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Development of a Rapid Titration Method for Predicting Optimal Coagulant Concentration for Filled Tofu

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A rapid titration method was developed for predicting the optimal coagulant concentration for making filled tofu. Cooked soymilk (350 mL, 20 °C) in a 400 mL beaker was stirred by a magnetic stirrer to form a swirl. The quick-acting coagulant solution (20.0 Brix) was added into the soymilk at 1.0 mL/min. The swirl depth decreased when the soymilk viscosity increased as a result of increasing the concentration of coagulant in the soymilk. At a suitable stirrer speed, the swirl finally disappeared but the soymilk still maintained rotation, and then the swirl reappeared after around 1 min. The critical point of coagulant concentration (CPCC) was calculated on the basis of the volume of coagulant consumed to get the swirl to disappear. The influences of several factors on the CPCC were investigated, including coagulant addition rate, soymilk temperature, soymilk concentration, soymilk volume, stir bar length, and container size. For validation, 33 soybean samples were used to determine their CPCCs and make filled tofus. The results indicated that CPCC was a characteristic parameter of soymilk and could be used as an effective indicator for predicting optimal coagulant concentration.

KEYWORDS: Rapid method; tofu; soymilk; coagulant; soybean; viscosity; swirl

INTRODUCTION

The amount of coagulant added into soymilk or the concentration of coagulant in soymilk affects profoundly the yield and textural and flavor properties of resulting tofu (*1*−*8*). The optimal coagulant concentration for tofu making is affected by soybean materials, which are affected by genetics and postharvest storage conditions (*2*, *9*, *10*). Maintaining a stable supply of the soybean materials with a constant quality is almost impossible for the tofu manufacturers (*11*). In practice, to make consistent quality and high yield tofu products, the amount of coagulant added to soymilk needs to be adjusted in accordance with varied soybean materials. This often results in wasting at least the first batch of soymilk caused by trial and error.

There are a few methods for determining the optimal coagulant concentration while making tofu. Since tofu quality usually depends on texture, tofu texture and tofu yield are the ultimate parameters that are used to determine the optimal coagulant concentration and other processing conditions. However, tofu making, even if it is performed at a small laboratory scale, is a time-consuming and labor-intensive procedure. In addition, a textural analyzer is needed for measuring objective textural properties.

For pressed tofu, whey is produced after soymilk coagulation or tofu pressing. The light transmittance, volume, or pH of the whey could be used as indirect parameters to judge whether the coagulant concentration used is optimal (*10*, *12*). The optimal concentration could be reached by a stepwise approach.

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Ohara and co-workers (*13*) found that the conductivity of coagulating soymilk linearly increased with a steady addition of 1 M calcium chloride to soymilk at 63 °C, and the curve of the conductivity as a function of calcium chloride volume consisted of two straight lines with different slopes. They defined the intersection or the break point as "point of critical concentration of coagulant" (*X* mL). By correlating *X* with the transparency of whey or the hardness of tofu, it was concluded that the *X*-value could be an effective indicator for determining the optimal coagulant concentration (*14*). Ohara and co-workers (*15*) also investigated the rotational viscosity change of soymilk at 60 °C while 1 M $CaCl_2$ solution was continuously added. MV (maximum viscosity) and MVT (time for MV, or $CaCl_2$ concentration for MV) were adopted to evaluate the coagulation process of soymilk for "kori" tofu. For a series of soymilk prepared from 19 varieties of soybeans, MVT correlated with *X* mL ($r = 0.86$, $p < 0.01$) and MV correlated with the hardness of tofu (0.5% GDL) ($r = 0.62$, $p < 0.01$). Compared to the conductivity method, the viscosity method is more practical (*16*). However, the continuous recording viscometer system as designed by Ohara and co-workers (15) for measuring and recording torque is costly and has not been used widely. A simple and rapid method for predicting optimal coagulant concentration without using costly equipment could benefit not only tofu makers but also tofu researchers and soybean breeders who want to accurately evaluate the tofu-making qualities of different soybean varieties.

Coagulants are classified into two groups (*17*). One is slow-acting coagulants such as $CaSO_4$ and glucono-δ-lactone (GDL),

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Table 1. Coagulant Solution Volumes Added to 100 mL of Soymilk for Making Filled Tofu and Equivalent Coagulant Concentrations in Soymilk[a]

	condition				
	C_{+2}	C_{+1}	C_0	C_{-1}	C_{-2}
coagulant volume (mL)	Y/3.5 + 0.2	Y/3.5 + 0.1	Y/3.5	Y/3.5 – 0.1	Y/3.5 – 0.2
equivalent concentration (mM)					
$MgCl_2$	CPCC + 2.82	CPCC + 1.41	CPCC	CPCC – 1.41	CPCC – 2.82
$CaCl_2$	CPCC + 2.50	CPCC + 1.25	CPCC	CPCC – 1.25	CPCC – 2.50

[a] Y is coagulant solution volume (mL) measured for 350 mL of soymilk by the titration method.

and the other is quick-acting coagulants such as $MgCl_2$ and $CaCl_2$. $MgCl_2$ can make the most delicious tofu, prized for subtly sweet flavor and aroma (*18*), which is preferred by worldwide consumers (*17*). It is well-known 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.

The production of filled tofu (or packed tofu), which has been increasing in recent years, has been the most automated among all types of tofu. No whey is produced during filled tofu making. Coagulant is added at a low temperature, and gelation is not expected during the addition of coagulant. The formation of curd takes place when the box filled with soymilk containing coagulant is heated at 85−90 °C for a period of time. Therefore, the reported methods, in which the whey, the conductivity, or the viscosity are measured during tofu making, are not applicable for determining the optimal coagulant concentration in the filled tofu system.

Our objective was to develop a rapid, simple, and reliable method based on the soymilk's swirl changes that were influenced by the apparent viscosity changes to predict the optimal concentrations of the quick-acting coagulants for making filled tofu.

