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Texture and Microstructure of Soybean Curd (Tofu) as Affected by Different Coagulants

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TEXTURE AND MICROSTRUCTURE OF SOYBEAN CURD (TOFU) AS AFFECTED BY DIFFERENT COAGULANTS

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Abstract

The coagulating properties of five coagulants and the nature of the curd obtained from soymilk was investigated. Viscosity changes during coagulation were studied using a Nametre Vibrating Sphere Viscometer and texture measurements were made by compression and computer assisted analysis. pH and amount of solids in the whey were determined. The microstructure of the tofu was examined by scanning electron microscopy. It was observed that $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ coagulated the milk instantly while $CaSO_4.1/2H_2O$, glucono delta lactone (GDL) and $MgSO_4.7H_2O$ acted comparatively slowly. The texture of the curd was greatly influenced by type and concentration of coagulant. Curd obtained with $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ was coarse, granular and hard, whereas $CaSO_4.1/2H_2O$ and GDL (fresh solution) gave a very smooth, soft and uniform curd. Among the five coagulants studied, 0.75% $CaSO_4$ and 0.4% GDL (fresh solution) appeared to be most suitable for making tofu of high bulk weight and smooth texture.

Key Words: Soybean curd, tofu, texture, viscosity, microstructure, coagulation, scanning electron microscopy, calcium salts, magnesium salts, glucono-δ-lactone.

Introduction

Tofu, a soybean derived curd, is a low-cost, high protein product which has been widely used in the Orient. In a study by Muto et al. (1963), tofu was judged to be nutritionally equivalent to the protein derived from a mixture of eggs, fish and liver. Depending on the kind 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%.

Making tofu is a relatively simple process but due to its bland nature, its textural properties play a big role in influencing quality and consumer acceptability. Shurtleff and Aoyagi (1984) presented a good review on the manufacturing of tofu. The variety of soybeans used may affect the quality of the tofu (Kamel and deMan, 1982; Skurray et al., 1980) and this is considered to be due to differences in protein content of the soybeans and the ratio of 7S and 11S proteins. Saio (1979) reported that higher solids in soymilk correlated with harder tofu and increasing coagulating temperature with increased hardness of tofu. Recently, Wang and Hesseltine (1982) investigated some of the coagulating conditions in tofu processing and reported that to obtain a good curd, the concentration required for commonly used salts was in the range of 0.01 to 0.1M.

One of the most important factors in determining the texture of tofu is the selection and addition of a coagulant at the proper concentration. This study was conducted to get more detailed information on the coagulating properties of different coagulants and the nature of the curd obtained under different conditions. The curds prepared in the laboratory were compared with some commercial tofu samples.

Materials and Methods

Preparation of tofu

The yellow hilum Ontario soybeans used in this study contained 10% moisture, 16.8% fat and 36.6% protein. The soybeans were made into milk by the following procedure: 300 g of beans were soaked overnight at 20°C. The soaked beans were drained, rinsed and blended for 4 min at high speed in a Waring blender with 750 ml of water. The resultant slurry was mixed with 800 ml of boiling water and strained through a filter cloth. The soymilk contained 10% total solids with 4.7% protein and 2.5% fat. Fresh soymilk was used to make tofu.

The following coagulants were used: $CaSO_4.1/2H_2O$ or plaster of Paris; $CaCl_2.2H_2O$; $MgCl_2.6H_2O$; $MgSO_4.7H_2O$ and glucono-δ-lactone (GDL). To make tofu, 300 ml of fresh soymilk was heated to near boiling and the required amount of coagulant dissolved or suspended in 7.5 ml of water. The hot soymilk and coagulant were poured simultaneously into a glass container ensuring good mixing without stirring. The curd was left to set for 15 min and then transferred to a perforated plastic container with a diameter of 9 cm and lined with a filter cloth. The curd was pressed by applying weights (31.4 g/cm^2) for 15 min. After pressing, the curd was left in running water for 1 h and then stored in a refrigerator.

