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Optimization of Coagulating Conditions for Preparation of Good Quality *Tofu* with Minimum Biochemical Loss through *Tofu* Whey

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ABSTRACT

***Tofu* or soybean curd is mainly made by coagulating soymilk. *Tofu* whey, a by-product of tofu manufacturing, is currently discarded by the food industry. The *Tofu* whey refuse is highly perishable and needs a quick treatment for effective utilization. *Tofu* whey which composition is related to optimum coagulating condition. The different coagulating conditions had great influence on tofu yield, texture, biochemical properties and whey composition. Different coagulants (calcium sulphate, calcium chloride and magnesium chloride and magnesium chloride) were used to optimize the coagulating parameters and optimal concentration of coagulation (OCC) at different temperatures for soymilk coagulation and tofu preparation. Each soymilk batch prepared with coagulants (20 mM to 90 mM) at 60^0 ,70^0 ,80^0 ,90^0 C. Coagulated batches were pressed to make tofu and measured whey volume, pH, transmittance tofu yield and coagulant efficiency. Whey transmittance and conductance correlated with coagulant concentration. Tofu yield, conductivity, pH and TDS data were also found to depend on coagulating conditions. Conductivity and transmittance could be used to optimize tofu coagulation. The OCC value was found to differ with coagulating conditions.**

Key word *Soybean, Tofu, Coagulants, OCC, Tofu whey, Texture*

Tofu is a very versatile and nutritious food that is made from soybean curds. While *Tofu* is gaining an increasing popularity in western countries, it remains the most important and popular food product in east and south-astern Asian countries (Oboh, 2006). It is produced traditionally by coagulating fresh hot soymilk with either salt (CaCl2 or CaSO4) or an acid (glucuno-d-lactone) (Oboh, 2006)). The coagulant produces a soy protein gel, which traps water, soy lipids and other constituents in the matrix forming curds. The quality of the beans, the amount of stirring, nature and concentration of used coagulants can have a high impact on the quality of the final product (Wang and Chang, 1995). Yield and quality of tofu have been reported to be influenced by soybean varieties, soybean quality, processing conditions and coagulants (Oboh, 2006, Cai, *et al.*, 1998). The coagulation of soymilk relies on the complex interrelationship between type of soybean, soymilk cooking temperature, soymilk volume, solid content, pH, coagulant type, amount and coagulation time (Cai and Chang,1998). To improve the texture and increase the yield of *Tofu*, researchers has been engaged to find better coagulation methods, concentration of coagulants and optimum temperature of coagulation. Studies have shown that the amount of soy protein used to make the soymilk is critical for *Tofu* yield and quality because tofu is a soy protein gel. Different coagulants produce *Tofu* with different textural and flavour properties. CS creates a bridge by which the soy proteins in the milk can aggregate. It may also interact with proteins to enhance the cross-linking of polymers (Beddows and Wong, 1987, Mullin, *et al.*, 2001). The combined heat- and calcium-induced mechanisms work to produce the *Tofu*. The resulting tofu product is affected by such things as pH, concentration of coagulate, and the rate at which the product is stirred (Beddows and Wong 1987). The Chinese have used the calcium salt mined from mountain quarries for 2000 years (Kohyama, *et al.*, 1992). The salt is the pure form of gypsum. The Japanese traditionally used sea salt in form of magnesium chloride to coagulate soymilk. Recently, CS and/or glucono-d-lactone (GDL) have been mostly used as coagulant for the production of tofu in Japan (Kohyama, *et al.*, 1995).The objective of this study was to evaluate the effect of four different coagulants, coagulation temperatures and coagulant concentration on yield, antioxidant and textural properties of tofu.

MATERIALS AND METHODS

Soybeans:

The soybean genotypes used were MAUS71, PK416, PS1347, JS335 (Bhopal), JS335 (Pune), JS9560, JS9305, NRC-12, NRC-37 and VL soya 47. The soybean samples were cleaned and stored in plastic containers in the dark at 4°C until they were processed for soymilk.

Preparation of soymilk:

One hundred grams of soybeans were first rinsed and soaked in 500 ml of deionized water for 4 h at room temperature. Hydrated soybeans were drained, rinsed and ground in a Waring blender for 2 min on high speed with hot water (1:6 soybean and water ratio). The slurry was boiled for 15 min at 90-94°C and filtered to remove the coarse material (okara) from the soymilk slurry. The volume of the final soymilk was set to 500ml using deionized water. In each experiment, the soymilk was freshly prepared daily.