MATERIALS AND METHODS

Materials. Soybeans of the Proto cultivar were provided by Sinner Brothers and Bresnahan Co. (Cassleton, ND, U.S.A.) and used for all of experiments in this study. For the experiment of validation, 33 soybean samples were used. They were different varieties and/or harvested in different years. Most of soybean samples were grown in North Dakota, Minnesota, or Iowa. After harvest, soybeans were stored in an air-conditioned laboratory at approximately 22−24 °C. Coagulant solutions of 20.0 Brix (20 °C) were prepared with reagent grade $MgCl_2 \cdot 6H_2O$ (EM Science, Gibbstown, NJ) or $CaCl_2 \cdot 2H_2O$ (Fisher Scientific, Fair Lawn, NJ). The molar concentrations of the 20.0 Brix coagulant solutions were 1.41 M for $MgCl_2$ and 1.25 M for $CaCl_2$.

Preparation of Soymilk. Soybeans were washed and soaked in tap water for 12−14 h at water temperatures varying from 18 to 21 °C. The hydrated beans were drained, rinsed, and ground with tap water (bean-to-water ratio was 1:7 unless otherwise specified) using a soymilk grinder (Chang-Seng Mechanical Company, Taoyuan, Taiwan), which was equipped with a centrifugal 100-mesh screen to separate raw soymilk automatically from the residues. Raw soymilk was cooked on a stove with constant stirring to boiling, kept boiling for 5 min, and then cooled to 20 °C for determining the critical point of coagulant concentration. The other portion of the cooked soymilk was cooled further to 4 °C in an ice−water bath for making filled tofu. The cooked soymilk was referred to as soymilk hereafter in this manuscript.

Chemical Compositions. The soymilk Brix was measured at 20 °C by an Auto Abbe Refractometer (model 10500, Leica Inc., Buffalo, NY). The soymilk solid content was measured by drying 10 g of soymilk at 105 °C until a constant weight. The soybeans were ground with a Tekmar (A-10) Analytical Mill (Tekamar Company, Cincinnati, OH) to pass through a 60-mesh sieve. Soybean moisture content was measured by a vacuum oven method (AOAC 925.09, 1990). The crude protein content was determined by the Kjeldahl method (AOAC 955.04, 1990) using a factor of 6.25 to convert nitrogen to crude protein content.

Determination of Critical Point of Coagulant Concentration (CPCC). A portion of 350 mL of cooked soymilk (20 °C) was placed in a 400 mL beaker (PYREX) with a flat and smooth bottom and stirred at varying speeds from 350 to 600 rpm to form a swirl by a magnetic stirrer (Digital Hot Plate/Stirrer, model 721, PMC, San Diego, CA) with a Teflon-coated magnetic stirring bar (8 mm $\phi \times$ 50 mm). The coagulant solution of 20.0 Brix was added continuously into soymilk at a constant rate (1.0 mL/min unless otherwise specified) by a peristaltic pump (model tris, ISCO, Lincoln, NE), which was connected to a coagulant-holding buret (capacity 10 mL, graduation accuracy 0.05 mL, KIMAX brand). It was important to make sure that there was no air bubble between the upper surface of the solution in the buret and the outlet of the tubing. At the exact moment the soymilk swirl disappeared, the pump was turned off, so that the volume of coagulant solution consumed (Y mL) could be read from the scale of the buret. CPCC was calculated from Y value as follows:

$$\text{CPCC (mM)} = 1000 \times \frac{Y}{350 + Y} \times \text{molar concn of coagulant solution}$$

Determination of Viscosity of Coagulating Soymilk. A viscometer (Synchro-Lectric, model LVT, Brookfield, Stoughton, MA) was used in conjunction with the Brookfield Helipath, which permits the measurement of apparent viscosity of undisturbed structure by slowly lowering a T-shaped spindle at 12 rpm through the coagulating soymilk. The coagulating soymilk was prepared as described in the measurement of CPCC. $MgCl_2$ was added continually by the peristaltic pump to 350 mL of soymilk at 20 °C in a 400 mL beaker, and the coagulating soymilk was stirred by a magnetic stirrer at 500 rpm. After addition of a certain amount of $MgCl_2$ solution, the T-shaped spindle was immediately immersed into the coagulating soymilk at the center position, and the viscometer and helipath stand was turned on. The dial reading was taken at 20 s after completion of the addition of $MgCl_2$ solution. The dial readings were converted to centipoises values by multiplying by a factor of 15.6 (*19*). Data are reported as "apparent viscosity" in view of the thixotropic properties of the coagulating soymilk. After viscosity measurement, the content in the beaker was discarded. The experiment was repeated with gradually increasing amount of $MgCl_2$ to establish the curve of apparent viscosity vs $MgCl_2$ concentration.

Preparation of Filled Tofu. A portion of 100 mL of soymilk in a 250 mL beaker (KIMAX) was cooled to 4 °C in an ice bath and stirred at 400 rpm by a magnetic stirrer (Digital Hot Plate/Stirrer, 7 model 721, PMC, San Diego, CA) with a 8 mm $\phi \times$ 50 mm stir bar. A certain amount of coagulant solution (20.0 Brix) was added to the soymilk by a pipettor within 2−3 s. As shown in **Table 1**, the coagulant solution volumes added or the equivalent coagulant concentration in soymilk were based on Y or CPCC determined by the titration method developed. The soymilk was mixed with the coagulant solution for 1 min. After mixing, the beaker with soymilk was immediately covered using aluminum foil and then immediately heated for 1 h in a water bath at 80 °C with the level of water exceeding the level of soymilk in the beaker. After coagulation, the filled tofu in the beaker was cooled to about 20 °C in an ice−water bath and then kept at the room temperature until next day for texture measurement.