Viscosity

A Nametre vibrating sphere viscometer (Nametre Co., Edison, N.J.) was used to follow changes in viscosity. The hot soymilk and coagulant were poured simultaneously into a glass container ensuring good mixing, and the vibrating sphere was immediately immersed to the mark. The kinematic viscosity was measured as a function of time. This provides a non-destructive method of measuring changes in viscosity.

Texture

For texture evaluation, the mechanical part of an Instron Universal Testing Machine was used. The original load sensing mechanism was replaced with a Daytronic load cell (cap. 12 kg) and a Daytronic 9000 strain gage amplifier-indicator (Daytronic Corp., Miamisburg, OH). The signal voltage was fed to an A-D converter (AII3 Interactive Structures, Bala Cynwyd, PA) and from there to an Apple IIe computer. The instrument output was stored on floppy disks and analyzed using a program developed by the Statistical and Engineering Research Institute, Agriculture Canada, Ottawa, Ont. (Buckley et al., 1984). The information obtained by this system included: peak force (N), time to peak (s), deformation to peak (mm), firmness (N/mm), and force at different points (N). Cylindrical samples were prepared from the curd with a boring tube and wire cutter, sample dimensions were 20 mm diameter and 20 mm height. Samples were compressed by a flat plate to 50% deformation using a crosshead speed of 10 mm/min. Peak force at 50% compression was measured as well as force at 25% deformation.

Moisture

For moisture determination about 100g of tofu was homogenized in a blender and 3–5g dried on a steam bath for 15 min followed by forced air oven drying at 98°-100°C overnight. Total solids in the whey was determined by drying for 15 min on a steam bath and 3 h in the oven at 98°-100°C.

pH

pH of the whey was measured using a Fisher Accumet pH meter model 825 MP.

Scanning Electron Microscope Observations

A scanning electron microscope (ETEC Autoscan) was used to examine the fine structure of tofu coagulated with different coagulants. The procedure used for sample preparation was that of Saio (1981) with some modifications. Small pieces of (< 2 mm cube) were fixed at room temperature with 5% glutaraldehyde in 0.1M phosphate buffer (pH 6.7) for 90 min. After five washes in 0.1M phosphate buffer (pH 6.7) at 10 min intervals, they were postfixed in 1% osmium tetroxide in the same buffer for 90 min at room temperature. The fixed samples were rinsed five times with phosphate buffer at 10 min intervals. Dehydration was done using a 10% incremental ethanol series, leaving samples at each concentration for 15 min followed by

three rinses with 100% ethanol. The samples were then rinsed three times with chloroform. Critical point drying (CPD) was conducted using CO_2.

For freeze drying, the samples were dehydrated using the ethanol series, frozen in liquid nitrogen and transferred to a Polaron E5300 freeze drier and dried for 24 h.

All of the samples were mounted on stubs and sputter coated with 20–30 nm of gold palladium (60:40) using a Technics Hummer V Sputter Coater. The observations were made at 10 kV.

Making of commercial tofu

Tofu was made in a commercial tofu plant (Victor Food Products Ltd., Toronto, Ont.) by a semiautomatic process using the optimum concentration of coagulants based on laboratory experience.

Results and Discussion

Results are reported only for those concentrations of coagulants which gave curds with clear or nearly clear whey. The minimum coagulant concentration required was 0.5% $CaSO_4.1/2H_2O$, 0.15% $CaCl_2.2H_2O$, 0.3% $MgSO_4.7H_2O$, 0.2% $MgCl_2.6H_2O$ or 0.3% GDL.

Viscosity

The coagulation rates as measured with the Nametre vibrating sphere viscometer are shown in Table 1. The coagulation rate was very rapid with $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$ and visible whey separation occurred at an early stage. A more gradual increase in viscometer readings was obtained with $CaSO_4.1/2H_2O$, $MgSO_4.7H_2O$ and GDL. With these coagulants up to 10 min were required for curd formation and no whey separation was observed. The recordings of viscosity when using different concentrations of $CaSO_4$ are presented in Fig. 1.

