Calcium Coagulation Properties of Hydrothermally Processed Soymilk

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ABSTRACT: The effects of hydrothermal cooking (HTC), a steam-injection process otherwise known as jet cooking, on the calcium salt coagulation properties of soymilk were determined. Full-fat soymilk was processed at five different conditions (traditional kettle cooking at 100°C for 5 min, HTC at 100°C for 20 s, HTC at 134°C for 26 s, HTC at 154°C for 31 s, and HTC at 162°C for 35 s) and coagulated at four calcium chloride concentrations (0.05, 0.10, 0.20, and 0.30%). Tofu yields and recoveries of dry matter and protein in the coagulated curd followed similar trends with increasing calcium chloride concentration, namely, an initial increase rising to a peak followed by a decrease. HTC-processed soymilks, especially those processed at high temperature (162°C), gave lower tofu yields and lower recoveries of dry matter and protein in tofu. HTC-processed soymilks, especially those processed at 134°C or higher, resulted in very soft, fragile, and adhesive tofu. The high calcium salt tolerance of HTC-processed soymilk might be used to improve dispersion stability of calcium-fortified soy-based dairy analogs.

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KEY WORDS: Coagulation, jet cooking, protein, soybeans, soymilk, soy protein, tofu.

Tofu, also known as soybean curd, has been widely consumed in the Orient for many centuries, providing an important source of protein and calcium in Asian diets. Soybean curd is prepared by coagulating soymilk, an aqueous extract of soybeans, by using coagulating agents and subsequently molding the coagulum (1,2). Coagulation properties of soymilk are critical to achieving high yields and the desired texture of tofu. Calcium sulfate is the most commonly used coagulating agent, but other calcium sources may be used, such as calcium chloride, calcium lactate, calcium acetate, calcium carbonate, calcium phosphate, calcium gluconate, and glucono-Δ-lactone (1).

Preparing soymilk is the first step in tofu manufacturing. Traditionally, soybeans are soaked overnight, then ground with water and kettle-cooked at ambient pressure at 100°C for 3–10 min. The resultant solution was filtered to remove insoluble matter, primarily fiber (2,3). The soymilk thus produced is usually adjusted to 5–6% dry matter content by adding water before coagulating. Traditionally prepared soymilk has painty and beany flavors owing to lipid oxidation catalyzed by lipoxygenase during soaking and grinding. In Western societies, this flavor is unacceptable to most consumers and is the major obstacle to widespread acceptance of almost all soy food products, especially soymilk and tofu.

A steam-infusion cooking process, known as hydrothermal cooking (HTC), was developed to produce soymilk continuously from ground full-fat soy flour (4–6). HTC-processed soymilk had less beany flavors because of the much shorter time for lipoxygenase to be active and because steam flashing stripped volatiles. The process also increased recoveries of dry matter and protein in the soymilk from 60 and 70% for traditional soymilk to 87 and 90%, respectively (7). It is not known whether HTC-processed soymilk can be used for tofu manufacturing despite its superior flavor characteristics and yield. However, the degree of heat treatment of soymilk affects gel formation and gel quality (8).

Another significant feature of the coagulation properties of soymilk is the sensitivity of soy protein to calcium fortification of soymilk. Compared with bovine milk, soymilk has a much lower calcium content (12 mg/100 mL for soymilk vs. 120 mg/100 mL for bovine milk) (9). Efforts to fortify soymilk with calcium have been largely unsuccessful because calcium tends to coagulate the proteins, forming precipitates and causing gelation of the soymilk during storage. Weingartner *et al.* (9) were able to fortify soymilk (6% dry matter) to a calcium level comparable to bovine milk by using mixtures of calcium citrate and tricalcium phosphate. Addition of these calcium salts did not adversely affect protein stabilities of the soy beverages. Zemel and Shelef (10) also patented a method for fortifying soymilk with calcium in which an alkali metal polyphosphate salt was added to suppress aggregation between soymilk constituents and the added calcium ions. All of these methods, though, require addition of special and more expensive forms of calcium.