Determination of Textural Properties of Filled Tofu. The textural properties were measured using an Instron Universal Testing Machine (model 1011, Instron, Canton, MA). The tofu was carefully removed

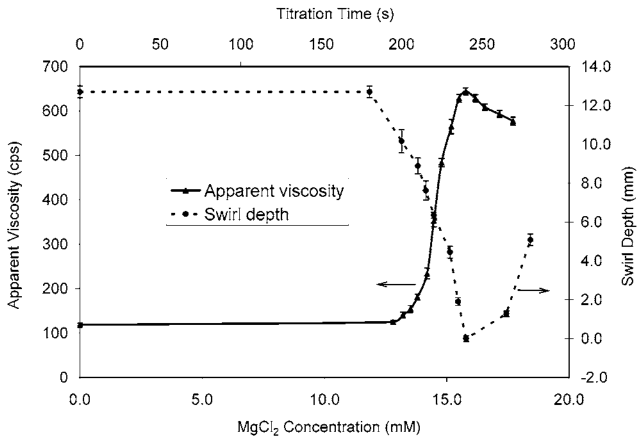

Figure 1. A typical relationship between the swirl depth and soymilk viscosity during titration with $MgCl_2$. Soymilk volume, 350 mL; $MgCl_2$ addition rate, 1.0 mL/min; soymilk temperature, 20 °C; soymilk concentration: 10.5 Brix; soybean, Proto (2001).

from the beaker. A sample of 44 mm diameter and 15 mm height was cut from the central portion of the tofu cake with a stainless steel cylindrical cutter. A cylindrical plunger of 100 mm diameter and a load transducer of 5 kg were used. The speed of the crosshead was set at 200 mm/min. A uniaxial compression test was performed. Breaking strength (or breaking stress) of the tofu samples was calculated from the load value at a breaking point divided by the initial cross-sectional area of the tofu sample. Breaking strain was determined as the ratio of the deformation at a breaking point to the initial height. The apparent Young's modulus was defined as the ratio of the breaking strength to breaking strain (*20*).

Statistical Analysis. All experiments were conducted in duplicate. Analysis of variance (ANOVA) was conducted by the SAS software package (*21*), and significant differences between group means were analyzed by the Duncan Multiple Range Test ($P = 0.05$). Pearson's correlation coefficients were used to measure the strength of the linear correlation between two variables.

RESULTS AND DISCUSSION

Development of Titration Method. *Swirl Depth and Coagulating Soymilk Viscosity.* Swirl flows are found in nature and are utilized in a very wide range of applications. A swirl could be created by rotating soymilk in a cylindrical container using a magnetic stirrer. While the soymilk was stirred, $MgCl_2$ solution was continually added as described in the measurement of CPCC. As the time of $MgCl_2$ addition increased, the swirl depth decreased and finally disappeared. The soymilk viscosity and the swirl depth were measured as a function of $MgCl_2$ concentration during the course of the titration. As shown in **Figure 1**, the soymilk viscosity and the swirl depth did not change at the initial stage of $MgCl_2$ addition. That was because the latent time for coagulation increases with decreasing coagulant concentration (*20*) and temperature (*22*), and the soymilk would not coagulate immediately in such a low $MgCl_2$ concentration (0.0−12.0 mM) at such a low temperature (20 °C). After 180 s of addition or above 12.0 mM concentration, the viscosity increased, and the swirl depth decreased with increasing $MgCl_2$ concentration. The increase in viscosity was the result of protein aggregation induced by Mg^{2+} (*23*) and was responsible for the decrease in swirl depth.

It should be noted that the coagulation or protein aggregation phenomenon that was being studied during addition of $MgCl_2$ can only be regarded as the early phase of protein gelation. Even at the time the swirl disappeared, the soymilk dispersion only thickened and had not gelled. Brookfield viscometer equipped with spindles was reported to be suitable for viscosity measurement at this early stage of protein gelation *(24)*.

Since the coagulation proceeded during the swirling, and coagulating soymilk was a kind of thixotropic fluid, it was difficult to analyze the quantitative relation between the swirl depth and the soymilk viscosity in theory. However, it was not difficult to understand the effect of the viscosity on the swirl depth. Sabersky and Acosta (*25*) reported that the swirl depth depended on the fluid rotating velocity, and a higher velocity would produce a deeper swirl. The increase in soymilk viscosity and hence the soymilk resistance to motion resulted in the decrease in the rotation velocity since the magnetic stirrer's power was set as a constant value. Therefore, the swirl depth decreased with the increase in soymilk viscosity.

As shown in **Figure 1**, the apparent viscosity of the soymilk increased to the maximum at 15.5 mM $MgCl_2$, and the swirl disappeared simultaneously (swirl depth = 0). Above 15.5 mM, the viscosity decreased with the increase of $MgCl_2$ concentration. Shortly after the swirl disappearance, the swirl reappeared and the swirl depth increased with further addition of $MgCl_2$.

Ohara and co-workers (*15*) reported at certain concentration of coagulant the coagulating soymilk viscosity was maximal. Our result agreed with their report, even though our mixing method, coagulant, soymilk temperature, and viscosity measuring method were different from theirs. The soymilk viscosity changes could be explained by the mechanism of coagulation. Because the pH of natural soymilk is greater than its major storage protein's isoelectric points and the denatured soybean proteins were negatively charged, Mg^{2+} ions neutralized the net charge of the proteins. As a result, the hydrophobic interactions among the neutralized protein molecules became more predominant to induce the aggregation of proteins (*26*). When more Mg^{2+} ions were added, more proteins were aggregated and soymilk viscosity was higher. At a certain concentration of $MgCl_2$, all of the protein molecules were neutralized and took part in the aggregation, and thus the soymilk viscosity was maximal. The decrease in viscosity was probably caused by the extra $MgCl_2$ that changed the ion strength of the system and by the continued mixing (shear action) which caused a unique irreversible breakdown of protein−protein interaction at room temperature. It is well-known that soymilk curd (gels) breaks down by continuous stirring even at a high temperature. This is why the stirring is only applied for a short period of time to disperse the coagulant during tofu making.

The most important result in our study showed that the soymilk viscosity was maximal at the time of swirl disappearance. Further, the point at which the viscosity was maximal could be easily judged by the visual observation of the swirl disappearance. The operation of this method to determine the critical point of coagulant concentration (CPCC) at which the swirl disappears was similar to a common chemical titration method. It was simple to operate.