Saio (1979) reported that GDL only coagulates soymilk when heated. GDL activity is influenced by temperature and time of preparation. Curd obtained by addition of fresh cold GDL solution to hot soymilk (near boiling) was very smooth and similar to curd obtained with $CaSO_4$. When the GDL solution was left at room temperature for 30 min, a very hard curd was obtained and even harder curd resulted with a hot (90–95°C) GDL solution. In the latter case, the curd was grainy, less cohesive and similar to curd made with $MgSO_4$. The active coagulant in GDL is gluconic acid and when a freshly prepared GDL solution is aged more gluconic acid is formed. The pH of a 0.4% GDL solution dropped from 3.0 after 10 min to 1.7 after 4 h. GDL has been reported to recover more protein in the tofu (Shurtleff and Aoyagi, 1984) and is used for making silken tofu.

Texture

Results of textural evaluation are presented in Table 2. Reported values are means of six replicates. Peak force values for $CaSO_4.1/2H_2O$ produced curd are not reported because the samples fell apart before reaching 50% compression. The hardest curd was obtained with 0.5% $MgCl_2.6H_2O$. It appears from these data that curd firmness can be affected by using various coagulants at different concentrations.

pH

According to Lu et al. (1980) pH, not the calcium ion concentration, is by far the most important factor in the precipitation of soy protein. These authors reported that protein starts to coagulate when the pH drops to about 6.0, therefore, according to Lu et al. (1980), the addition of salt should be stopped when the pH approaches 6.0. In this study, with all the coagulants except GDL, the pH of whey was in the range of 5.89 to 6.25
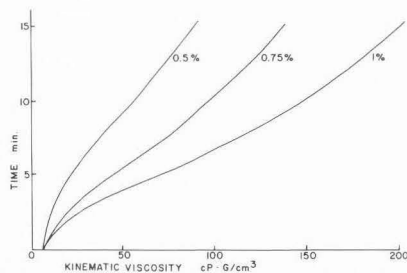
Fig. 1. Development of kinematic viscosity with time of soy-milk with 0.5%, 0.75% and 1.0% added $CaSO_4.1/2H_2O$.

Table 1. Effect of coagulant type and concentration on viscosity of soymilk.

Coagulant	Concentration		Kinematic viscosity $(cP.g/cm^3)$ at time (min)			
	%	M	0	5	10	15
$CaSO_4.1/2H_2O$	0.50	0.034	7.3	29.8	72.8	106.3
	0.75	0.052	8.5	45.9	98.3	135.3
	1.00	0.069	9.3	70.5	153.0	213.5
$CaCl_2.2H_2O$	0.15	0.010	20.9	19.6	24.4	29.8
	0.20	0.014	23.5	22.0	25.1	30.1
$MgSO_4.7H_2O$	0.30	0.012	27.8	41.8	55.0	68.4
	0.40	0.016	45.0	83.5	105.0	128.0
$MgCl_2.6H_2O$	0.20	0.010	12.2	24.5	39.0	52.1
	0.30	0.015	31.2	27.8	35.8	44.6
	0.50	0.025	40.3	33.7	42.2	51.8
GDL (heated)	0.30	0.017	12.0	28.3	52.0	75.0
	0.40	0.022	13.0	53.5	91.0	117.5
GDL (fresh solution)	0.40	0.022	3.9	81.7	172.0	244.0

(Table 3) and even with the same pH of whey, the coagulants behaved differently. For example, the curd obtained with 0.3% $MgCl_2.6H_2O$ was three times harder than the curd obtained with 0.5% $CaSO_4.1/2H_2O$ although the pH of whey was 6.03 in both cases.

Moisture content of tofu and solids in whey

Results in Table 3 show that the coagulant used affects the amount of whey liberated and, therefore, the weight and moisture content of the final product. With increase in coagulant concentration, there was a decrease in moisture content of the tofu. With increase in coagulant concentration, the structure of tofu became more porous separating more whey and leaving less moisture in the tofu.