Preparation of *Tofu* using chemical coagulants:

One hundred ml of freshly prepared soymilk (20°C) was transferred to a plastic container and maintained in a 96°C water bath for 15 min. The hot soymilk was removed from the water bath and transferred to a beaker. The following coagulants were used: magnesium chloride, calcium chloride, calcium sulfate and magnesium sulfate at concentrations of 20,30,40,50 60, 70, 80 and 90 mM. The coagulant was added to soymilk at 90°C, 80°C, 70°C and 60°C for each concentration of coagulant and the mixture was stirred five times. The solution was allowed to coagulate undisturbed for 5 minutes at the room temperature. The curd was then transferred into a laboratory-designed tofu box (steel mould) lined with Miracloth for molding and tofu whey removal. The cloth was folded over the top of the curd and pressing was achieved with a weight (the weight covered the entire top surface of the folded cloth of curd while pressing), for 1 hours. The tofu was then unwrapped from the Miracloth and the weight of the fresh tofu brick recorded. Textural analysis was conducted promptly.

Determination of *Tofu* yield and moisture:

Tofu yield was determined by weighing the tofu brick immediately after removing from the tofu-box and was expressed as weight in grams of fresh tofu produced per 100 grams of soymilk. An approximate 3 g portion was used for dry matter determination. The total solid content of tofu sample was determined by drying the sample to constant weight at 85°C in an air oven.

Physical characteristics determination of tofu whey:

1. **Soluble solids / brix %**; Total solid contents of tofu whey was measured by using the lab hand refractometer (Model ATAGO N1, brix 0-32%, made in Japan). With samples equilibrated at 20°C prior to taking measurement, 1 ml of each sample was poured on refractometer prism and readings taken. Values were expressed as brix %.
2. **pH measurement;** ph measurements of tofu whey were conducted by using a hand held pH/mV/Temperature meter (Model pH 323, Ser.-Nr. 63260002, WTW 82362 Weilheim, Germany) attached to a stainless steel pH/ Temperature probe. Prior to taking measurements, the instrument was calibrated with distilled water pH 7.
3. **Conductometric analysis;** Conductometric analysis of tofu whey was performed by conductivity meter (H12300, microprocessor conductivity meter, Hanna instrument Inc., Woonsocket, Romania, USA.)

Proximate analysis:

Chemical composition of the soybean, soymilk and *Tofu* was done. The crude moisture, protein and fat content were determined by vacuum oven method, Kjeldahl method using a protein conversion factor of 6.25 and Soxhlet extraction method, respectively (AOAC, 1995). The ash contents were determined using the method of AOAC 1990. Results were expressed on dry-matter basis.

Determination of antioxidant activity (2, 2- diphenyl-1-picrylhydrazyl (DPPH) inhibition):

DPPH is a stable free radical in a methanolic solution. In its oxidized form, the DPPH radical has an absorbance maximum centered at about 520 nm.Take 0.5 gm dried sample and add 4 ml methanol for extraction for over night in the test tube. After extraction filter it using what man no. 1 filter paper and maintain 5 ml using methanol.Take 0.2 ml extracted sample in the test tube and add 3.8 ml DPPH reagent and shake well. The samples allow incubating at room temperature for 30 min. The absorbance of the DPPH solution was measured at 517 nm against blank and control (methanol + DPPH reagent).

Determination of textural properties of *Tofu*:

The textural properties of *Tofu* were determined using the Texture Profile Analysis (TPA) curve. Texture Profile Analysis uses mechanical parameters of texture, which imitate the action of jaws, and the texture analyser is programmed to compress a bite-size piece twice in a reciprocating motion. Texture analysis was carried out using a laboratory-developed system consisting of a moving probe and a digital balance connected to a computer equipped with software recording the actual force produced by compressing the tofu sample. Samples for texture measurement were 2x2 cm dia. cylinders of tofu, cut from the main block with a cork borer, then trimmed to length by cutting with fine wires set in a frame 2 cm apart. A two-cycle compression test was used, in which the probe touched the sample and compressed it to 75% of the brick height (15 mm) at a cross-head speed 18 mm/min, returned and repeated the test using the same parameters. Parameters recorded from each test curve were fracturability, hardness, cohesiveness, gumminess, springiness, chewiness and resilience. These values represent standard calculations of curve attributes of Texture Profile Analysis described by Bourne, 1978. All the peaks were recorded as forces.Each tofu sample was analyzed in duplicate.

