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# Effect of Soy Milk Characteristics and Cooking Conditions on Coagulant Requirements for Making Filled Tofu

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The amount of coagulant added to soy milk is a critical factor for tofu-making; particularly it affects the textural properties of tofu. Earlier research indicated that the critical point of coagulant concentration (CPCC) is a characteristic parameter of soy milk and could be used as an effective indicator of optimal coagulant concentration (OCC) for making filled tofu. The objective of this study was to investigate the possible correlations between CPCC and the characteristics of soy milk made from various soybean samples and the effect of soy milk cooking and dilution conditions on CPCC. CPCC was determined by a titration method. Calcium chloride and magnesium chloride were used as coagulants. Soy milk characteristics including solid, protein, phytate, pH, titratable acidity, mineral content, and 11S/7S protein and these characteristics as affected by heating rate, heating time, and sequence of dilution and heating were studied. The results showed that the CPCC was significantly ($p < 0.05$) positively correlated with phytate content (grams per gram of protein), pH, and 7S protein content but negatively correlated with protein content, 11S protein content, 11S/7S ratio, titratable acidity, and original calcium content. Within the same soybean material, more proteins required more coagulant, but higher protein concentration during cooking resulted in less coagulant required by each gram of protein during coagulation. The CPCC decreased with increasing soy milk heating time or decreasing heating rate. The sequence of heating and diluting for preparing soy milk also had an effect on CPCC.

**KEYWORDS: Tofu; soybean; soy milk; cooking condition; coagulant**

## INTRODUCTION

Coagulation of soy milk is the most important step and the most difficult to master in the tofu-making process because it depends on the complex interrelationship of many variables (*1*, *2*), which are classified into four categories: soybean characteristics, soy milk processing variables, coagulant characteristics, and coagulating variables. Soybean characteristics include soybean cultivar, growing environment, and storage conditions. Soy milk processing variables include but are not limited to soybean soaking conditions, water-to-bean ratio, grinding conditions, and soy milk cooking conditions. Coagulant characteristics include coagulant type and coagulant solution concentration. Coagulating conditions include methods of mixing coagulant solution into soy milk, soy milk temperature, and amount of coagulant added into soy milk. The amount of coagulant added into soy milk, or concentration of coagulant in soy milk, is a critical variable during soy milk coagulation. It affects profoundly the yield and textural properties of the resulting tofu (*2*−*9*). Therefore, it is desirable to use coagulants at the optimal concentration during the coagulating operation. However, optimal coagulant concentrations (OCC) are affected by soybean characteristics, soy milk processing conditions, and coagulating conditions (*1*−*4*, *10*, *11*). It is a challenge to automate intelligently the mechanical operation of coagulation, especially when quick-acting coagulants such as $CaCl_2$ and $MgCl_2$ are used.

Under fixed mechanical conditions for coagulation, the variance in OCC is apparently due to soy milk characteristics. Information on the relationships between OCC and soy milk characteristics is limited in the literatures (*4*, *11*). Soy milk cooking is a prerequisite for tofu-making, and cooking conditions affect soy milk properties. However, the effect of soy milk cooking conditions on OCC has not been reported. To a great extent, the reason for a lack of results is probably due to the inconvenience of measuring OCC. Recently, a rapid titration method for determining the critical point of coagulant concentration (CPCC) was developed in our laboratory (*10*). The availability of this convenient and reproducible method has made it possible for us to make an extensive study of coagulant requirements for a number of soybean materials. Our previous study shows that the CPCC is a characteristic parameter of soy milk and could be used as an effective indicator of OCC for making filled tofu (*10*).

Depending on methods of preparation, textural properties, and moisture content, commercial tofus are generally classified as dry tofu (Doufugan), firm tofu (Momen), soft tofu, silken tofu (Kinu), and filled (packed) tofu. In filled tofu's preparation,

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coagulant is normally added to cooled soy milk, followed by heating without mixing to initiate and finish the coagulation process of the proteins to form curd in the package. The unique processing feature makes filled tofu preparation the most easily controlled. In addition, curd does not need to be broken and transferred. Therefore, filled tofu is a good model for monitoring changes in tofu texture due to soy milk or coagulant.

This study's objective was to examine the relationships between the CPCC and soy milk compositions/properties and to investigate the effect of soy milk cooking and dilution conditions on CPCC. This type of information will be helpful for understanding the mechanism of tofu-making and ultimately enable tofu manufacturers to easily control the coagulation process to improve end-product quality.

## MATERIALS AND METHODS

**Materials.** Thirty-seven soybean samples were used in this study. Most of the soybean samples were obtained through local soybean dealers (Sinner Brothers and Bresnahan Co., Casselton, ND; Earthwise Co., Moorhead, MN). Low-phytate cultivars (CX 1834 and CX 1836) were obtained from Dr. J. R. Wilcox of the USDA, Agricultural Research Service, Midwest Area, Purdue University. Soybeans were stored in an air-conditioned laboratory at ~18−24 °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 Soy Milk.** Soybeans were washed and soaked in tap water for 12 h at room temperature. The hydrated beans were drained, rinsed, and ground with tap water (the ratio of water to dry bean was 7:1 unless otherwise specified), using a soy milk grinder/extractor (Chang-Seng Mechanical Co., Taoyuan, Taiwan). The grinder/extractor, equipped with a centrifugal 100 mesh screen, could separate soy milk automatically from the residue.