There was a general trend towards decrease in solids in the whey with increase in coagulant concentration. However, the difference was not significant. The solids content of the whey increased dramatically when the coagulant concentration used was lower than the minimum concentration listed in Table 3.

Scanning electron microscopy

Figures 2a and 2b are micrographs of the critical-point dried

Table 2. Effect of coagulant type and concentration on texture of curd.

Coagulant	Conc. %	Peak force (N)	Force at 25% compression (N)	Firmness (N/mm)
$CaSO_4.1/2H_2O$	0.50	− *	0.47	0.11
	0.75	−	0.54	0.14
	1.00	−	0.87	0.21
$CaCl_2.2H_2O$	0.15	1.09	0.54	0.14
	0.20	2.66	1.34	0.30
$MgSO_4.7H_2O$	0.30	0.84	0.44	0.11
	0.40	1.97	0.96	0.25
$MgCl_2.6H_2O$	0.20	0.80	0.44	0.09
	0.30	2.88	1.43	0.36
	0.50	3.25	2.37	0.62
GDL (heated)	0.30	1.13	0.58	0.15
	0.40	2.52	1.26	0.32
GDL (fresh solution)	0.40	1.24	0.76	0.19

*Samples disintegrated before 50% compression was reached.

Table 3. Effect of coagulant type and concentration on pH of whey, solids in whey, amount of whey and % moisture of tofu.

Coagulant	Conc. %	pH of whey	Solids in whey %	Whey %	Moisture of tofu %
$CaSO_4.1/2H_2O$	0.50	6.04	3.1	16.17	91.0
	0.75	5.97	3.2	17.50	89.4
	1.00	5.94	3.0	18.93	89.1
$CaCl_2.2H_2O$	0.15	5.98	3.3	36.57	87.4
	0.20	5.94	3.3	45.33	86.4
$MgSO_4.7H_2O$	0.30	6.09	3.2	33.11	88.2
	0.40	6.07	3.2	47.83	86.4
$MgCl_2.6H_2O$	0.20	6.25	3.2	40.00	88.4
	0.30	6.03	3.1	56.23	85.0
	0.50	5.89	2.7	59.67	82.8
GDL (heated)	0.30	5.52	3.5	42.10	89.2
	0.40	5.27	3.5	49.33	84.8
GDL (fresh solution)	0.40	5.41	3.3	19.43	88.5

(CPD) and freeze-dried (FD) tofu coagulated with 0.4% fresh GDL. The network structure appeared to be similar in the two pictures, although the CPD sample seemed to have shrunken considerably. CPD has been shown to cause shrinkage (Cohen, 1977). Freeze drying appeared to be more appropriate for observing tofu structure. In Figures 3 and 4, SEM micrographs of tofu coagulated with different coagulants show clearly different fine structures. The microstructures as indicated in these pictures can be easily related to the visually observed texture. Tofu obtained with GDL (fresh solution) was judged best in texture on the basis of smoothness, and the micrograph showed a fine and uniform honeycomblike structure (Fig. 3a). The structure was very uniform with smaller holes than those prepared
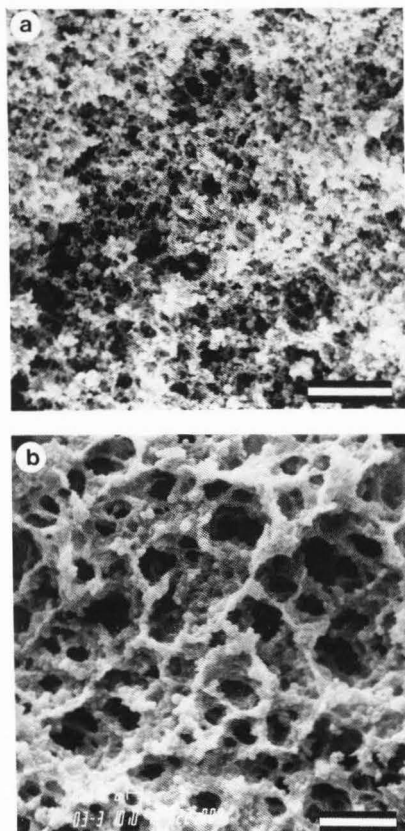
Fig. 2. Comparison of critical point drying (a) and freeze drying (b) of tofu coagulated with 0.40% GDL (fresh solution.) (Bar = 5 μm).

