



Comparative Study on Uttarakhand's Indigenous Black Soybean Variety (*Bhat*) for Tofu Production

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ABSTRACT

Background: Tofu is increasingly captivating the food industry as a milk-based product alternative. Diverse soybean varieties worldwide are harnessed to create various milk substitutes. This study assesses the potential of Uttarakhand's indigenous bhat variety for producing soymilk and tofu, comparing its commercial viability and quality with conventional counterparts.

Methods: The study focused on Uttarakhand's indigenous variety, *bhat* and the commercially popular PS-1347 for soymilk extraction. Preliminary investigations were conducted to optimize the process to maximize recovery and minimize beany flavor, grinding soybeans for 6 minutes and boiling for 20 minutes. Tofu was produced using CaSO_4 as a coagulant at varying concentrations (0.2%, 0.4%, 0.6%) and coagulating temperatures (75°C, 85°C, 95°C).

Result: Tofu produced from *bhat*, coagulated with 0.4% CaSO_4 at 85°C, exhibited superior acceptability, making it suitable for large-scale commercial production. Its quality matched commercial counter parts, with protein at 16.45%, total mineral at 6.23%, calcium at 152.66 mg/100 g and iron at 14.62 mg/100 g.

Key words: Beany flavor, Calcium sulphate, Coagulant, PS-1347.

INTRODUCTION

The black seeded soybean [*Glycine max* (L.) Merrill], belongs to the family *Fabaceae*, subfamily *Faboideae*, genus *Glycine* and subgenera *Soja* (Kumudini, 2010; Vyas and Chandel, 2013). In recent decades, black soybean is becoming more popular as compared to yellow, due to its richness in various essential nutrients and bioactive compounds (Joshi and Rahal, 2018). Yellow varieties of soybean are cultivated throughout India (Rehal *et al.*, 2017) while black varieties of soybean are traditionally grown on a small scale in Himachal Pradesh, *Kumaon* hills of Uttarakhand, Eastern Bengal and Khasi hills. In Uttarakhand, black varieties of soybean are commonly known as *bhat* or *bhatmash* (Dobhal and Raghuvanshi, 2018). According to Zhou *et al.* (2017) black soybean is a rich source of phenolic compounds which include various secondary metabolic groups, ranging from simple molecules such as phenolic acids, flavonoids, to polymerized compounds such as lignin. Phytochemicals present in black soybean are potentially effective in human health, including treatment of cancer, diabetes, cardiovascular diseases and neurodegenerative diseases (Ganesan and Xu, 2017). Although black soybean is widely used since ancient times in China, Korea, Japan, Indonesia and Northwestern Himalayan hills of India as a remedial food, but its potential is still undervalued due to its black colored seed coat, which is not readily acceptable in many parts of world. Therefore, its cultivation and consumption remained confined to almost very small part of the globe.

Black soybean, an annual legume has a brief life cycle of 3-4 months and thrives in diverse environmental condition (Gunathilake *et al.*, 2019). It plays a vital role in ensuring food and nutritional security, especially in hilly

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areas plagued by deficiencies, benefiting rural, tribal and backward populations practicing subsistence farming in marginal rainfed terrains. Black soybean cultivation is cost-effective and resilient, surviving adverse conditions, making it preferable to other crops during the rainy season. Soybeans, with various seed coat colors, serve as a rich source of vegetable oil, protein and animal feed. The protein content of soybean is considered complete, supplying essential amino acids for tissue building and repair. Notably, the only distinction lies in the black hull, signifying the presence of anthocyanin compounds, granting soy the highest antioxidant activity. This nutritional profile emphasizes the significance of black soybeans in promoting sustainable agriculture and addressing nutritional challenges in diverse communities.

The total area in Uttarakhand under black soybean production is 5,734 hectares, yet its potential for value-

added products remains largely untapped. Although, several studies have been reported on nutritional and medicinal value of black soybean variety indigenous to Uttarakhand, but, very little information is available on its utilization for development of value added products. Its use is meagre due to lack of information on varieties (suitable for soymilk and tofu production), handling and processing. Unlike studies in Korea and Japan, Uttarakhand's black soybean lacks exploration in product development and nutritional benefits. To address this gap, our study focuses on *bhat*'s suitability for tofu making, emphasizing quality attributes. The primary technological challenge involves optimizing the preparation method to align sensory and physicochemical characteristics with existing market offerings, promoting the utilization of this indigenous variety in the food industry.