The objectives of the present study were to investigate the effects of HTC processing conditions on coagulation properties of soymilk and to explore the possibilities of utilizing the HTC process to manufacture tofu.

MATERIALS AND METHODS

Soymilk processing. Soymilk was prepared by using full-fat soy flour, which was obtained by grinding Vinton 81

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soybeans (West Central Cooperative, Jefferson, IA). The same processing methods used in our previous study on the effects of HTC processing on functionalities of soy protein products (11) were followed. The ground soybean slurry was processed at 8% dry matter in the present study. Four different HTC temperatures (100, 130, 154, and 162°C) were used. Only one holding tube length (9.3 m) was used, which resulted in four different cooking times (20, 26, 30, and 35 s) for the four temperatures, respectively. In HTC processing, steam is injected under pressure directly into the product. Different cooking temperatures are achieved by controlling the back pressure; however, increasing the back pressure inherently increases holding time. It is not possible to achieve the same holding time at different cooking temperatures without changing the holding tube length (it would be almost impossible to do this reliably). Traditional soymilk prepared by kettle boiling for 5 min was used as a control. The cooked slurries were centrifuged at 1000 × *g* for 10 min, and the supernatants were decanted and collected as soymilk. Dry matter and protein contents were determined using the same procedures as we reported previously (11).

Tofu coagulation. Protein coagulation was carried out by modifying the tofu-making procedure used by Saio *et al.* (12). Instead of calcium sulfate, calcium chloride was used as the coagulating agent because of its high water solubility, which increases reproducibility (1). The soymilk was adjusted to 5.0% dry matter by adding appropriate quantities of water. Samples (39 mL) were placed in 50-mL centrifuge tubes and incubated in a 70°C waterbath for 30 min. Then 1 mL of various calcium chloride solutions of different concentrations (0.02, 0.04, 0.08, and 0.12 g/mL) was added to the warm soymilk. The samples were promptly mixed for 10 s with a vibrating mixer (Barnstead/Thermolyne, Dubuque, IA). The final calcium chloride concentrations in the soymilk were 0.05, 0.10, 0.20, and 0.30%. The samples were then placed in the waterbath for 30 min. The samples were cooled by placing them in an ice/water bath for 20 min before centrifuging at 1000 × *g* for 10 min. The resulting curds at the bottom of the tubes were tofu. The test-tube tofu procedure required less sample and gave reproducible mass-balance information.

Tofu evaluation. The amounts of supernatant and tofu were measured gravimetrically. Tofu yield was calculated as the percentage of wet precipitate weight after centrifuging divided by the starting soymilk weight ("as-is" basis). Dry matter and protein contents of the supernatant were determined analytically, and dry matter and protein recoveries in the tofu fractions were calculated by mass balance.

Texture profiles of curds were evaluated using a Voland Stevens Texture Analyzer (Voland Corporation, Hawthorne, NY). Curds from the test tubes were taken and carefully cut into blocks (2 × 2 cm base, 1.5 cm height) by using a razor blade. These blocks were uniform in reference to their geometric location in the test tubes. Care was taken to ensure that the texture analyzer probe pressed and penetrated the center of the block. A TA58 probe was used, and a 5-mm travel distance and a speed of 0.5 mm/s in the cycle mode were chosen as operating parameters. Texture profiles were recorded with a chart recorder. The chart speed and sensitivity were set to 10 cm/min and 2 V, respectively. The analyzer was calibrated so that the full-scale force was 100 g. Hardness, fracturability, and adhesiveness were read from the profile according to the definitions of Saio *et al*. (12).

Experimental design. A complete random design was used. The treatments included combinations of processing conditions (heat treatment involving combinations of temperature and time) and calcium concentration. Five levels of processing conditions and four levels of calcium concentrations were used. The treatments were repeated three times. The yields, solid and protein recoveries, and texture data were analyzed by using the GLM (general linear models) procedure of the SAS (13) system. The means for all treatment combinations (processing condition and calcium concentration) were compared with Duncan's multiple range test ($P < 0.05$), which was used to determine whether differences between any two treatment combinations were statistically significant. The comparisons were necessary to answer practical questions regarding this processing technology.