A portion of 950 g of raw soy milk in a 3 qt stainless steel pot (diameter = 18 cm) was heated with continual stirring to ensure homogeneous heating on a stove to boiling (it took ~10 min to reach boiling), kept boiling for 5 min (unless otherwise specified), and then cooled to 20 °C in a water bath for the determination of CPCC.

For the experiment on the heating rate effect, a portion of 500 g of raw soy milk was heated from 20 to 97 °C and kept for 5 min at 97 °C by an ohmic heater, which had a volume of ~690 mL. Two large identical stainless steel plate electrodes with height and width of 12 and 10 cm, respectively, were used to ensure a homogeneous heating of the soy milk without the need of stirring. The distance between the two plate electrodes was 5.8 cm. Heating rates of 0.2, 0.5, and 1.5 °C/s were achieved by adjusting the voltage to 55, 110, and 220 V, respectively. For the experiment on the effect of sequence of heating and diluting, three different concentrations of original raw soy milks were prepared using 8:1, 7:1, and 6:1 water-to-bean ratios. The soy milk that was prepared by heating original raw soy milk and then diluting the cooked soy milk was designated HD soy milk. The soy milk that was prepared by diluting original raw soy milk and then heating the diluted raw soy milk was designated DH soy milk. During the dilutions, HD soy milk and DH soy milk concentrations were adjusted to 8.4 °Brix with an Auto Abbe refractometer (model 10500, Leica Inc., Buffalo, NY).

**Determination of Critical Point of Coagulant Concentration.** The CPCC was determined by a rapid titration method (*10*). A portion of 350 mL of cooked soy milk (20 °C) was placed in a 400 mL beaker (Pyrex) with a flat and smooth bottom and stirred 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$\varphi$ × 50 mm). The coagulant solution of 20.0 °Brix was added continuously into soy milk at 1.0 mL/min 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). At the exact moment the soy milk 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. The suitable stirrer speed was chosen in such a way that the soy milk rotates slowly at a disappearance of the swirl and then the swirl reappears after ~1 min. CPCC was calculated using the following equation:

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

**Chemical Composition.** Samples of cooked soy milk were freeze-dried. Moisture (vacuum oven method 925.09), crude protein (Kjeldahl nitrogen method 955.04), and ash (dry ashing method 924.05) were determined according to the AOAC (*12*). Calcium and magnesium were determined by atomic absorption spectrophotometry according to AOAC method 965.09 (*12*).

**Titratable Acidity.** The method of determining titratable acidity was modified from AOAC method 935.57 (*12*). Lyophilized soy milk (2 g each experiment) were well mixed with 50 mL of $CO_2$-free water and titrated with 0.1 N NaOH. The titratable acidity was calculated using citric acid as a reference acid.

**Phytate Content.** Phytate in soy milk was extracted and analyzed by anion exchange column chromatography according to AOAC method 986.11 (*12*). Lyophilized soy milk was extracted with 2.4% HCl. After filtration, the filtrate was applied onto an anion exchange column (1.0 × 20 cm; resin AG1-X4, 100−200 mesh, chloride form, Bio-Rad Laboratory, Hercules, CA). Phytate was eluted from the column with 0.7 M NaCl and then digested with $H_2SO_4$ and $HNO_3$. The phosphorus content was determined colorimetrically using molybdate solution and sulfonic acid reagent. The absorbance was read at 640 nm. The phytate content was calculated from the phosphorus by assuming that one molecule of phytic acid contained six molecules of phosphorus.

**Determination of 11S and 7S Proteins.** Protein was extracted according to a procedure modified from the method of Cai and Chang (*13*). One gram of freeze-dried soy milk was defatted twice in 15 mL of acetone in a tube for 30 min with vortexing every 5 min. The supernatant was removed by a pipet, and the precipitate was dried in a vacuum oven at <40 °C to dryness. A 15 mL portion of 1% SDS solution containing 50 mM 2-mercaptoethanol was added and homogenized with a Tekmar tissumizer (model T25-S1, Tekmar, Cincinnati, OH). The mixture was sonicated at 60 °C for 90 min to extract the proteins. The mixture was then centrifuged at 17600*g* for 30 min. To analyze protein content according to the Bradford method (*14*), the supernatant was diluted by 50 times with distilled water. Bovine serum albumin was used as a standard.

The protein extract was diluted to 2 mg/mL with distilled water, and 0.5 mL of diluted extract was mixed with 0.5 mL of SDS sample buffer containing 5% 2-mercaptoethanol. After 2 min of boiling, 30 $\mu$L of the cooled solution containing equivalent to 30 $\mu$g of protein was loaded onto a gradient gel containing 8−16% polyacrylamide. Electrophoresis was performed in a Bio-Rad Protean II chamber at 40 V for 8 h by SDS−polyacrylamide gel electrophoresis (SDS-PAGE) based on the procedure of Laemmli (*15*). At the end of electrophoresis, gels were stained with Coomassie Brilliant Blue R-250. The relative proportion of 11S and 7S globulin protein subunits was determined with an imaging densitometer (model GS 670, Bio-Rad Laboratories, Molecular Bioscience Group, Hercules, CA). The ratios of 11S to 7S proteins were calculated from the sum of the peak areas of their subunits according to the methods of Nagano and others (*16*) and Wang and Chang (*17*).