Tofu gel texture is formed by coagulating soymilk at 60−85 °C. The optimal coagulant concentration for making "kori" tofu, a pressed firm tofu, could be determined by the characteristic rise in the coagulating soymilk viscosity, which was recorded during addition of $CaCl_2$ to soymilk at 63 °C (16). Since soymilk could reach the maximal viscosity at CPCC under our titration conditions, it was logical that CPCC could be used as an indicator to predict the optimal concentration for making filled tofu.

Effect of Stirrer Speed on CPCC. CPCC was measured at a series of stirrer speeds for some different varieties of soybeans, respectively. It was found that CPCC was affected by not only

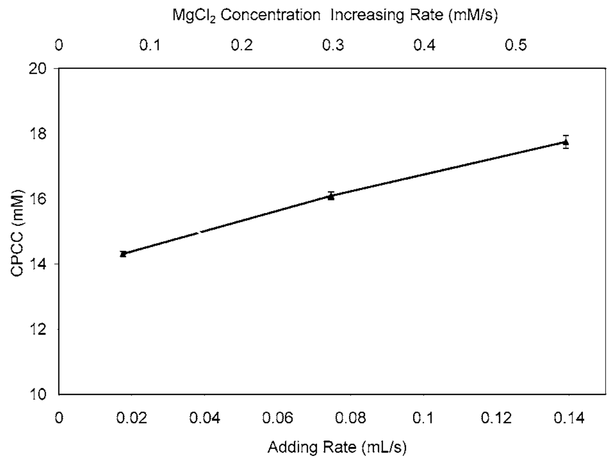

Figure 2. Effect of $MgCl_2$ addition rate or $MgCl_2$-concentration increasing rate on CPCC. Soymilk volume, 350 mL; soymilk temperature, 20 °C; Soymilk concentration, 10.5 Brix; soybean, Proto (1999)

soybean varieties but also stirrer speeds (data not shown). Although stirrer speed did not significantly affect CPCC within a relative wide range of speeds for some soybean varieties, it was desirable to simplify the procedure by providing a guideline for choosing a suitable stirrer speed for different soybean materials. For this purpose, the rotation condition of the soymilk at the time of swirl disappearance and the time to get the swirl to reappear were recorded. At a lower speed, the soymilk stopped rotating when the swirl disappeared, and it took a longer time to get the swirl to reappear. When the stirrer speed was set high enough, the soymilk still maintained rotating at the time of swirl disappearance, and the time to get the swirl to reappear became shorter. When the stirrer speed was too high, the swirl never disappeared.

When the soymilk did not rotate any more at the time of swirl disappearance, there was no way to mix more coagulant with soymilk. When soymilk rotated quickly at the time of swirl disappearance, it was not easy to visually judge the point at which the swirl disappeared. Thus, the suitable stirrer speed (V_s) should be chosen in such a way that the soymilk rotates slowly when the swirl disappears and the swirl reappears after around 1 min.

Therefore, to measure CPCC for an unknown soybean material, the stirrer speed should be adjusted according to the soymilk rotating conditions during last measurement until a suitable stirrer speed is found. Since one measurement takes less than 5 min, 15 min is enough for determining one sample's CPCC.

Effect of Coagulant Addition Rate on CPCC. **Figure 2** shows the changes in CPCC and the titration time as a function of $MgCl_2$ addition rate or $MgCl_2$ concentration increasing rate. The increase in CPCC was due to a reduction in the titration time (faster titration). When the addition rate was lower, the titration time was longer, and therefore the reaction between Mg^{2+} ions and the protein molecules was more complete and fewer $MgCl_2$ molecules were needed to attain the maximal viscosity. When the addition rate was higher and the titration time was shorter, the reaction between Mg^{2+} ions and the protein molecules were less complete and more $MgCl_2$ molecules were needed to attain the maximal viscosity. Therefore, the rate of coagulant addition or more accurately the coagulant concentration increasing rate had an important effect on CPCC.

Effect of Soymilk Temperature on CPCC. As shown in **Figure 3**, the increase in soymilk temperature resulted in a decrease in CPCC. This was consistent with the Ohara and co-worker's

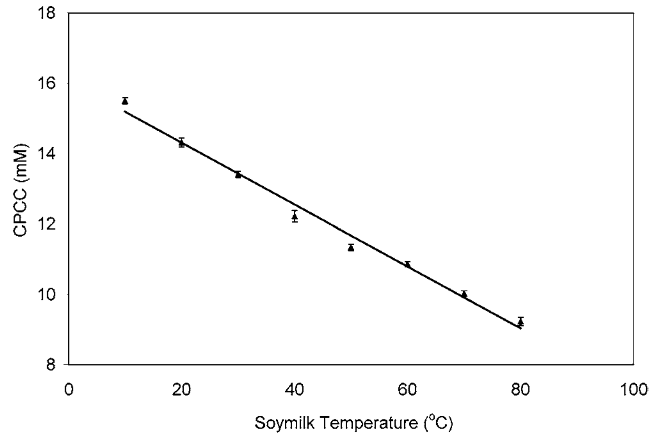

Figure 3. Effect of soymilk temperature on CPCC. $MgCl_2$ addition rate, 1.0 mL/min; soymilk volume, 350 mL; soymilk concentration, 10.5 Brix; soybean, Proto (1999)

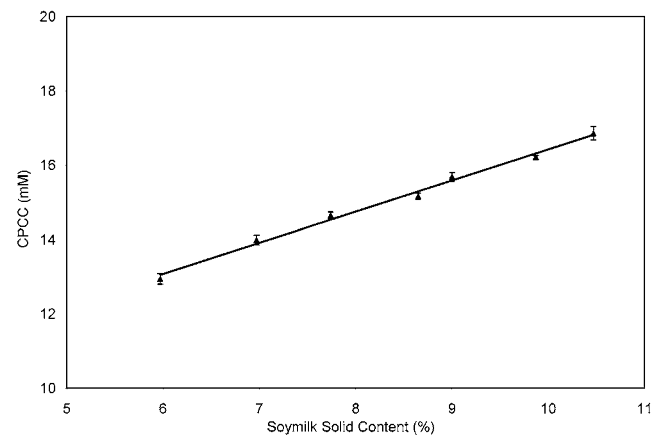

Figure 4. Effect of soymilk solid content on CPCC. $MgCl_2$ addition rate, 1.00 mL/min; soymilk volume, 350 mL; soymilk temperaure, 20 °C; soybean: Proto (1999).

result obtained by recording the rotational viscosity of coagulating soymilk (*15*). Dynamic viscoelasticity study showed that the rate constant of soybean protein coagulation depended on the coagulant $CaSO_4$ concentration and soymilk temperature (*25*). Undoubtedly, a higher temperature induced a larger rate constant, and thus less $MgCl_2$ was needed to get the maximal viscosity. For making tofu, it was known that the hotter the soymilk at the time of coagulation, the less the amount of coagulant was required (*9*); thus the effect of soymilk temperature on CPCC supported our assumption that CPCC could be an indicator for predicting optimal coagulant concentration for making tofu.