RESULTS AND DISCUSSION

Soymilk is essentially a water extract of soybeans and there are many variations on the basic soymilk processing steps. *Tofu* was made by coagulating soy milk, and then pressing the resulting curds into blocks. Four tested chemical coagulants with 10 soybean genotypes were used to coagulate the soymilk and the results showed that the concentration of soymilk and type of coagulant had a great influence on the properties of the *Tofu* gel. The results also confirmed that the use of a suitable concentration of the quick-acting coagulants is more critical than that of the slow-acting coagulants in tofu making. It was found that different coagulants produce tofu with different textural and flavour properties. Calcium Sulphate creates a bridge by which the soy proteins in the milk can aggregate.

Proximate composition of Soybean:

Figure 1 shows the proximate biochemical composition of different soybean varieties. The moisture contents of different soybean varieties were between 3.9 % to 10.0%. On a dry weight basis, the protein content of different varieties ranged from 31- to 36%. There was no clear relationship between tofu yield and soybean protein content; however, the firmness of tofu prepared from these varieties decreased as the protein content of the soybean decreased. These results are in general agreement with other findings, wherein it has been reported that soybean varieties high in protein content produced tofu with high yield and firmer texture (Shen, *et al.*, 1991).

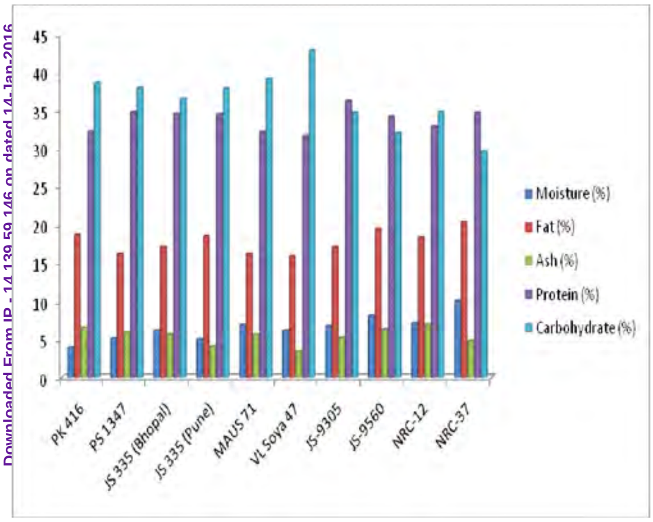

Fig. 1. Proximate composition of soybean genotypes used in preparation for *tofu*

Soymilk preparation using soybean genotypes:

Soymilk was prepared with hot grinding method using bean to water ratio of 1:8 and 1:6 for grinding. The soymilk prepared with 1:6 beans to water ratio was found to more suitable for tofu preparation and minimum loss through tofu whey. Under identical conditions of extraction, yield of soymilk from various varieties was different (Table1). Genotypes 'JS335' and 'PK416' gave maximum and minimum yield of soymilk, respectively**.** The total solids and protein content of soymilk prepared from different varieties differed significantly. Genotypes PS 1347yielded soymilk with highest total solids whereas protein content was maximum in soymilk prepared from genotype JS9305 followed by PS1347 and JS9305. The fat contents of various soymilk samples were statistically different. Soymilk prepared from genotype NRC 37 exhibited maximum fat content and variety VLsoya contains minimum fat content. Results obtained in this investigation demonstrated that soybeans containing greater amounts of protein, in general, yielded soymilk with higher protein content. Min, *et al.* 2005 reported the correlation coefficients between soybean protein and Nearly 27-39 percent of fat present in whole soybean is lost along the residue (okara), which is a by product of soymilk industry. The values in the present study are similar to those obtained by Wang, *et al.*, 1983, Lim, *et al.*, 1990 and Shen, *et al.*, 1991.

***Tofu* preparation by using chemical coagulants:**

Tofu was prepared by using 10 soybean genotypes with salt coagulants (magnesium chloride, calcium chloride, calcium sulfate and magnesium sulfate) at concentrations of 20,30,40,50 60, 70, 80 and 90 mM.Table 2 shows the optimal coagulating conditions for highest average yield of different soybean varieties. The highest tofu yield of different soybean varieties are between 232.6 to307 (g/100gm). The result indicates that magnesium chloride is effective with most of the varieties. Higher temperature above 80^0C is most suitable for coagulation in most of the processing conditions. Antioxidant properties of tofu prepared with different coagulants with soybean varieties were between 25 to 45 %. Highest antioxidant activity

Table.1. Yield, physicochemical properties and organoleptic characteristics of soymilk prepared from different soybean varieties*

