymilk were different for making ideal soytofu. The optimized levels of coagulants and coagulating temperature for making quality soytofu was 0.6, 86.55, 0.57, 84.9, 0.6, 87.23, 0.46, 86.18, 0.46, 0.35, 89.33, 0.3, 80.25 and 0.15%, 87.45°C, respectively for CaCl_2 , CaSO_4 , MgCl_2 , MgSO_4 , lactic acid, acetic acid and citric acid coagulants. Preparation of soytofu sample from RSM optimized level and their organoleptic evaluation indicated that among the seven coagulants calcium chloride was best for making quality soytofu with higher and it contained 68.59, 50.3, 12, 5.9 and 15.35% moisture, protein, crude fat, ash and carbohydrates, respectively.

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