Effect of Soymilk Concentration on CPCC. To investigate the effect of soymilk concentration on CPCC, soymilks were made using different ratios of soybeans to water. **Figure 4** shows that CPCC increased with the increase in soymilk solid content. This agrees with the result obtained by recording the rotational viscosity of coagulating soymilk (*15, 16*). For making tofu, it had been reported that the more concentrated the soymilk, the more the coagulant required (*9*); thus the effect of soymilk concentration on CPCC was another evidence to support our assumption that CPCC could be an indicator of the optimal coagulant concentration.

Effects of Soymilk Volume, Container Diameter, and Stir Bar Length on CPCC. The effects of soymilk volume, container diameter, and stir bar length on CPCC were investigated (data not shown). The results show that the decrease in soymilk

Table 2. Character of 33 Soybean Samples and Their CPCC Values Determined Using 350 mL of Soymilk at 1.00 mL/min Addition Rate at 20 °C

		soybean[a]		soymilk concentration[a]		V_s (rpm)		CPCC (mM)[b]	
variety	harvest year	protein (%)	moisture (%)	solid (%)	Brix	$MgCl_2$	$CaCl_2$	$MgCl_2$	$CaCl_2$
Proto	1995	43.2	6.60	9.27	11.0	500	600	11.8 ± 0.2	9.9 ± 0.1
Proto	1996	48.0	6.29	8.51	10.6	500	600	12.3 ± 0.1	9.8 ± 0.1
Proto	1997	46.5	7.21	8.56	10.3	450	500	12.2 ± 0.0	9.9 ± 0.1
Proto	1998	43.7	6.38	8.59	10.5	450	550	13.0 ± 0.1	10.7 ± 0.0
Proto	1999	42.5	6.82	8.66	10.6	450	550	14.2 ± 0.1	11.6 ± 0.1
Proto	2000	43.7	6.94	8.80	10.6	425	525	14.7 ± 0.1	11.9 ± 0.1
Vinton	1996	45.3	6.51	8.86	10.8	500	550	11.9 ± 0.1	9.5 ± 0.0
Vinton	2000	46.0	6.87	8.21	10.5	375	400	12.1 ± 0.0	10.2 ± 0.1
Norpro	1998	41.4	6.64	9.02	10.6	400	500	16.4 ± 0.2	12.4 ± 0.1
Norpro	1999	41.7	6.67	8.85	10.7	450	500	15.8 ± 0.1	12.1 ± 0.1
Norpro	2000	41.7	7.29	8.91	10.9	400	500	16.6 ± 0.0	12.9 ± 0.1
Toyopro	2000	44.7	9.40	8.48	10.5	475	550	13.6 ± 0.2	10.8 ± 0.1
Soyapro	2000	43.5	7.80	8.76	10.6	450	550	12.2 ± 0.1	9.7 ± 0.0
NL02	2000	48.2	8.58	8.40	10.3	500	600	13.3 ± 0.1	10.7 ± 0.0
NL06	2000	43.0	8.45	8.92	10.8	450	550	13.9 ± 0.1	10.8 ± 0.1
NL08	2000	41.3	7.70	8.90	10.8	450	550	14.0 ± 0.1	11.1 ± 0.1
NL12	2000	41.9	8.22	8.70	10.6	450	550	13.9 ± 0.1	10.8 ± 0.1
NL17	2000	46.4	8.49	8.61	10.5	500	600	13.2 ± 0.1	10.5 ± 0.1
NL18	2000	46.9	8.70	8.55	10.4	500	600	12.5 ± 0.1	10.2 ± 0.1
NL19	2000	47.6	7.99	8.71	10.7	500	600	14.3 ± 0.1	11.3 ± 0.1
NL20	2000	41.7	9.20	8.75	10.6	400	550	13.3 ± 0.1	10.3 ± 0.1
SB2450	2000	42.4	6.59	8.79	10.5	450	550	15.9 ± 0.1	12.4 ± 0.0
Surge	2000	38.6	8.91	8.37	10.4	350	450	14.1 ± 0.2	10.7 ± 0.0
P9071	2000	36.7	6.65	8.59	10.7	325	400	15.8 ± 0.1	12.4 ± 0.1
Korada	2000	39.1	6.58	8.55	10.8	350	400	15.5 ± 0.1	12.2 ± 0.1
Raydor	1999	36.7	6.57	8.72	10.7	350	400	15.9 ± 0.1	12.1 ± 0.1
Kandi	1999	40.1	6.78	8.90	10.8	400	450	14.4 ± 0.0	11.6 ± 0.1
Goodwine	1999	37.3	6.54	8.97	10.8	400	500	14.1 ± 0.1	11.3 ± 0.1
Windsor	1999	38.8	6.90	8.60	10.7	380	480	15.4 ± 0.2	11.8 ± 0.0
Baxter	1999	38.7	6.56	8.87	10.9	400	500	14.3 ± 0.0	11.3 ± 0.1
Biscay	1999	38.8	6.46	8.78	11.0	350	450	15.8 ± 0.1	12.4 ± 0.1
X5715	1999	37.8	6.43	8.76	10.8	350	400	16.4 ± 0.1	12.7 ± 0.1
Soaord	1999	39.2	6.62	8.78	10.7	375	500	14.8 ± 0.0	11.3 ± 0.1
Minimum	1995	34.2	6.29	8.21	10.3	325	400	11.8	9.5
Maximum	2000	44.9	9.40	9.27	11.1	500	600	16.6	12.9
mean		39.1	7.28	8.70	10.7	427	514	13.9	11.1
std dev		3.03	0.94	0.21	0.20	55	65	1.5	1.0