with $CaCl_2.2H_2O$, $MgCl_2.6H_2O$ and $MgSO_4.7H_2O$. Coagulation with $CaSO_4.1/2H_2O$ gave a structure similar to that obtained with GDL but less uniform. The SEM pictures taken at higher magnification (Fig. 4) of curd made with $MgCl_2.6H_2O$ and $CaCl_2.2H_2O$ appear to show similar structures. The networks of these samples were not as fine and continuous as those obtained with GDL and $CaSO_4.1/2H_2O$. $MgSO_4.7H_2O$ gave a more continuous and uniform structure than curd prepared with $CaCl_2.2H_2O$ and $MgCl_2.6H_2O$.

Commercial tofu

The process used in the plant was basically the same as used in the laboratory. However, in the plant the curd is broken up

Table 4. Texture and moisture content of some commercial tofu samples.

Sample	Coagulant	Peak force (N)	Force at 20% compression (N)	Firmness (N/mm)	Moisture %
A	$CaSO_4$	1.47	0.93	0.22	87.57
B	$MgSO_4$	3.08	1.76	0.45	83.41
C	$CaSO_4$	1.12	0.82	0.17	88.68
D	$MgSO_4$	3.68	2.06	0.45	83.60
E	$CaCl_2-$ $CaSO_4$	11.19	6.23	1.63	70.05
F	$CaCl_2-$ $CaSO_4$	10.63	5.44	1.64	73.79

during the transfer to the press. Due to this difference, the moisture content of tofu made in the plant was slightly lower than tofu made in the laboratory and turned out to be harder (Table 2 and Table 4).

For good curd production and clear whey formation, the concentration of coagulants on a molarity basis ranged from 0.01 to 0.02M except for $CaSO_4.1/2H_2O$ which required a minimum of 0.03M. It is not feasible to decide on a common optimum concentration for all of the coagulants as has been pointed out in some other studies (Wang and Hesseltine, 1982; Tsai et al., 1981). Tsai et al. (1981) noticed a dramatic change in the texture of tofu when increasing the concentration of coagulants above 0.03N (0.015M). The present study reemphasizes the different behaviour of various coagulants.

Table 4 lists results of texture and moisture analyses of some commercial tofu samples. Texture and moisture content of samples A, B, C and D fall in the range obtained with the laboratory made tofu.

It has been suggested that the coagulation of soymilk is due to the crosslinking between protein molecules by divalent cations (Saio et al., 1967). However, the site of crosslinking is still under debate. Saio et al. (1967) suggested that the free carboxyl group of soybean protein is the major site of calcium binding and phytic acid also acts as a binding site. According to Appurao and Narasinga Rao (1975) a probable binding site on the protein molecules is the imidazole group. In addition to uncertainties about the binding sites of the soy proteins, there is a lack of understanding of the mechanism of coagulation with GDL.