**Statistical Analysis.** All experiments were conducted in duplicate. Analysis of variance (ANOVA) was conducted by the SAS software package (*18*), and significant differences between group means were analyzed by Duncan's multiple-range test. Pearson's correlation coefficients were used to measure the strength of the linear correlation between two variables.

## RESULTS AND DISCUSSION

**Soy Milk Characteristics.** As shown in **Table 1**, the 37 soybean samples included different cultivars harvested in different years. Although all soybean samples were ground with

**Table 1.** Cultivar and Harvest Year of 37 Soybean Samples and Resultant Soy Milk Characteristics[a]

| variety | harvest year | soy milk concn (%) solid | soy milk concn (%) protein | mineral content (mg/g) ash | mineral content (mg/g) Ca | mineral content (mg/g) Mg | phytate (mg/g) | titratable acidity (mg of citric acid/g) | 11S/7S ratio | pH of soy milk | pH of coagulum $CaCl_2$ | pH of coagulum $MgCl_2$ | CPCC[b] $CaCl_2$ | CPCC[b] $MgCl_2$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Proto | 1995 | 9.27 | 4.90 | 5.79 | 0.225 | 0.332 | 1.60 | 2.38 | 1.81 | 6.60 | 6.15 | 6.26 | 9.9 ± 0.1 | 11.8 ± 0.2 |
| Proto | 1996 | 8.51 | 4.69 | 5.00 | 0.180 | 0.277 | 1.65 | 2.29 | 1.86 | 6.61 | –[c] | – | 9.8 ± 0.1 | 12.3 ± 0.1 |
| Proto | 1997 | 8.56 | 4.81 | 4.94 | 0.166 | 0.272 | 1.62 | 2.08 | 1.71 | 6.60 | 6.14 | 6.26 | 9.9 ± 0.1 | 12.2 ± 0.0 |
| Proto | 1998 | 8.59 | 4.65 | 5.07 | 0.133 | 0.286 | 1.53 | 1.78 | 1.86 | 6.66 | 6.24 | 6.33 | 10.7 ± 0.0 | 13.0 ± 0.1 |
| Proto | 1999 | 8.66 | 4.48 | 5.20 | 0.161 | 0.290 | 1.75 | 1.87 | – | – | – | – | 11.6 ± 0.1 | 14.2 ± 0.1 |
| Proto | 2000 | 8.80 | 4.66 | 4.89 | 0.178 | 0.286 | 1.63 | 1.69 | 1.63 | 6.68 | – | – | 11.9 ± 0.1 | 14.7 ± 0.1 |
| Vinton | 1996 | 8.86 | 4.23 | 5.34 | 0.267 | 0.287 | 1.10 | 1.94 | 1.86 | 6.55 | – | – | 9.5 ± 0.0 | 11.9 ± 0.1 |
| Vinton | 2000 | 8.21 | 4.27 | 4.10 | 0.195 | 0.250 | 0.88 | 1.48 | – | – | – | – | 10.2 ± 0.1 | 12.1 ± 0.0 |
| Norpro | 1998 | 9.02 | 4.24 | 5.53 | 0.153 | 0.278 | 1.82 | 2.11 | | 6.68 | 6.06 | 6.19 | 12.4 ± 0.1 | 16.4 ± 0.2 |
| Norpro | 1999 | 8.85 | 4.47 | 5.47 | 0.147 | 0.265 | 1.85 | 1.86 | – | 6.69 | 6.06 | 6.20 | 12.1 ± 0.1 | 15.8 ± 0.1 |
| Norpro | 2000 | 8.91 | 4.55 | 5.56 | 0.158 | 0.259 | 1.91 | 1.88 | | 6.72 | 6.00 | 6.14 | 12.9 ± 0.1 | 16.6 ± 0.0 |
| Toyopro | 1998 | 8.38 | 4.48 | 4.55 | 0.176 | 0.235 | 1.12 | 1.94 | – | – | – | – | 10.4 ± 0.1 | 12.9 ± 0.0 |
| Toyopro | 2000 | 8.48 | 4.57 | 4.76 | 0.171 | 0.227 | 1.51 | 1.81 | 1.44 | 6.64 | 6.1 | 6.18 | 10.8 ± 0.1 | 13.6 ± 0.2 |
| Soyapro | 2000 | 8.76 | 4.57 | 4.61 | 0.176 | 0.253 | 1.26 | 2.28 | – | 6.65 | 6.17 | 6.27 | 9.7 ± 0.0 | 12.2 ± 0.1 |
| NL02 | 2000 | 8.40 | 4.85 | 5.13 | 0.196 | 0.272 | 1.52 | 1.96 | 1.64 | 6.64 | 6.18 | 6.25 | 10.7 ± 0.0 | 13.3 ± 0.1 |
| NL06 | 2000 | 8.92 | 4.69 | 4.70 | 0.161 | 0.226 | 1.56 | 1.84 | 1.54 | 6.61 | 6.09 | 6.16 | 10.8 ± 0.1 | 13.9 ± 0.1 |