[a] Data are means of two replicates and on dry basis. [b] Data are means ± SD of two replicates.

volume without changing $MgCl_2$ addition rate resulted in an increased CPCC. The reason might be that decreasing soymilk volume increased $MgCl_2$-concentration increasing rate, which affected CPCC as shown in **Figure 2**. To keep a constant $MgCl_2$ concentration increasing rate, $MgCl_2$ addition rate was adjusted from 0.98 mL/min for 350 mL of soymilk to 0.70 mL/min for 250 mL of soymilk. As a result, there was no significant difference between CPCC values measured with 250 and 350 mL soymilk. Neither beaker diameter nor stir bar length significantly influenced the CPCC, although the suitable stirrer speeds (V_s) were greatly affected. The results from the studies of the effects of soymilk volume, container diameter, and stir bar length indicate that CPCC is a characteristic parameter of soymilk.

Validation of Titration Method. Thirty-three soybean samples were used to validate the titration method. **Table 2** lists soybean varieties, harvest years, soybean protein content, soymilk concentration, and CPCC, as well as V_s. The CPCC varied from 11.8 to 16.6 mM for $MgCl_2$ and 9.5 to 12.9 mM for $CaCl_2$. It was interesting to note that it seems that age of beans (soybean cultivar Proto) affected CPCC. The older beans seemed to have significantly ($P < 0.05$) decreased CPCC. The V_s varied with soybean samples from 325 to 500 rpm for $MgCl_2$ and 400 to 600 rpm for $CaCl_2$. The correlation of CPCC and soymilk composition will be reported in another paper.

Because of tofu's bland taste, the texture properties of tofu play an important role in influencing its quality and consumer acceptability (*27*). Previous study indicated that filled tofu's breaking strength and apparent Young's modulus increased with increasing quick-acting coagulant concentration up to a certain point (*28*). When the concentration passed that point, serious syneresis occurred and the coagulum lost tofu character. For verifying the relationship between CPCC and the optimal coagulant concentration, filled tofus were made with different concentrations of coagulants based on CPCC (**Table 2**). **Table 3** shows that, for all of the 33 soybean samples tested, serious syneresis occurred when the coagulant concentration was CPCC + 1.41 (mM) for $MgCl_2$ and CPCC + 1.25 (mM) for $CaCl_2$. The optimal $MgCl_2$ concentration range was from CPCC to CPCC − 1.41 (mM), and the optimal $CaCl_2$ concentration range was from CPCC to CPCC − 1.25 (mM). Therefore CPCC was a reliable indicator of the optimal coagulant concentration for making filled tofu.

The optimal coagulant concentrations for making tofu was affected by not only soybean materials but also processing conditions such as coagulant mixing condition and soymilk temperature (*9*, *29*). As shown in the above studies, CPCC was also affected by coagulant addition rate and soymilk temperature. For a specific tofu manufacturer or research laboratory, as long as those conditions are kept consistent, the mathematical relationships between CPCC and the optimal coagulant concentration should be constant all the time.

There are many types of tofu products, and the method of adding coagulant also varies. The optimal temperature and

Table 3. Textures of Filled Tofu Made from 33 Soybean Samples at Different Coagulant Concentration[a]