Acknowledgements

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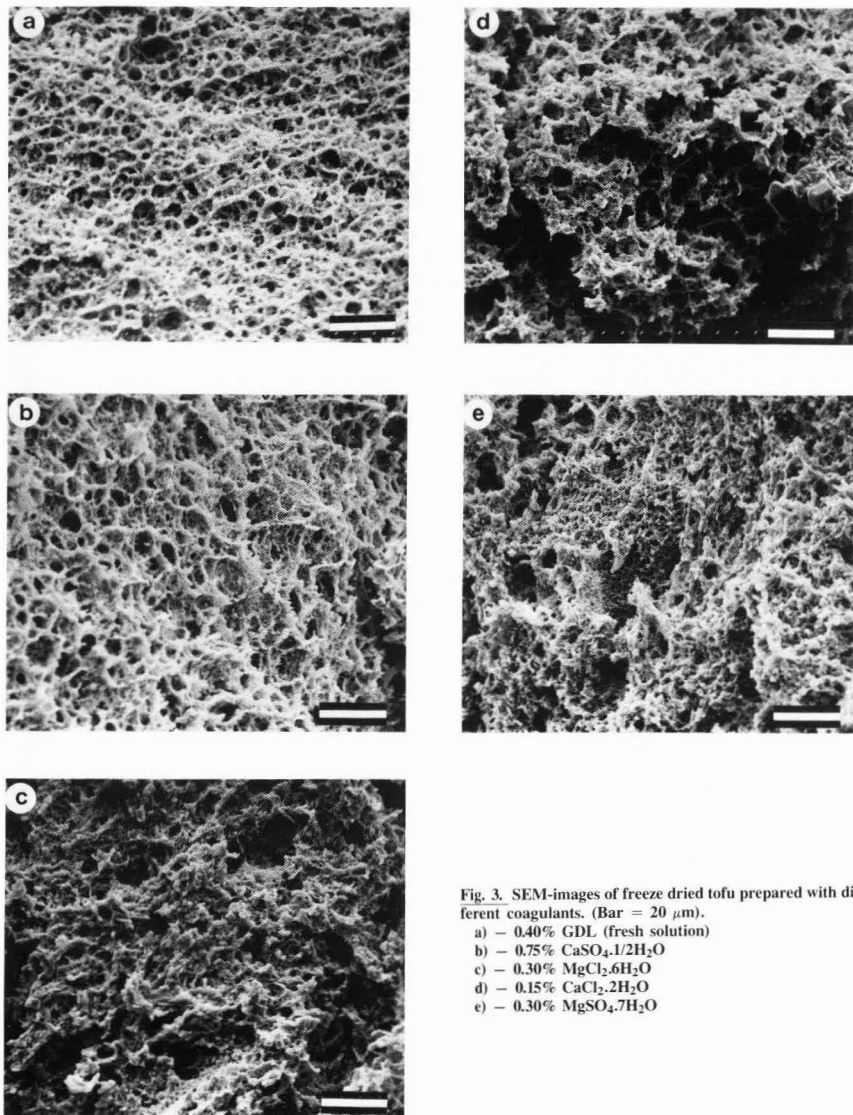
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Fig. 3. SEM-images of freeze dried tofu prepared with different coagulants. (Bar = 20 μm).
a) — 0.40% GDL (fresh solution)
b) — 0.75% $CaSO_4.1/2H_2O$
c) — 0.30% $MgCl_2.6H_2O$
d) — 0.15% $CaCl_2.2H_2O$
e) — 0.30% $MgSO_4.7H_2O$
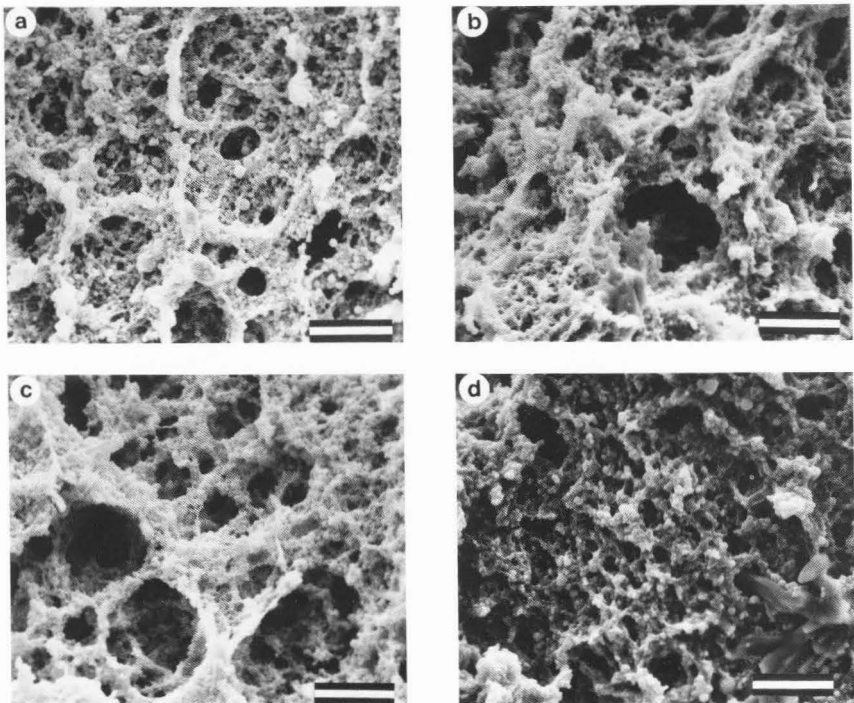
Fig. 4. SEM-images of freeze dried tofu prepared with different coagulants. (Bar = 5 μm).
a) – 0.75% $CaSO_4.1/2H_2O$
b) – 0.30% $MgCl_2.6H_2O$
c) – 0.15% $CaCl_2.2H_2O$
d) – 0.30% $MgSO_4.7H_2O$