| NL08 | 2000 | 8.90 | 4.52 | 5.16 | 0.191 | 0.263 | 1.78 | 1.91 | 1.68 | 6.61 | 6.06 | 6.16 | 11.1 ± 0.1 | 14.0 ± 0.1 |
| NL12 | 2000 | 8.70 | 4.43 | 4.98 | 0.188 | 0.282 | 1.54 | 1.64 | 1.61 | 6.63 | 6.08 | 6.22 | 10.8 ± 0.1 | 13.9 ± 0.1 |
| NL17 | 2000 | 8.61 | 4.86 | 5.46 | 0.208 | 0.277 | 1.97 | 1.98 | – | 6.60 | 6.04 | 6.15 | 10.5 ± 0.1 | 13.2 ± 0.1 |
| NL18 | 2000 | 8.55 | 4.81 | 4.98 | 0.184 | 0.247 | 1.63 | 1.94 | 1.64 | 6.66 | 6.08 | 6.24 | 10.2 ± 0.1 | 12.5 ± 0.1 |
| NL19 | 2000 | 8.71 | 4.72 | 5.06 | 0.122 | 0.226 | 1.72 | 1.95 | 1.50 | 6.66 | 6.05 | 6.21 | 11.3 ± 0.1 | 14.3 ± 0.1 |
| NL20 | 2000 | 8.75 | 4.41 | 4.89 | 0.160 | 0.253 | 1.33 | 1.83 | 1.52 | 6.61 | 6.06 | 6.17 | 10.3 ± 0.1 | 13.3 ± 0.1 |
| SB2450 | 2000 | 8.79 | 4.39 | 5.94 | 0.203 | 0.297 | 2.10 | 1.76 | – | – | – | – | 12.4 ± 0.0 | 15.9 ± 0.1 |
| Surge | 2000 | 8.37 | 3.77 | 4.69 | 0.145 | 0.258 | 1.31 | 7.96 | 1.62 | – | – | – | 10.7 ± 0.0 | 14.1 ± 0.2 |
| P9071 | 2000 | 8.59 | 3.78 | 4.49 | 0.188 | 0.260 | 1.20 | 1.54 | 1.19 | – | – | – | 12.4 ± 0.1 | 15.8 ± 0.1 |
| Korador | 2000 | 8.55 | 3.98 | 4.69 | 0.185 | 0.246 | 1.29 | 1.87 | 1.39 | – | – | – | 12.2 ± 0.1 | 15.5 ± 0.1 |
| Raydor | 1999 | 8.72 | 3.92 | 4.58 | 0.173 | 0.250 | 1.28 | 1.49 | 1.45 | 6.64 | 6.15 | 6.24 | 12.1 ± 0.1 | 15.9 ± 0.1 |
| Kandi | 1999 | 8.90 | 4.25 | 4.66 | 0.202 | 0.249 | 1.19 | 1.62 | 1.41 | 6.61 | 6.16 | 6.24 | 11.6 ± 0.1 | 14.4 ± 0.0 |
| Goodwine | 1999 | 8.97 | 4.13 | 4.77 | 0.177 | 0.269 | 1.31 | 1.45 | 1.38 | 6.65 | 6.17 | 6.24 | 11.3 ± 0.1 | 14.1 ± 0.1 |
| Windsor | 1999 | 8.60 | 4.11 | 5.00 | 0.152 | 0.220 | 1.48 | 1.91 | – | 6.69 | 6.11 | 6.23 | 11.8 ± 0.0 | 15.4 ± 0.2 |
| Baxter | 1999 | 8.87 | 4.28 | 4.74 | 0.167 | 0.262 | 1.29 | 1.61 | 1.42 | 6.61 | – | – | 11.3 ± 0.1 | 14.3 ± 0.0 |
| Biscay | 1999 | 8.78 | 4.03 | 4.43 | 0.155 | 0.239 | 1.19 | 1.45 | – | 6.64 | – | – | 12.4 ± 0.1 | 15.8 ± 0.1 |
| X5715 | 1999 | 8.76 | 4.04 | 4.30 | 0.128 | 0.214 | 1.00 | 2.12 | 1.25 | 6.65 | – | – | 12.7 ± 0.1 | 16.4 ± 0.1 |
| Soaord | 1999 | 8.78 | 3.98 | 4.77 | 0.107 | 0.245 | 1.43 | 2.13 | 1.48 | 6.57 | – | – | 11.3 ± 0.1 | 14.8 ± 0.0 |
| Bygland | 1999 | 8.44 | 3.77 | 4.76 | 0.144 | 0.240 | 1.49 | 1.62 | – | – | – | – | 13.3 ± 0.2 | 18.4 ± 0.1 |
| CX1834 | 2000 | 8.35 | 4.21 | 4.50 | 0.197 | 0.237 | 0.60 | 1.85 | 1.65 | 6.56 | 6.48 | 6.5 | 10.3 ± 0.0 | 11.9 ± 0.1 |
| CX1836 | 2000 | 7.67 | 3.79 | 4.25 | 0.243 | 0.258 | 0.59 | 1.48 | 1.59 | 6.72 | 6.58 | 6.56 | 11.8 ± 0.1 | 14.0 ± 0.1 |
| min | | 7.67 | 3.77 | 4.10 | 0.107 | 0.214 | 0.59 | 1.45 | 1.19 | 6.55 | 6.00 | 6.14 | 9.5 | 11.8 |
| max | | 9.27 | 4.90 | 5.94 | 0.267 | 0.332 | 2.10 | 2.38 | 1.86 | 6.72 | 6.58 | 6.56 | 13.3 | 18.4 |
| mean | | 8.66 | 4.36 | 4.91 | 0.175 | 0.259 | 1.43 | 1.85 | 1.57 | 6.64 | 6.15 | 6.25 | 11.2 | 14.2 |
| SD | | 0.28 | 0.34 | 0.42 | 0.032 | 0.024 | 0.34 | 0.24 | 0.18 | 0.04 | 0.14 | 0.10 | 1.0 | 1.60 |