Attributes	Genotypes									
	PK 416	PS 1347	JS 335 (Bhopal)	JS 335 (Pune)	MAUS 71	VL Soya 47	JS-9305	JS-9560	NRC-12	NRC-37
Yield of milk, (ml / 100g)	625	641	640	645	630	635	631	638	640	640
Total solids, %	10.95	11.23	11.12	11.20	10.75	10.30	11.38	11.40	10.82	10.95
Protein, %`	3.18	3.19	3.08	3.11	3.12	3.02	3.32	3.11	3.17	3.19
Fat , %	1.89	1.72	1.78	1.89	1.71	1.65	1.78	1.92	1.90	1.94
pH	6.23	6.29	6.21	6.22	6.27	6.34	6.37	6.29	6.23	6.31
TDS (g/l))	2.07	2.05	2.09	2.05	2.08	2.09	2.04	2.05	2.06	2.09
Flavour	7.17	7.13	7.17	7.1	6.7	6.8	6.9	6.8	7.11	7.12
Overall acceptability	7.3	6.9	7.07	6.7	6.5	6.7	6.3	6.3	6.8	6.9

*Results are the means of triplicates

Table. 2. Optimum coagulating conditions for tofu yield in soybean varieties*

Genotypes	Coagulating conditions	Moist Yield (g/100g soybean)	Antioxidant activity (% inhibition)
PK 416	MgCl2 (80 mM 90^0 C)	285.67	26.45
	MgCl2 (50 mM 60^0 C)	263.67	23.59
	MgCl2 (60 mM 70^0 C)	248.44	25.90
PS 1347	MgCl2 (40 mM 60^0 C)	246.20	33.66
	MgCl2 (90 mM 70^0 C)	223.32	29.34
	MgCl2 (30 mM 60^0 C)	203.60	32.67
JS 335 (Bhopal)	MgCl2 (80 mM 90^0 C)	297.67	24.5
	MgCl2 (90 mM 70^0 C)	279.81	23.66
	MgSO4 (30 mM 90^0 C)	262.17	20.50
JS 335 (Pune)	Ca SO4 (40 mM 90^0 C)	308.67	45.45
	Ca SO4 (40 mM 90^0 C)	288.41	39.25
	Ca SO4 (60 mM 60^0 C)	279.88	42.30
MAUS 71	MgSO4 (40 mM 90^0 C)	283.18	25.20
	MgSO4 (40 mM 90^0 C)	264.93	27.80
	MgSO4 (70 mM 90^0 C)	245.67	26.30
VL Soya 47	CaCl2 (90 mM 60^0C)	262.20	36.33
	Ca SO4 (30 mM 80^0 C)	237.94	32.00
	CaCl2 (90 mM 60^0C)	208.83	31.80
JS-9305	Mg SO4 (50 mM 70^0 C)	299.61	31.26
	Mg SO4 (60 mM 70^0 C)	273.46	32.90
	MgCl2 (30 mM 90^0 C)	258.33	29.60
JS-9560	Ca SO4 (90 mM 70^0 C)	252.31	33.00
	Ca SO4 (40 mM 70^0 C)	246.15	33.25
	Ca SO4 (50 mM 70^0 C)	231.33	30.78
NRC-12	MgCl2 (60 mM 70^0 C)	234.86	34.50
	MgCl2 (50 mM 70^0 C)	226.71	33.75
	MgCl2 (90 mM 70^0 C)	216.20	34.33
NRC-37	MgCl2 (70 mM 90^0 C)	263.88	39.25
	Mg SO4 (80 mM 90^0 C)	246.38	37.90
	Ca SO4 (80 mM 80^0 C)	227.23	41.75

*Results are the means of triplicates

is found in tofu prepared with varieties JS335 (Pune).The tofu was processed from the same soybean and the same batch of soymilk with all salt coagulants. A significant ($P < 0.05$) increase and decrease of protein and fat, respectively, was observed when soybean was processed into soymilk. Similar trend of significant increase and decrease in the content of protein and fat, respectively, was observed when the soymilk was coagulated into tofu irrespective of the source of coagulant. It could be assumed that the coagulants allow the release of fats during processing, probably suggesting that the processing method considerably decreases the fat-binding capacity of protein.