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Discussion with Reviewers

W.J. Wolf: Is pH a factor in the coagulation of tofu with GDL? The pH values in Table 3 for GDL are all significantly lower

than for any of the other coagulants. Moreover, pH values of whey obtained by GDL coagulation are all below 6 and are approaching the isoelectric range for the proteins.

Authors: GDL acts by opening of the lactone ring to form gluconic acid. This occurs when GDL is dissolved in water even at room temperature as is demonstrated in the paper by monitoring the pH of a GDL solution at room temperature. Saio (1979) reported that GDL-solution should be added to cold soymilk and then reheated. In industry this means cooling of the soymilk and then reheating with GDL solution. It is better to dissolve the required amount of GDL in water just before addition to a batch of hot soymilk coming from the production line as is done with the other coagulants. The release of gluconic acid at that temperature results in a very uniform curd as shown in the SEM photograph. When the GDL solution is left at room temperature for a longer time the reaction with hot soymilk is like acid precipitation and produces a coarse curd.

Reviewer III: How do you define texture and what proof is there that "peak force," "force at 25% compression" and "firmness" measure texture?

Authors: Texture can be defined as "The way in which the various constituents and structural elements are arranged and combined into a micro- and macrostructure and the external manifestations of this structure in terms of flow and deformation." Instrumental analysis of texture involves measurement of mechanical properties such as resistance to deformation, in this case peak force and force at 25% compression and also the stress/strain ratio which is defined as firmness.

K. Saio: As shown in Fig. 4 the hardness of tofu is influenced by the concentration and kinds of coagulants. Japanese like tofu because of its texture and bland flavor. Different coagulants are used for various tofu types, e.g., $CaCl_2$ for kori tofu, $CaSO_4$ or $MgSO_4$ or $Mg\ Cl_2$ (with phosphoric or citric acid) for hard tofu and GDL and $CaSO_4$ (alone or with GDL) for silken tofu. It was mentioned in the paper that tofu coagulated with GDL had the best smooth, soft and uniform texture but do you think North Americans prefer such silken tofu to the hard kind?

Authors: From our experience it appears that North Americans prefer the firmer styles of tofu. These seem to be more suitable for western style cooking and are preferred in salads.

K. Saio: Is it possible to distinguish the differences of coagulation state among soybean varieties with a Nametre vibrating sphere viscometer?

Authors: We have tested this on 17 different varieties of soybeans grown in Ontario, and found no significant differences between them. However, these soybeans were harvested at the same time and stored under identical conditions. We suspect that storage conditions have a greater effect than varietal differences and this is now being investigated.