		tofu texture ($MgCl_2$)							tofu texture ($CaCl_2$)						
			breaking strength (kPa)			apparent Young's modulus (kPa)				breaking strength (kPa)			apparent Young's modulus (kPa)		
variety	year	CPCC + 1.41 (mM)	CPCC (mM)	CPCC − 1.41 (mM)	CPCC − 2.82 (mM)	CPCC (mM)	CPCC − 1.41 (mM)	CPCC − 2.82 (mM)	CPCC + 1.25 (mM)	CPCC (mM)	CPCC − 1.25 (mM)	CPCC − 2.50 (mM)	CPCC (mM)	CPCC − 1.25 (mM)	CPCC − 2.50 (mM)
Proto	1995	*b*	10.3a(0.0)	9.5a(0.5)	8.6b(0.0)	17.4a(0.4)	16.1ab(1.1)	14.3b(0.0)	*b*	6.5b(0.0)	7.1a(0.2)	5.8c(0.0)	12.1a(0.4)	12.7a(0.3)	10.4b(0.0)
Proto	1996	*b*	10.2a(0.3)	9.3ab(0.5)	8.6b(0.2)	17.1a(0.3)	15.7b(0.1)	14.9c(0.3)	*b*	6.1b(0.2)	7.2a(0.3)	5.8b(0.1)	11.5ab(0.7)	12.6a(0.4)	10.3b(0.0)
Proto	1997	*b*	9.7a(0.5)	8.3a(0.6)	8.3a(0.1)	16.4a(1.2)	13.7b(0.7)	13.7b(0.0)	*b*	6.2a(0.4)	6.0a(0.0)	5.0b(0.2)	11.5a(0.5)	10.8a(0.2)	9.3b(0.3)
Proto	1998	*b*	9.7a(0.5)	9.2a(0.5)	9.3a(0.2)	16.0a(1.1)	15.4a(0.3)	15.0a(0.1)	*b*	6.0a(0.4)	6.6a(0.1)	5.9a(0.3)	10.9a(0.4)	11.6a(0.3)	10.6a(0.4)
Proto	1999	*b*	8.3b(0.2)	10.1a(0.6)	8.9ab(0.3)	13.8b(0.3)	16.5a(1.0)	15.2ab(0.3)	*b*	6.0a(0.3)	5.9a(0.1)	5.6a(0.1)	11.4a(0.7)	10.5a(0.2)	10.3a(0.2)
Proto	2000	*b*	8.7a(0.2)	9.7a(0.7)	8.2a(0.2)	15.1a(0.1)	15.8a(0.7)	14.4a(0.3)	*b*	7.3b(0.2)	8.5a(0.1)	7.6b(0.2)	13.1b(0.1)	14.3a(0.5)	12.7b(0.2)
Vinton	1996	*b*	7.9a(0.5)	8.9a(0.5)	8.5a(0.4)	13.6a(0.6)	14.5a(0.9)	13.2a(0.2)	*b*	4.8b(0.0)	5.6a(0.1)	4.6b(0.2)	9.6ab(0.1)	10.5a(0.3)	8.9b(0.4)
Vinton	2000	*b*	8.6ab(0.2)	8.0a(0.2)	7.7b(0.1)	15.0a(0.1)	13.5b(0.8)	12.5b(0.1)	*b*	6.4b(0.1)	7.0a(0.1)	5.8c(0.2)	11.2b(0.2)	12.5a(0.3)	9.7c(0.0)
Norpro	1998	*b*	6.9a(0.3)	6.4a(0.0)	6.7a(0.1)	12.5a(0.3)	11.4a(0.5)	11.7a(0.2)	*b*	4.7a(0.3)	4.6a(0.2)	4.1a(0.3)	9.1a(0.9)	9.2a(0.4)	7.6a(0.7)
Norpro	1999	*b*	8.6a(0.3)	8.1ab(0.1)	7.6b(0.2)	14.8a(0.0)	13.7b(0.4)	12.6c(0.4)	*b*	5.2a(0.3)	5.7a(0.2)	5.3a(0.2)	9.8a(0.7)	10.4a(0.0)	9.7a(0.3)
Norpro	2000	*b*	8.1a(0.1)	7.8ab(0.3)	7.4b(0.2)	14.1a(0.0)	13.4b(0.2)	12.5c(0.1)	*b*	5.5ab(0.2)	5.9a(0.1)	5.2b(0.0)	10.7a(0.2)	10.6a(0.1)	9.9b(0.2)
Toyopro	2000	*b*	11.7a(0.3)	11.1a(0.3)	10.7a(0.3)	19.1a(0.6)	17.7a(0.1)	17.3a(0.8)	*b*	7.6ab(0.2)	7.8a(0.2)	7.2b(0.0)	14.0a(0.1)	13.5a(0.5)	11.9b(0.2)
Soyapro	2000	*b*	7.9a(0.4)	7.4a(0.1)	7.4a(0.3)	13.2a(0.7)	12.3a(0.2)	12.2a(0.3)	*b*	5.6ab(0.1)	5.7a(0.2)	5.0b(0.3)	10.5a(0.4)	10.2a(0.3)	9.1b(0.2)
NL02	2000	*b*	11.9b(0.1)	14.8a(0.1)	11.1c(0.0)	19.2b(0.5)	22.9a(0.1)	18.7b(0.0)	*b*	7.9ab(0.1)	8.7a(0.0)	7.3b(0.6)	13.9ab(0.1)	15.2a(0.0)	12.8b(0.8)
NL06	2000	*b*	10.3a(0.4)	10.3a(0.4)	9.0b(0.0)	17.6a(0.7)	16.9a(0.5)	14.9b(0.2)	*b*	5.9b(0.1)	6.4a(0.2)	5.2c(0.1)	11.2a(0.1)	11.6a(0.1)	9.2b(0.2)
NL08	2000	*b*	9.7a(0.5)	9.6a(0.1)	8.9a(0.1)	16.0a(0.0)	15.6ab(0.3)	14.6b(0.7)	*b*	5.9b(0.1)	6.4a(0.1)	6.0ab(0.0)	11.2ab(0.1)	11.7a(0.2)	10.5b(0.4)
NL12	2000	*b*	8.8a(0.1)	8.7a(0.3)	8.1a(0.4)	15.3a(0.3)	14.8ab(0.5)	13.5b(0.7)	*b*	5.8ab(0.4)	5.9a(0.1)	4.9b(0.3)	10.8a(0.8)	10.7a(0.1)	8.9b(0.3)
NL17	2000	*b*	11.7a(0.3)	12.4a(0.2)	9.8b(0.4)	18.6a(0.4)	19.6a(0.1)	15.8b(0.9)	*b*	7.4a(0.4)	6.8a(0.2)	5.2b(0.5)	14.2a(0.8)	13.7a(0.1)	10.5b(0.5)
NL18	2000	*b*	11.6ab(0.4)	12.2a(0.3)	10.5b(0.5)	19.3a(0.6)	19.9a(0.6)	17.0b(0.5)	*b*	7.5a(0.1)	7.5a(0.4)	5.6b(0.8)	13.7a(0.1)	13.6a(0.9)	10.2b(1.2)
NL19	2000	*b*	11.1a(0.2)	10.5b(0.0)	10.2b(0.1)	17.7a(0.8)	17.3a(1.4)	16.5a(0.6)	*b*	6.6a(0.0)	6.4ab(0.4)	5.9b(0.1)	12.6a(0.3)	11.8ab(0.5)	11.2b(0.5)
NL20	2000	*b*	9.1a(0.0)	8.4b(0.0)	7.9b(0.3)	15.7a(0.3)	13.9b(0.5)	13.2b(0.4)	*b*	5.3b(0.2)	5.9a(0.1)	5.1b(0.0)	10.3a(0.2)	10.6a(0.3)	9.3b(0.2)
SB2450	2000	*b*	9.1a(0.6)	8.6ab(0.1)	7.9b(0.2)	15.7a(0.2)	14.4b(0.3)	12.7c(0.2)	*b*	5.5a(0.1)	5.7a(0.2)	4.8b(0.0)	10.0b(0.2)	11.3a(0.2)	9.3c(0.2)
Surge	2000	*b*	7.4a(0.0)	5.9b(0.3)	5.9b(0.6)	12.4a(0.2)	10.7b(0.3)	9.9b(0.8)	*b*	4.6b(0.0)	5.1a(0.2)	3.6c(0.0)	8.8a(0.3)	9.2a(0.2)	7.3b(0.3)
P9071	2000	*b*	6.2a(0.1)	4.3b(0.1)	3.9c(0.0)	11.0a(0.2)	8.3b(0.1)	6.7c(0.0)	*b*	5.0b(0.1)	5.6a(0.3)	4.6b(0.1)	9.2b(0.3)	10.0a(0.1)	8.1c(0.1)
Korada	2000	*b*	8.5a(0.1)	7.9b(0.1)	7.2c(0.2)	10.0a(0.1)	9.1a(0.5)	8.1b(0.2)	*b*	6.5c(0.1)	8.1a(0.1)	7.5b(0.2)	8.7b(0.1)	9.9a(0.0)	8.6b(0.0)
Raydor	1999	*b*	5.4a(0.1)	4.6b(0.3)	4.0b(0.1)	9.8a(0.1)	9.1a(0.0)	8.5a(0.8)	*b*	4.1b(0.1)	4.5a(0.0)	3.7c(0.0)	8.2a(0.3)	8.3a(0.3)	7.3b(0.0)
Kandi	1999	*b*	7.7a(0.1)	7.4a(0.1)	5.8b(0.3)	13.8a(0.2)	12.9b(0.3)	10.4c(0.1)	*b*	5.1a(0.1)	5.4a(0.3)	4.6a(0.3)	10.0a(0.1)	10.3a(0.5)	8.6b(0.3)
Goodwin	1999	*b*	5.9a(0.5)	6.2a(0.1)	5.1a(0.4)	10.6a(1.0)	11.4a(0.1)	9.5a(0.9)	*b*	4.6ab(0.3)	4.9a(0.1)	4.3b(0.1)	9.1a(0.1)	9.5a(0.1)	8.2b(0.3)
Windsor	1999	*b*	7.3a(0.1)	6.9b(0.2)	6.2c(0.1)	12.9a(0.1)	12.4a(0.3)	11.3b(0.2)	*b*	4.9a(0.1)	4.9a(0.5)	4.4a(0.3)	9.7a(0.2)	9.2a(0.7)	8.4a(0.5)
Baxter	1999	*b*	7.8a(0.5)	6.7ab(0.3)	6.0b(0.3)	13.7a(1.1)	11.5ab(0.4)	10.8b(0.6)	*b*	5.4a(0.3)	5.6a(0.2)	4.6b(0.0)	10.1ab(0.6)	10.6a(0.4)	9.2b(0.2)
Biscay	1999	*b*	10.9a(0.7)	9.9ab(0.2)	9.6b(0.1)	12.3a(0.2)	11.5b(0.4)	11.1b(0.1)	*b*	5.5b(0.1)	6.5a(0.1)	5.6b(0.1)	10.5a(0.2)	11.3ab(0.2)	10.0b(0.4)
X5715	1999	*b*	6.9a(0.3)	5.2b(0.1)	4.8b(0.1)	12.1a(0.4)	9.8b(0.2)	8.9c(0.2)	*b*	5.1b(0.1)	5.8a(0.2)	4.7b(0.1)	9.6ab(0.7)	10.4a(0.2)	8.7b(0.1)
Soaord	1999	*b*	11.1a(0.4)	10.3ab(0.3)	8.6b(0.8)	13.1a(0.3)	12.5a(0.5)	10.3b(0.4)	*b*	6.6ab(0.4)	7.2a(0.0)	6.0b(0.1)	8.6b(0.3)	9.1a(0.0)	8.0c(0.1)
average			8.9	8.6	7.8	14.7	14.1	12.8		5.9	6.3	5.3	10.8	11.1	9.5