[a] Data are expressed as means of duplicate analyses on a wet basis. [b] Critical point of coagulant concentration. [c] –, sample not available.

an identical water-to-soybean ratio, the resultant soy milk compositions varied significantly. The solid content varied from 7.67 to 9.27%. The protein content varied from 3.77 to 4.90%. The ash content ranged from 4.10 to 5.94 mg/g of soy milk. The content of calcium and the content of magnesium naturally presented in soy milk ranged from 0.107 to 0.267 mg/g of soy milk and from 0.214 to 0.332 mg/g of soy milk, respectively. The phytate content varied from 0.59 to 2.10 mg/g of soy milk. The titratable acidity varied from 1.45 to 2.38 mg of citric acid/g of soy milk. The ratios of 11S/7S were determined for 25 available samples and ranged from 1.19 to 1.86. The pH values of 29 soy milk samples were determined and varied from 6.55 to 6.72.

**Critical Point of Coagulant Concentration.** As shown in **Table 1**, for 37 soy milk samples, the critical concentrations of $CaCl_2$ varied from 9.5 to 13.3 mM and the critical concentrations of $MgCl_2$ varied from 11.8 to 18.4 mM. On average, the critical concentration of $CaCl_2$ was 3.0 mM lower than that of $MgCl_2$. Therefore, $CaCl_2$ seems to be more potent than $MgCl_2$. This observation is consistent with our previous study (*19*). Because the conditions for soy milk preparation were controlled, the variations of CPCC among soybean samples could be attributed to the variation of soy milk characteristics.

**Table 2.** Significant Correlation Coefficients among Soy Milk Compositions/Properties (on a Wet Basis)

| relationship | coagulant | $R$[a] | no. of samples |
|---|---|---|---|
| phytate/protein | | 0.55** | 37 |
| phytate/ash | | 0.81** | 37 |
| phytate/coagulum pH | $CaCl_2$ | –0.89** | 22 |
| | $MgCl_2$ | –0.85** | 22 |
| phytate/pH drop | $CaCl_2$ | 0.89** | 22 |
| | $MgCl_2$ | 0.85** | 22 |
| coagulum pH/pH drop | $CaCl_2$ | –0.96** | 22 |
| | $MgCl_2$ | –0.93** | 22 |

[a] *, significant at $p < 0.05$; **, significant at $p < 0.01$.

**Interrelationships among Soy Milk Characteristics.** The interrelationships among soy milk characteristics were examined. The significant correlation coefficients are reported in **Table 2**. The phytate content was found to be significantly correlated to protein and ash contents, whereas the titratable acidity did not significantly correlate with original soy milk pH.

When coagulant was added to soy milk at 20 °C, coagulum was formed during titration. After titration, the coagulum pH was measured (**Table 1**). The difference in pH between soy

**Table 3.** Correlation Coefficients between Soy Milk Compositions/Properties and Critical Point of Coagulant Concentrations

| soy milk compositions/properties | CPCC[a] | | no. of samples |
|---|---|---|---|
| | $CaCl_2$ | $MgCl_2$ | |
| ash | −0.01 | 0.04 | 37 |
| Ca content | −0.35* | −0.45** | 37 |
| Mg content | −0.23 | −0.26 | 37 |
| titratable acid | −0.42** | −0.34* | 37 |
| pH of soy milk | 0.60** | 0.55** | 29 |
| protein content | −0.53** | −0.55** | 37 |
| 11S protein content | −0.79** | −0.81** | 25 |
| 7S protein content | 0.79** | 0.81** | 25 |
| 11S/7S ratio | −0.79** | −0.81** | 25 |
| solid content | 0.09 | 0.15 | 37 |
| phytate (g/g of soy milk) | 0.14 | 0.23 | 37 |
| phytate (g/g of protein) | 0.35* | 0.46** | 37 |

[a] *, significant at $p < 0.05$; **, significant at $p < 0.01$.

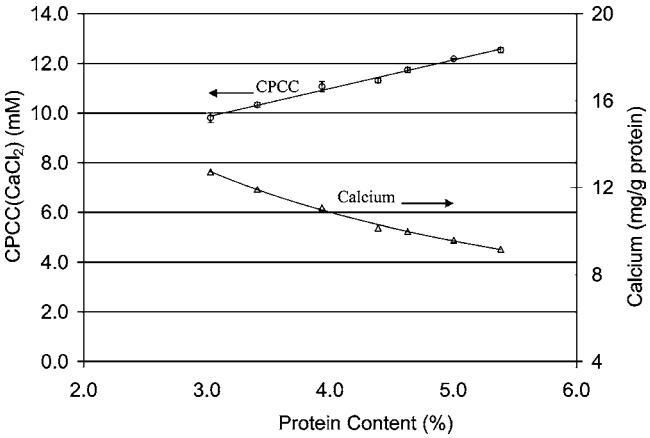


**Figure 1.** Critical point of coagulant concentration (CPCC) and amount of calcium required by 1 g of protein as influenced by protein content of soy milk made from the Proto (2000) soybean.