MATERIALS AND METHODS

In this study, mature *bhat* (black soybean) seeds and sugar were obtained from Pantnagar's local market, Uttarakhand, India. Commercially popular yellow soybean seeds (variety PS-1347), were sourced from the Crop Research Centre, GB Pant University of Agriculture and Technology, Pantnagar. Analytical grade coagulants for tofu preparation and chemicals for quality evaluation of raw soybean seeds and tofu were purchased locally. The investigation took place at the Department of Food Science and Technology, College of Agriculture, GB Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India.

Tofu was prepared following Saini's (2014) method, with minor adjustments based on the soybean variety. Soymilk was derived from *bhat* and PS 1347, optimizing grinding for 6 minutes and boiling for 12 minutes, according to preliminary studies (Fig 1). The soymilk was then cooled to various coagulating temperatures (75, 85, 95°C), with calcium sulphate added at concentrations of 0.2%, 0.4% and 0.6% as a coagulant. After coagulation, the soymilk stood undisturbed for 30 minutes at room temperature. Whey was removed by filtering through double-layered cheesecloth and the resulting curd was pressed at a constant pressure of 28 gm/cm² for 15 minutes in a wooden box. Subsequently, the curd was stored for further analysis.

Whole grains of black soybean (*Bhatt*) and yellow soybean (variety PS 1347) were analyzed for various physico-chemical characteristics (Table 1).

Physical characteristics of soybean varieties were analyzed using standard deviation. Analysis of variance employed a completely randomized design (CRD) for various treatments of tofu, (Gomez and Gomez, 1985). Sensory evaluation data used a randomized block design (RBD), with the standardization experiment replicated three times.

RESULTS AND DISCUSSION

Physicochemical characteristics of *bhat* and PS 1347

Table 2 represents the physical properties, while Table 3 outlines the chemical characteristics of *bhat* and PS-1347 soybean varieties. Past research highlights the significant

impact of varietal differences on tofu recovery and nutritional composition. (Shen *et al.*, 1991; Cai *et al.*, 1997; Bhardwaj *et al.*, 1999; Poysa *et al.*, 2002; Guo and Ono, 2005; Min *et al.*, 2005; Poysa *et al.*, 2006).

Effect of concentration of CaSO₄ and temperature of coagulation on yield of tofu

Tofu is derived from soymilk through acid or salt coagulation. Factors like soymilk quality, coagulant type, concentration and temperature affect tofu yield and quality. This study used calcium sulphate as a coagulant, varying its level and coagulation temperature. The optimal combination was determined based on yield and sensory evaluation.

Table 4 presents the impact of coagulant concentration and coagulation temperature on tofu yield. The study indicates a non-significant effect of coagulant concentration and temperature on tofu yield for two soybean varieties. Regardless of coagulant concentration and variety, the highest tofu yield occurred at 85°C. The increase in temperature from 75 to 85°C resulted in higher yield, likely due to less loss of soluble protein (whey protein) along with water as more bonding occurs thus making the protein matrix more compacted. However, further temperature increase (95°C) led to reduced yield, attributed to protein structure changes, causing loss of soluble protein and water.

There was significant effect of coagulant concentration on the yield of tofu. With the increase in coagulant concentration from 0.2 to 0.4%, the recovery of tofu increased (122.79 to 123.43 g), which decreased (122.89 g) with further increase in concentration (0.6%). Gel strength of tofu depends on coagulating temperature and concentration of coagulant (Mathare *et al.*, 2009). The coagulation of protein was highest at 0.4% of CaSO₄ and the coagulated protein was less soluble in residual water due to its compact protein matrix. As the concentration of coagulant increased to 0.6% CaSO₄, the structure of coagulated protein changed, thereby changing its functionality (solubility) and rendering it to be more soluble in residual water and consequently affecting the yield of tofu. Therefore, it can be concluded that the 0.4% of CaSO₄ at 85°C coagulating temperature is appropriate for the maximum recovery of tofu, irrespective of the variety of soybean.