Tofu whey analysis:

The tofu whey is the byproduct *Tofu* and it composition is important for optimization of coagulating condition for higher yield, greater quality and least soya components loss through whey. The value of per cent transmittance of *Tofu* whey is directly indication of loss of soy compounds through tofu whey. pH, per cent transmittance, conductivity, total solid content and total dissolve solid (TDS) were determined for optimization of coagulating condition (Table 3 and Fig.2).The

Table.3. Physico-chemical analysis of *Tofu* whey prepared by using different coagulants*

Genotyes	Optimal Coagulating conditions	pH	Solid contents (%)	Conductivity (mS)	TDS (g/l)	Transmittance (%)
PK 416	MgCl2 (80 mM 90^0 C)	5.58	3.0	4.88	2.42	93
PS 1347	MgCl2 (40 mM 60^0 C)	5.38	3.2	7.38	3.48	92
JS 335 (Bhopal)	MgCl2 (80 mM 90^0 C)	5.63	4.8	7.46	2.45	97.5
JS 335 (Pune)	Ca SO4 (40 mM 90^0 C)	5.87	3.2	4.77	2.72	97
MAUS 71	MgSO4 (40 mM 90^0 C)	4.9	3.0	4.76	2.39	97
VL Soya 47	Ca SO4 (40 mM 80^0 C)	5.87	4.0	5.6	3.3	94
JS 9305	MgCl2 (90 mM 90^0 C)	5.49	5.0	21.87	6.93	76
JS 9560	Ca SO4 (30 mM 60^0 C)	6.15	4.8	4.11	2.76	66
NRC 12	MgCl2 (60 mM 70^0 C)	5.60	5	7.56	3.87	95
NRC 37	MgCl2 (70 mM 90^0 C)	5.64	3.1	10.54	5.26	92

*Results are the means of triplicates

whey volumes from each soymilk batch coagulated at different coagulants at concentrations (20mM to 90 mM) were compared. Whey from the batches with low concentration of $CaSO_4.2H_2O$ was cloudy and/or contained small fragile curd fragments which, when undisturbed, would settle to the bottom of the beaker. At an optimum coagulation condition amount of whey being pressed out of the tofu was lowest and the *Tofu* yield was highest.

The pH of the whey was found to affect with increases in coagulant concentrations with soybean genotypes. The change was slower after most of the protein bodies had coagulated. The pH of tofu whey was not dependent on the

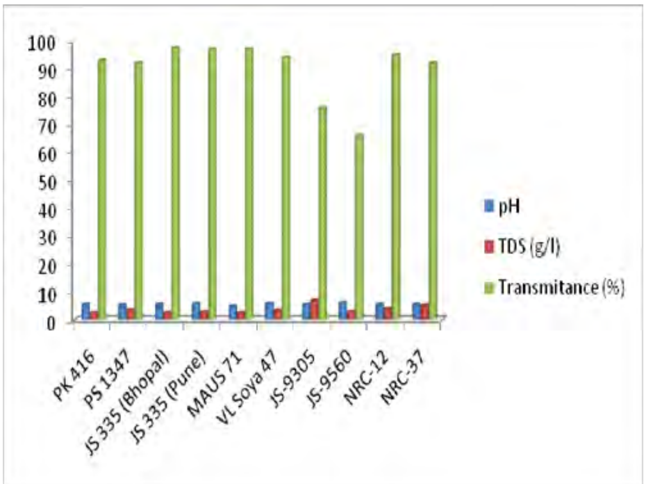

Fig. 2. Effect of coagulants on physic-chemical parameters of discharge tofu whey

temperature at which the coagulant was added. The transparency of the whey was measured at 400 nm with increasing concentration of coagulants. Per cent transmittance of the whey was not similar for all the coagulating conditions (concentration and temperature). Optimum clarity of whey transmittance, with cultivars of soybeans, depended on coagulant concentrations and the percent solids of soymilk.

Tofu prepared using the coagulants gave clear whey, indicating that the level of coagulants added was sufficient for complete coagulation of soy proteins. The solids in whey are most probably soluble sugars and low molecular weight protein (Lee and Rha. 1978). The variation in the whey volume was most likely due to a change in water holding capacity of tofu, which may be affected by coagulants (Lim, *et al.,* 1990). The coagulation and pressing process removes some carbohydrates, which results in an increase in protein content.