milk and the coagulum was calculated as pH drop value. Ono and co-workers reported that the decrease in soy milk pH upon the addition of $CaCl_2$ or $MgCl_2$ might be due to the formation of calcium phytate or magnesium phytate (*20*), which is insoluble above pH 6 (*21*, *22*). As shown in **Table 2**, phytate content significantly correlated with the coagulum pH ($r = -0.89$ for $CaCl_2$, $r = -0.85$ for $MgCl_2$; $p < 0.01$) and also significantly ($r = 0.89$ for $CaCl_2$, $r = 0.85$ for $MgCl_2$; $p < 0.01$) correlated with the pH drop value. These observations indicated that with an increase in phytate content in soy milk, the coagulum pH would decrease and the value of the pH drop would increase. These results agreed with those of Tezuka and others (*23*), who reported that the pH decrease by the addition of calcium in phytate-rich soy milk was greater than that in low-phytate soy milk.

**Correlation between Critical Point of Coagulant Concentration and Soy Milk Characteristics.** The correlation coefficients between the CPCC and soy milk characteristics are shown in **Table 3**. Ash content did not correlate significantly with the critical concentration of either $CaCl_2$ or $MgCl_2$. The content of calcium naturally presented in soy milk was negatively correlated with the critical concentrations of both coagulants ($r = -0.35$ for $CaCl_2$, $p < 0.05$; $r = -0.45$ for $MgCl_2$, $p < 0.01$). This is reasonable because $Ca^{2+}$ is an effective ion that reacts with protein during soy milk coagulation. However, magnesium content did not correlate with the critical concentration of either coagulant. The reason probably is that most of the magnesium may have not been present in free ion form in soy milk. Our observations are not completely consistent with the results of Ohara and others (*11*), who reported that optimal coagulant concentration significantly correlated with soy milk ash content ($p < 0.01$, $n = 19$) but did not significantly correlate with calcium or magnesium content in soy milk ($p < 0.05$, $n = 19$).

As shown in **Table 3**, the titratable acidity negatively correlated with CPCC ($r = -0.42$ for $CaCl_2$, $p < 0.01$; $r = -0.34$ for $MgCl_2$, $p < 0.05$). This is because acidic compounds could be used as acid coagulants by reducing the negative charges on the soy protein molecules by the protonation of the $-COO^-$ of the acidic amino acid residues. The correlation analysis also shows that soy milk pH was positively correlated with CPCC ($r = 0.60$ for $CaCl_2$ and $r = 0.55$ for $MgCl_2$, $p < 0.01$). This could be understood with the knowledge that with increasing soy milk pH, protein negative charges would increase and, therefore, more coagulants would be needed to reduce the negative charges of soy proteins. On the other hand, it had been reported (*24*) that pH strongly affected the extent of $Ca^{2+}$ binding to soy protein, because hydrogen ions compete with calcium ions for the same binding sites on the protein. Our results agreed with those of Ohara and others (*11*), who reported that optimal coagulant concentration increased with increasing pH of the soybean acid-precipitated protein dispersion.

**Table 3** shows a negative correlation between soy milk protein content and the critical concentration of either coagulant ($r = -0.53$ for $CaCl_2$ and $r = -0.55$ for $MgCl_2$, $p < 0.01$). This observation agrees with the results of Skurray and others (*4*), who reported that there was a negative relationship ($r = -0.87$, $n = 12$, $p < 0.01$) between the protein content of various bean cultivars and the calcium sulfate concentration required for good-quality tofu. A contrary result, however, was reported by Ohara (*11*), who found a positive correlation between OCC and soy milk protein content ($r = 0.626-0.695$, $p < 0.01$). To clarify the effect of soy milk protein content on the CPCC, a series of soy milks with different protein contents were prepared from a specific soybean material. As shown in **Figure 1**, the critical concentration of $CaCl_2$ increased with increasing protein content of the soy milk. This observation is in accordance with Watanabe and others (*3*) and Ohara and others (*11*). It was also noted from **Figure 1** that the amount of $CaCl_2$ consumed by each gram of protein decreased with increasing soy milk protein content. However, this observation did not eliminate the discrepancies that existed among the various correlations between soy milk protein content and coagulant requirement found by various researchers. Apparently, coagulant requirements are affected by various other biochemical factors in addition to protein properties. The effect of protein concentration during cooking on CPCC was further investigated by another approach and will be discussed later.

Our previous investigation (*10*) showed that CPCC increased with an increase in the solid content of soy milk made from a specific soybean material. In the present study, no significant correlations existed between CPCC and the solid content of soy milk made from various soybean materials (**Table 3**). This result could be interpreted to indicate that the variations in CPCC did not result from the variation in solid contents among the 37 soy milk samples.