Effect of coagulation temperature and coagulant concentration on the sensory attributes of tofu

The effect of variety, coagulating temperature and coagulant concentration on the sensory attributes such as colour, odor, taste, texture and overall acceptability are presented in Table 5-9. There were no significant differences between the tofu samples made by both varieties. But there are significant differences in score of tofu color due to different concentration of calcium sulphate at different temperature. The tofu prepared by both the varieties, coagulated with 0.4% CaSO₄ at 85°C got the highest score for color as 7.5 and 7.8, respectively (Table 5). Color of tofu depends on color of soymilk as well as surface properties of tofu, type and amount of coagulant and coagulating temperature.

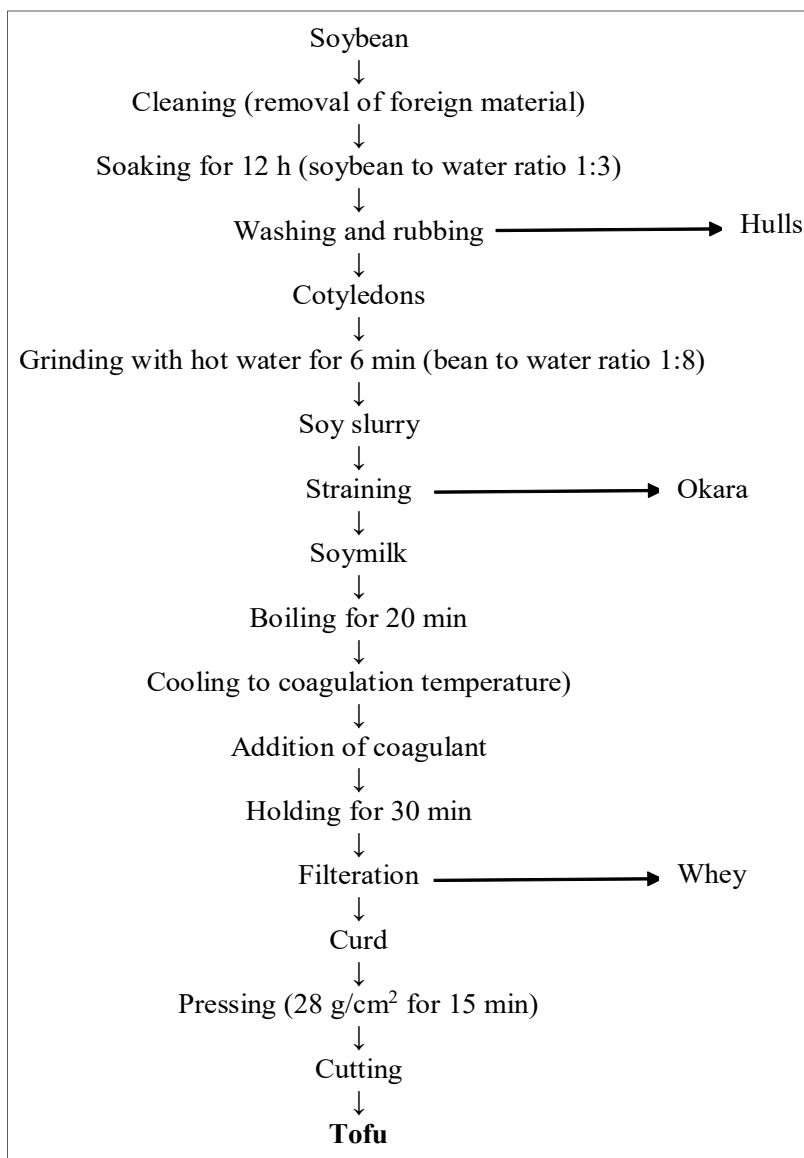


Fig 1: Standardized method for the preparation of tofu.

Table 1: Method used for analysis of physico-chemical and sensory parameters of raw material and tofu.