RESULTS AND DISCUSSION

Tofu mass balances. The effects of calcium chloride concentration and HTC-processing conditions on tofu yields were dramatic (Table 1). When 0.05% calcium chloride was added to HTC soymilk processed at 132, 154, and 162°C, there was little coagulation of tofu (1.2–2.3%). There were significantly greater amounts of tofu coagulation (37.8%) in traditional soymilk, although the precipitates were in a semiliquid form. When the calcium chloride concentration was increased to 0.1%, tofu yields from all soymilks increased significantly. Traditionally prepared soymilk had the highest yield (34.4%), followed by HTC soymilk processed at 100, 132, and 154°C (31.2–32.4%). The HTC soymilk processed at 162°C had the lowest tofu yield (25.8%). When the calcium chloride concentration was increased to 0.2% and above, tofu yields tended to decrease, but most of the differences were not statistically significant. However, traditionally prepared soymilk declined to a greater extent than HTC-processed soymilk so that HTC soymilk processed at 100, 132, and 154°C had

TABLE 1
Effects of HTC Processing on Tofu Yield[a] (%)

Processing conditions		$CaCl_2$ concentration (%)			
Temperature (°C)	Time	0.05	0.10	0.20	0.30
HTC					
100	20 s	9.3[f]	31.2[c,d]	29.7[d]	30.6[c,d]
132	26 s	1.2[g]	32.1[c]	29.8[d]	31.6[c,d]
154	31 s	2.3[g]	32.4[b,c]	29.8[d]	31.1[c,d]
162	35 s	1.9[g]	25.8[e]	25.3[e]	25.3[e]
Traditional					
100	5 min	37.8[a]	34.4[b]	27.1[e]	27.2[e]

[a]Tofu yield was calculated as wet tofu weight percentage of soymilk ("as-is" basis). HTC, hydrothermal cooking. Means with common superscripts are not significantly different at $P \leq 0.05$.

higher tofu yields than traditionally prepared soymilk when 0.2 or 0.3% calcium chloride was added. Overall, HTC-processed soymilk gave lower tofu yields than did traditionally prepared soymilk, especially when processed at a higher HTC temperature (162°C).

Dry matter recovery as tofu was a better indicator than tofu yield based on an "as-is" wet basis. The effects of HTC-processing conditions and calcium chloride concentrations on dry matter recoveries followed trends similar to those of wet tofu yields (Table 2).

Higher concentrations of calcium chloride generally led to higher dry matter recoveries. However, dry matter recoveries started to decline when the calcium chloride concentration exceeded critical levels. Traditionally prepared soymilk reached maximum dry matter recovery at 0.1% calcium chloride concentration. This optimal calcium chloride concentration compared favorably with an earlier study (1), where 0.09% calcium chloride was considered optimal. However, HTC soymilk processed at 162°C had its highest dry matter recovery at 0.2% calcium chloride. Although some differences were not statistically significant, the trend was obvious. HTC soymilk processed at lower temperatures (100, 132, and 154°C) exhibited maximal dry matter recoveries at either 0.1 or 0.2% calcium chloride.

When 0.05% calcium chloride was added, dry matter recoveries for HTC soymilk processed at 132, 154, and 162°C were very low (4–7%) compared with traditionally processed soymilk, which gave the highest dry matter recovery (53%). At higher calcium chloride concentrations, HTC soymilk processed at lower temperatures (100, 132, and 154°C) gave higher dry matter recoveries, followed by traditional soymilk and then HTC soymilk processed at 162°C. The more extreme the HTC-processing condition was (higher temperature and longer time), the lower the dry matter recovery in tofu.