Glycinin (11S) and $\beta$-conglycinin (7S) are the major protein fractions in soybeans. Saio and Watanabe (*25*) and Yuan and others (*26*) reported that 11S protein precipitated in a lower $CaCl_2$ concentration than 7S protein. It was also observed that the tofu-gel prepared from an 11S-rich soybean variety was more sensitive to calcium than that from a 7S-rich soybean variety

**Table 4.** Effect of the Sequence of Heating and Diluting on Critical Point of Coagulant Concentrations[a]

| original raw soy milk[b] | | HD soy milk[c,d] | | DH soy milk[d,e] | | |
|---|---|---|---|---|---|---|
| water-to-bean ratio | protein content (%) | protein content (%) | CPCC (mM) | protein content (%) | CPCC (mM) | differences in CPCC[f] (mM) |
| 8 | 4.01 | 3.32 ± 0.04x | 14.52 ± 0.03b | 3.35 ± 0.01x | 14.69 ± 0.02a | 0.17 |
| 7 | 4.57 | 3.34 ± 0.03x | 14.11 ± 0.04c | 3.34 ± 0.02x | 14.41 ± 0.05b | 0.30 |
| 6 | 5.31 | 3.36 ± 0.03x | 13.50 ± 0.06e | 3.33 ± 0.06x | 13.94 ± 0.09d | 0.44 |

[a] Data are expressed as means ± SD of duplicate analyses on a wet basis. [b] Raw soy milk was made from the Proto (2000) soybean. [c] Soy milk was prepared by heating original raw soy milk and then diluting cooked original soy milk. [d] Values with different letters (a–e) are significantly different ($p < 0.05$). Values with the same letter (x) are not significantly different ($p < 0.05$). [e] Soy milk was prepared by diluting original raw soy milk and then heating diluted original raw soy milk. [f] Difference in CPCC between HD soy milk and DH soy milk.

(*27*). Tezuka and co-workers (*23*) reported that the concentrations of $CaCl_2$ or $MgCl_2$ at which the protein solubility of the soy milk made from an 11S-rich soybean variety decreased sharply were lower than that for a 7S-rich soybean variety. Kohyama and Nishinari (*28*) reported that the rate of gelation for 7S with glucono-$\delta$-lactone (GDL) was much slower than that for 11S. In the present study, a positive correlation between CPCC and 7S protein and a negative correlation between CPCC and 11S protein or 11S/7S ratio were observed (**Table 3**). This agrees with the results obtained by other researchers mentioned above. All of these observations suggest that higher calcium or magnesium concentrations are required for the 7S globulin coagulation than for 11S globulin coagulation. The difference in CPCC between 7S and 11S was attributed to the difference in isoelectric points between 7S and 11S proteins. Because the isoelectric point of the 7S protein is lower than that of the 11S protein, a larger amount of calcium or magnesium ions is required to remove more negative charges on 7S than on 11S at the natural soy milk pH of ~6.5.

The phytate effect on tofu-making was studied by several researchers. Saio and co-workers (*29*) reported that the higher content of phytic acid in soy milk resulted in the slower coagulative reaction between soybean protein and calcium, thereby producing a higher yield of a softer tofu. They hypothesized that added calcium coagulant was bound preferentially by phytic acid rather than by the soy protein. In contrast, Schaefer and Love (*30*) found a positive correlation coefficient between phytic acid content and tofu hardness and a negative correlation coefficient between phytic acid content and tofu yield. Hou and Chang (*31*) reported that tofu yield increased and its texture became softer when the phytate content in soy milk was selectively reduced with a phytase treatment. However, Kamel and deMan (*32*) reported that the soybean phosphorus content was not significantly correlated with either bean curd hardness ($r = 0.54$) or curd yield. Skurray and co-workers (*33*) prepared tofu with the optimal concentration of calcium sulfate and concluded that there were no significant correlations between the soybean phosphorus content and tofu quality.

**Table 3** shows the amount of phytate per gram of protein was significantly correlated with the critical concentration of either $CaCl_2$ ($r = 0.35$, $p < 0.05$) or $MgCl_2$ ($r = 0.46$, $p < 0.01$), indicating more coagulant was needed when more phytate was present in soy milk on a per gram of protein basis. This result is consistent with the report of Saio and co-workers (*29*). However, the amount of phytate per gram of soy milk did not significantly correlate with CPCC (**Table 3**), and the magnitudes of the correlation coefficients between CPCCs and the amount of phytate per gram of protein were not substantial. Ohara and co-workers (*11*) showed the contribution of soybean nonprotein constituents to OCC was only 35%. Therefore, it may be concluded that phytate did influence the amount of CPCC among soybean samples, but its effect was not great.

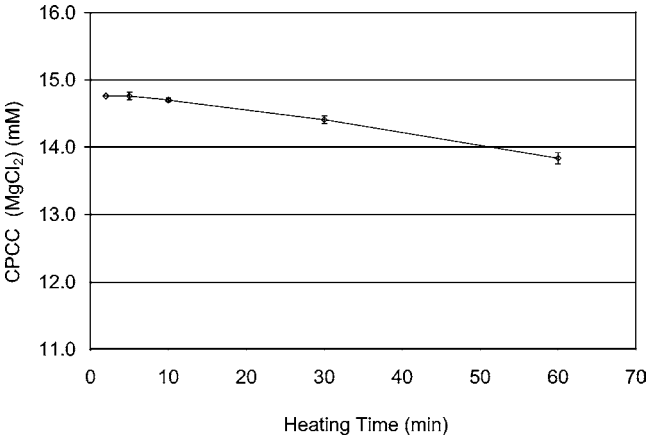


**Figure 2.** Critical point of coagulant concentration (CPCC) as influenced by heating time. Soy milk: 4.62% protein, Proto (2000).