Parameters	References/equipment/method
Physical parameters	
Thousand grain weight	Sumangala and Kulkarni, 2019
Bulk density, True density, percent porosity	Mangaraj <i>et al.</i> , 2005
Seed size	Digital vernier calipers
Hull and cotyledon content	Ali-Khan, 1993
Chemical parameters	
Moisture	AOAC, 1986
Crude protein	AOAC, 2005
Crude fat, ash, titratable acidity	AOAC, 1984
Carbohydrate	Difference method
Calcium, iron	Atomic absorption spectroscopy ((Ranganna, 2007).
pH	Digital pH meter
Sensory evaluation	Panel of semi-trained members on Nine point Hedonic Rating Scale (Larmond, 1977).

The temperature of coagulation influences the gel development as well as the surface properties of tofu, which subsequently effects the reflection, absorption and transmission of light. It may also be attributed to different hilum colour of soybean seed coat which could migrate to tofu during grinding (Goel *et al.*, 2018).

Tofu prepared from both the varieties of soybean by employing 0.4% calcium sulphate at 85°C obtained concentration on microstructure of tofu and protein content in tofu (Table 9). The firm tofu made with 0.4% concentration of calcium sulphate was found most uniform in microstructure.

Table 2: Physical characteristics of seeds of soybean varieties.

Characteristics	Average±SD	
	Yellow soybean (PS 1347)	Black soybean (<i>Bhat</i>)
Seed coat color	Light yellow	Black
Cotyledon color	Creamy yellow	Yellow
Bulk density (g/ cc)	0.63±0.01	0.62±0.01
True density (g/ cc)	1.16±0.01	1.12±0.01
Porosity (%)	44±0.01	44±0.00
Length (mm)	5.93±0.57	5.33±0.57
Width (mm)	4.23±0.57	2.33±0.57
1000 grain weight (g)	111.8±0.60	85.6±0.62
Hull (%)	9.20±0.07	9.87±0.06
Cotyledon (%)	90.79±0.07	90.12±0.06

*SD= Standard deviation.

Table 3: Chemical characteristics of seeds of soybean varieties.

Characteristics	Average±SD*	
	Bhatt	PS 1347
Moisture (%)	9.56±0.96 (10.57)**	9.34±0.58 (10.30)**
Crude protein (%)	43.83±0.62 (48.46)**	40.95±0.58 (40.95)**
Crude fat (%)	19.46±0.76 (21.46)**	23.04±0.71 (25.41)**
Ash (%)	6.08±0.27 (6.70)**	5.25±0.09 (5.79)**
Carbohydrates (%)	21.06±0.54 (23.22)**	21.42±0.75 (23.62)**
Calcium (mg/100 g)	279±2.12 (307.74)**	258±2.12 (284.57)**
Iron (mg/100 g)	11.88±0.22 (13.10)**	11.05±0.91 (12.18)**

*SD= Standard deviation.

**Values in parenthesis indicates values on dry weight basis.

Table 4: Effect of concentration of CaSO₄ and temperature of coagulation on yield (g) of tofu.

Temperature (°C)	Variety (V)	Concentration of coagulant (g/100 g of soymilk)			Mean	CD _{0.05}
		0.2	0.4	0.6		
75	PS 1347	118.91	136.80	123.89	126.53	Factor CD _{0.05}
	<i>Bhat</i>	117.99	136.68	121.94	125.54	
	Mean	118.45	136.74	122.915		
	Grand mean (Temperature)	125.53				
85	PS 1347	122.01	139.81	125.95	129.25	Factor CD _{0.05}
	<i>Bhat</i>	124.18	141.07	127.13	130.37	
	Mean	123.095	140.44	126.54		
	Grand mean (Temperature)	130.79				
95	PS 1347	126.57	137.20	119.95	127.91	Factor CD _{0.05}
	<i>Bhat</i>	127.07	135.20	121.73	128.00	
	Mean	126.82	136.20	120.84		
	Grand mean (Temperature)	128.09				
Grand mean	(Concentration of coagulant)	122.79	123.43	122.89		

Chemical characteristics of standardized soymilk and tofu

The physico-chemical parameter tofu prepared from black soybean variety *i.e.* *bhat* is presented in Table 10. maximum scores for odour (8.3). Possibly the influence of coagulating temperature and variety on the odor (Table 6) of tofu might be due to presence of odorous substances in soybean as well as their entrapment in tofu during formation

of gel. The maximum score (8.7) for taste was obtained by tofu prepared from *bhat* using 0.4% CaSO_4 at 85°C (Table 7). Variety, coagulating temperature and concentration of coagulant significantly affected the taste score of tofu as they affect formation of soy gel and ultimately leads to difference in the entrapment of flavor substances in the gel which impart taste to the tofu.