Antioxidant properties of *Tofu*:

Antioxidant property, especially radical scavenging activity is important in foods and in biological systems due to its deleterious role on free radicals. Excessive formation of free radicals accelerates the oxidation of lipids in foods and decreases food quality and consumer acceptance (Min, *et al.*, 2005). The increase in antioxidant activity may be due to the polyphenolic compounds present in acid coagulants which may contribute to increase in antioxidant activity of tofu. The antioxidant activity of tofu prepared with different varieties was found to different (Table 3). Soybean is a polyphenolic rich legume consumed worldwide ,and tofu is a widely consumed soybean product (Wu, *et al.*, 2004). Researchers have postulated that the health benefit of tofu may be due to a specific group of phenolic compounds found uniquely within soybean known as isoflavonoids. It may be due to its estrogenic effect or antioxidant activity (Lee, *et al.,*2004). Isoflavones are phytochemicals that exist in two basic categories, the aglycones and the glucosidic conjugates. The main glucosidic isoflavones are daidzin and genistin and the main aglycones are daidzein, and genistein. However, it is the aglycone (glucoside-free) form of isoflavonoids that is metabolically active, which also possesses higher antioxidant activity. Each gram of *Tofu* contains 0.532 mg of isoflavones. In another study, the total isoflavone content in raw *Tofu* and cooked *Tofu* was found to be 0.297 and 0.258 mg/g, respectively. Variation in isoflavone contents in tofu products was governed by the original content in soybeans and extent of loss in whey during recovery of soy curd.

Texture properties of *Tofu*:

For all used chemical coagulants, except for organic acids, significant correlations were found between tofu hardness and volume of separated whey and between the fracturability and gumminess of tofu (Table 4). On the basis of results coagulants can be classified into two groups – slow-acting coagulants such as CaSO4 and GDL, and quick-acting

Table. 4. Texture analysis of *Tofu* prepared from different coagulants*

Geno-types	Optimal Coagulating condition with highest yeild	Hard-ness (g)	Sprin-giness	Cohes-iveness	Chewi-ness (g)
PK 416	MgCl2 (80 mM 90^0C)	970	0.75	0.31	180
PS 1347	MgCl2 (40 mM 60^0C)	319	0.79	0.26	58
JS 335 (Bhopal)	MgCl2 (80 mM 90^0C)	855	0.68	0.33	196
JS 335 (Pune)	Ca SO4 (40 mM 90^0 C)	272	0.83	0.39	72
MAUS 71	MgSO4 (40 mM 90^0C)	690	0.80	0.36	112
VL Soya 47	Ca SO4 (40 mM 80^0 C)	208	0.59	0.31	76
JS-9305	MgCl2 (90 mM 90^0C)	720	0.73	0.28	213
JS-9560	Ca SO4 (30 mM 60^0 C)	158	0.62	0.26	78
NRC-12	MgCl2 (60 mM 70^0C)	335	0.75	0.31	211
NRC-37	MgCl2 (70 mM 90^0C)	670	0.67	0.30	210

*Results are the means of triplicates

coagulants such as $MgCl_2$ and $CaCl_2$. $MgCl_2$-tofu had on an average the highest hardness compared to other used coagulants. $MgSO_4$ was also shown to be very effective in producing hard tofu; however this was valid only when the coagulant was added at high temperature. The results also confirmed the well-known fact that the use of a suitable concentration of the quick-acting coagulants is more critical than that of the slow-acting coagulants in tofu making. Increasing coagulation temperature and coagulant concentration increased tofu hardness but decreased tofu yield.

Tofu is one of the most popular soy-products and is prepared by coagulating soymilk. The quality of *Tofu* depends on several parameters such as coagulation method, processing condition, texture, the content of two storage protein components glycinin and â-conglycinin and their ratio. All coagulants (salts and acids) used in investigation were able to coagulate the soymilk at selected concentrations (20 mM to 90mM). In used coagulants the highest tofu yield (308.36g/100g seed) was found in genotypes JS335 procured from Pune with CaSO4 (40 mM 90^0C, moisture content 75%, antioxidant 45%) followed by yield (299.6g/100g seed) in variety JS335 procured from CIAE, Bhopal with $MgCl_2$ (80 mm 90^0C, moisture content 72.27%, antioxidant 24.5 %). The most suitable temperature for tofu preparation was found to 80^0 C and 90^0 C. Depending on the type and concentration of coagulant used, as well as stirring during coagulation and pressure applied to the curd, tofu ranges in hardness from soft to firm with a moisture content of 70 to 90% and protein content of 5 to 16%. The pH of tofu whey was found to independent on the temperature. *Tofu* prepared with $MgCl_2$ (salt coagulant) and citric acid (acid coagulants) had highest hardness compared to other used coagulants. Use of a suitable concentration of the quick-acting coagulants is more critical than that of the slow-acting coagulants in *Tofu* making.

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