[a] Data are means ± SD of two replicates (two tofu samples). Means of the same texture property for the same soybean sample and the same kind of coagulant followed by different letters are significantly different ($P < 0.05$). [b] No data for the textural property due to serious syneresis.

Table 4. Correlation Coefficient between Soybean Protein Contents, Suitable Stirring Speeds (V_s), Breaking Strength, and Apparent Young's Modulus[a]

	stirrer speed (V_s)		breaking strength		apparent Young's modulus	
	$MgCl_2$	$CaCl_2$	$MgCl_2$ CPCC − 1.41 (mM)	$CaCl_2$ CPCC (mM)	$MgCl_2$ CPCC − 1.41 (mM)	$CaCl_2$ CPCC (mM)
soybean protein content	0.84	0.72	0.74	0.69	0.82	0.79
stirrer speed (V_s) ($MgCl_2$)			0.72		0.85	
stirrer speed (V_s) ($CaCl_2$)				0.53		0.68

[a] $n = 33$, $p < 0.0001$.

concentration of soymilk are different for different types of tofu. However, soymilk coagulation mechanism is similar. Since CPCC is a characteristic parameter of soymilk, it is possible that CPCC could be used as an indicator to predict the optimal concentration of quick-acting coagulant for all types of tofu products. Further validation studies should be completed not only in the research laboratories but also through collaborative studies in various tofu factories in the future.

Correlations among the suitable stirrer speed, soybean protein content, tofu breaking strength, and apparent Young's modulus for 33 soybean samples were analyzed. The correlation coefficients between any two variables that were significantly ($p <$ 0.0001) correlated are listed in **Table 4**. The results showed that the suitable stirrer speed correlated significantly ($r = 0.72$ and 0.84 for $CaCl_2$ and $MgCl_2$, respectively) with soybean protein content. These positive correlations suggest that the suitable stirrer speed could be predicted by soybean protein content or soybean protein content could be estimated by the suitable stirrer speed. **Table 4** also showed that the suitable stirrer speed positively correlated with tofu texture parameters. This result indicated that the suitable stirrer speed, which was chosen for determining CPCC, could be used to predict tofu strength.

CONCLUSIONS

The aggregations of soy protein dispersions in the early stage of gelation changed the soymilk viscosity. The increase in viscosity changed the gross appearance of the swirl created by a magnetic stirrer. By the use of this principle, a rapid titration method was developed to determine the critical point of coagulant concentration (CPCC) at which the viscosity peaked and the swirl disappeared. CPCC was a characteristic parameter of soymilk and was affected by soybean variety, soymilk temperature, soymilk concentration, and coagulant concentration increasing rate during titration. CPCC could be used as a reliable indicator for predicting optimal coagulant concentrations of various soybean materials for making filled tofu.

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