Table 5: Effect of coagulant concentration and coagulating temperature on the colour of tofu.

Temperature (°C)	Variety (V)	Concentration of coagulant (g/100 g of soymilk)			Mean	CD _{0.05}	
		0.2	0.4	0.6		Factor	CD _{0.05}
75	PS 1347	4.4	4.6	5.3	4.7	Variety (V)	NS
	<i>Bhat</i>	4.4	4.6	4.9	4.6	Temperature (T)	0.23
	Grand mean (Temperature)	4.7				Concentration of (C)	NS
85	PS 1347	5.6	7.5	7.1	6.7	V × T	0.23
	<i>Bhat</i>	5.6	7.8	6.9	6.7	V × C	NS
	Grand mean (Temperature)	6.8				T × C	0.40
95	PS 1347	6.3	7.6	7.2	7.0	V × T × C	NS
	<i>Bhat</i>	5.4	6.6	6.3	6.2	Grand mean (variety)	
	Grand mean (Temperature)	7.0				PS 1347	6.1
Grand mean (Concentration of coagulant)		5.4	6.6	6.3		<i>Bhat</i>	6.2

Table 6: Effect of coagulant concentration and coagulating temperature on the odor of tofu.

Temperature (°C)	Variety (V)	Concentration of coagulant (g/100 g of soymilk)			Mean	CD _{0.05}	
		0.2	0.4	0.6		Factor	CD _{0.05}
75	PS 1347	6.5	6.4	6.7	6.5	Variety (V)	0.27
	<i>Bhat</i>	6.0	5.7	6.2	5.9	Temperature (T)	0.37
	Grand mean (Temperature)	6.2				Concentration of (C)	NS
85	PS 1347	7.0	7.6	8.3	7.6	V × T	0.34
	<i>Bhat</i>	7.3	8.2	8.3	7.9	V × C	0.48
	Grand mean (Temperature)	7.7				T × C	0.58
95	PS 1347	7.6	7.4	7.2	7.4	V × T × C	NS
	<i>Bhat</i>	7.2	8.1	7.7	7.6	Grand mean (variety)	
	Grand mean (Temperature)	7.7				PS 1347	7.4
Grand mean (Concentration of coagulant)		6.9	7.4	7.4		<i>Bhat</i>	7.0

Table 7: Effect of coagulant concentration and coagulating temperature on the taste of tofu.

Temperature (°C)	Variety (V)	Concentration of coagulant (g/100 g of soymilk)			Mean	CD _{0.05}	
		0.2	0.4	0.6		Factor	CD _{0.05}
75	PS 1347	4.5	5.7	5.6	5.2	Variety (V)	0.35
	<i>Bhat</i>	4.5	5.5	5.4	5.1	Temperature (T)	0.23
	Grand mean (Temperature)	5.2				Concentration of (C)	0.56
85	PS 1347	5.6	6.7	6.1	6.1	V × T	0.23
	<i>Bhat</i>	5.4	8.7	5.4	6.5	V × C	NS
	Grand mean (Temperature)	5.9				T × C	0.40
95	PS 1347	6.6	6.2	6.0	6.2	V × T × C	NS
	<i>Bhat</i>	6.4	6.6	4.9	5.9	Grand mean (variety)	
	Grand mean (Temperature)	6.6				PS 1347	5.8
Grand mean (Concentration of coagulant)		5.5	6.7	5.7		<i>Bhat</i>	5.8

Table 8: Effect of coagulant concentration and coagulating temperature on the texture of tofu.