**Effect of the Sequence of Heating and Diluting on Critical Point of Coagulant Concentration.** An experiment was carried out by changing the sequence of heating and diluting of soy milk. Soy milk was prepared from an original raw soy milk either by diluting first and then heating (DH) or by heating first and then diluting (HD). The dilution process was so controlled that there were no significant differences ($p < 0.05$) in solids and protein contents among all final soy milks (**Table 4**). Three different concentrations of original raw soy milks were tested. For each original raw soy milk, it was found that HD soy milk's CPCC was lower than that of DH soy milk, and the differences in CPCC between HD soy milk and DH soy milk increased with increasing original soy milk protein content (**Table 4**). It was found that heating first and then diluting the soy milk resulted in less coagulant required than the process of diluting first then heating. It had been reported that an increase in soy protein concentration caused the formation of higher molecular mass aggregates during thermal denaturation (*34*, *35*). We speculated that higher molecular mass protein aggregates might have been formed by heating first than that by diluting first, thereby leading to a reduced coagulant requirement. Therefore, the sequence of diluting and heating should be taken into consideration in tofu manufacturing. In addition, it was confirmed that a higher protein soy milk would require less coagulant on a per gram of protein basis.

**Effect of Heating Time on Critical Point of Coagulant Concentration.** The raw soy milk was heated to boiling and kept boiling for 2, 5, 10, or 30 min, respectively. CPCC was determined for the resulting soy milk. As shown in **Figure 2**, the CPCC decreased significantly ($p < 0.05$) with an increase in heating time. Thermal denaturation of soybean proteins is a prerequisite for the formation of tofu gel (*36*), but overheating does not benefit. In fact, overheating may damage tofu texture (*37*, *38*). Wang and Hesseltine (*6*) reported the destructions of

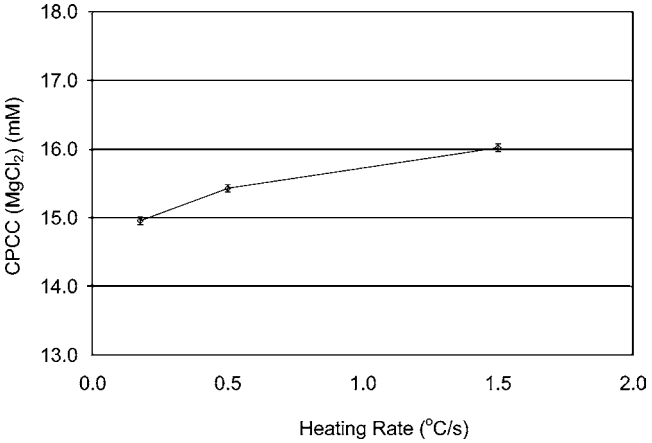


**Figure 3.** Critical point of coagulant concentration (CPCC) as influenced by heating rate. Soy milk: 4.60% protein, Proto (2000).

cystine and methionine after extended boiling of soy milk. Although the heat denaturation mechanism of soybean proteins in model systems has been studied by many researchers (*39*), there is still not enough knowledge about protein subunit behavior during soy milk heating to understand the mechanisms of how heating time affects CPCC.

**Effect of Heating Rate on Critical Point of Coagulant Concentration.** Soy milk samples were heated at different rates from room temperature to 97 °C. As shown in **Figure 3**, CPCC increased with an increase in heating rate. The increase in CPCC might have resulted from the differential interactions between 11S and 7S proteins during thermal denaturation of the different heating rates. As the denaturation temperature of the 11S protein (90−95 °C) is ∼20 °C higher than that of the 7S protein (70−75 °C) (*16*, *40*), the 7S protein would denature in advance of the denaturation of the 11S protein when the heating rate is slow. When the heating rate is high, 7S and 11S proteins would denature almost simultaneously. The denaturations of 7S and 11S proteins involve dissociation and association of protein subunits, and the interactions between 7S and 11S proteins occur during heating (*41*−*44*). We have observed (*45*) that the viscosity of soy milk increased when the soy milk was heated by a two-step process including heating first at 75 °C for 5 min followed by heating at 95 °C for 5 min. Therefore, we hypothesize that the heating rate would affect the interactions of 7S and 11S proteins, thereby modifying the soy milk coagulating properties.

**Conclusions.** Soy milk characteristics varied with soybean materials. The statistical correlation analyses between critical point of coagulant concentration and soy milk characteristics suggested that coagulant requirements for making tofu were significantly related to soy milk composition/properties. Among all of soy milk characteristics studied, soy protein content, its major protein components of 7S and 11S protein, and their ratio showed the highest correlations with CPCC, whereas pH and titratable acidity also were substantially correlated with CPCC. When soy milk was made from one specific soybean material, more protein in the soy milk needed more coagulant, but a higher protein concentration during cooking resulted in less coagulant required by each gram of protein during coagulation. Less coagulant was required when soy milk heating time was increased, and more coagulant was required when soy milk was heated more quickly. Different sequences of heating and diluting resulted in different coagulant requirements. The findings from this study can be readily applied in the tofu manufacturing industry to improve end-product quality.

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