Temperature (°C)	Variety (V)	Concentration of coagulant (g/100 g of soymilk)			Mean	CD _{0.05}	
		0.2	0.4	0.6		Factor	CD _{0.05}
75	PS 1347	4.3	5.6	5.8	5.2	Variety (V)	0.19
	<i>Bhat</i>	4.8	5.8	5.2	5.2	Temperature (T)	0.23
	Grand mean (Temperature)	5.2				Concentration of (C)	0.33
85	PS 1347	5.1	6.6	5.4	5.7	V × T	0.23
	<i>Bhat</i>	6.3	8.7	4.8	6.6	V × C	NS
	Grand mean (Temperature)	6.8				T × C	0.40
95	PS 1347	6.3	6.2	5.0	5.7	V × T × C	NS
	<i>Bhat</i>	5.9	7.2	4.9		Grand mean (variety)	
	Grand mean (Temperature)	6.6				PS 1347	5.5
Grand mean (Concentration of coagulant)		5.6	7.0	5.2		<i>Bhat</i>	5.9

Table 9: Effect of coagulant concentration and coagulating temperature on the overall acceptability of tofu.

Temperature (°C)	Variety (V)	Concentration of coagulant (g/100 g of soymilk)			Mean	CD _{0.05}	
		0.2	0.4	0.6		Factor	CD _{0.05}
75	PS 1347	5.8	6.5	5.4	5.9	Variety (V)	0.24
	<i>Bhat</i>	4.6	5.5	6.0	5.3	Temperature (T)	0.30
	Grand mean (Temperature)	5.6				Concentration of (C)	0.41
85	PS 1347	6.5	7.1	6.1	6.5	V × T	0.29
	<i>Bhat</i>	5.1	8.1	6.1	6.4	V × C	NS
	Grand mean (Temperature)	6.3				T × C	0.51
95	PS 1347	7.0	6.9	6.2	6.7	V × T × C	NS
	<i>Bhat</i>	6.2	5.9	5.7	5.9	Grand mean (variety)	
	Grand mean (Temperature)	6.8				PS 1347	6.5
Grand mean (Concentration of coagulant)		5.8	7.0	5.9		<i>Bhat</i>	5.8

Table 10: Physico-chemical characteristics of tofu from black soybean variety (*bhat*).

Characteristics	Average±SD*
Yield	123.43 g/100 g soymilk
Moisture, %	59.89±0.27
Crude protein, %	16.45±0.43
Crude fat, %	9.13±0.38
Total mineral, %	6.23±0.76
Carbohydrates, %	8.3±0.80
Calcium, mg/100 g	152.66±0.53
Iron, mg/100 g	14.62±0.40
pH	6.5
Titrateable acidity, %	0.14±0.63

*SD= Standard deviation.

It can be observed that tofu samples prepared by *bhat* using 0.4% calcium sulphate at 85°C showed maximum scores as 8.7 for texture (Table 8). The texture score increased with increased concentration of calcium sulphate from 0.2 to 0.4%. It also increased with increasing in temperature from 75 to 85°C but at 95°C the texture of tofu

found to be firm, which decreased its score. Texture of soy gel depends upon several factors and important ones are type and content of proteins, type and level of coagulants and temperature. Appropriate proportion along with ideal processing parameters are essential to form a well set gel with ideal textural properties. Hence, the differences in texture score of tofu samples as prepared in present investigation may be due to differences in variety and concentration of coagulant and temperature of soymilk at the time of coagulation.

Tofu prepared from *bhat* by using 0.4% calcium sulphate at 85°C was found most acceptable by panelist. Kao *et al.* (2003) studied the effects of calcium sulphate.

CONCLUSION

The present study investigated the suitability of black indigenous variety of soybean of Uttarakhand for the preparation of tofu. The soymilk prepared after 6 minutes of grinding and 20 minutes of boiling can be efficiently utilized for development of tofu using 0.4% CaSO₄ as coagulating agent at 85°C coagulation temperature from *bhat* indicating that the prepared product was comparable

to their commercial counterpart in terms of sensory attributes. In general, tofu samples had good nutritional composition and fell within acceptable limit.

Conflict of Interest

The authors have declared no conflict of interest for this article.

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