Protein recovery was calculated as the percentage of protein in the original soymilk that was recovered in the tofu fraction (Table 3). The effects of HTC processing conditions and calcium chloride concentration followed trends similar to those of tofu yield and dry matter recovery.

The higher the calcium chloride concentration, the more protein was recovered in tofu. Traditionally prepared soymilk reached the highest protein recovery at 0.10% calcium chloride; a higher concentration led to lower protein recovery in tofu. HTC soymilk processed at 100°C rose from a low value at 0.05% calcium chloride to a plateau at the other three concentrations. HTC soymilks processed at 132, 154, and 162°C reached their maximal protein recoveries at 0.20% calcium chloride.

When 0.05% calcium chloride was added to HTC-processed soymilk, especially soymilk processed at higher temperatures (132, 154, and 162°C), very little protein was recovered in the precipitated fraction, and the majority of the protein remained in the supernatant. However, about 42% of the protein was recovered in the precipitated fraction (tofu) from traditionally prepared soymilk. When the calcium chloride concentration was increased to 0.1%, traditionally prepared soymilk and HTC soymilk processed at 100°C had the highest protein recoveries as tofu. HTC soymilk processed at 162°C had the lowest protein recovery; more than 40% of the protein remained in the supernatant. When the calcium chloride concentration was increased to 0.2 and 0.3%, HTC soymilk processed at lower temperatures (100, 134, and 154°C) gave about the same protein recoveries as did traditionally prepared soymilk. However, HTC soymilk processed at 162°C gave significantly lower protein recovery as tofu than did the other treatments.

With increasing calcium chloride concentration, tofu yield and recoveries of dry matter and protein for soymilk processed differently followed a common trend, i.e., initial increases to maximum values followed by declines. This can be explained as salt-in effects, whereby excess salts can make protein more soluble (14). The manner in which the soymilk was processed affected the amounts of calcium chloride required to achieve maximal values. Traditionally processed soymilk reached maximal values at lower calcium chloride concentrations than did HTC-processed soymilk, and the higher the processing temperature, the higher the calcium chloride concentration required to maximize tofu yield and recoveries of dry matter and protein. The mechanism for this peak delay or high tolerance of HTC-processed soymilk to calcium salts is not known.

The high tolerance of HTC-processed soymilk to low calcium chloride concentration may prove to be useful in calcium fortification of soymilk. Soymilk with 6% dry matter

TABLE 2
Effects of HTC Processing on Dry Matter Recovery in Tofu[a] (%)

Processing conditions		$CaCl_2$ concentration (%)			
Temperature (°C)	Time	0.05	0.10	0.20	0.30
HTC					
100	20 s	21.1[j]	80.3[a]	79.3[a,b,c]	77.7cdef
132	26 s	4.4[l]	80.1[a,b]	79.1[a,b,c,d]	78.1[b,c,d,e]
154	31 s	6.2l[k]	77.4[c,d,e,f]	76.9[e,f]	76.1[f]
162	35 s	7.4[k]	65.5[h]	67.6[h]	66.5[h]
Traditional					
100	5 min	53.1[i]	77.1[d,e,f]	73.9[g]	73.2[g]

[a]Means with common superscripts are not significantly different at $P \leq 0.05$. For abbreviation see Table 1.

TABLE 3
Effects of HTC Processing on Protein Recovery (%) in Tofu[a]

Processing conditions		$CaCl_2$ concentration (%)			
Temperature (°C)	Time	0.05	0.10	0.20	0.30
HTC					
100	20 s	8.2[g]	77.9[a]	77.4[a]	77.1[a]
132	26 s	0.3[i]	73.6[b]	76.4[a]	77.3[a]
154	31 s	3.2[h]	70.9[c]	77.4[a]	76.6[a]
162	35 s	4.8[h]	58.0[e]	66.2[d]	66.9[d]
Traditional					
100	5 min	41.7[f]	78.8[a]	76.1[a]	76.6[a]

[a]Means with common superscripts are not significantly different at $P \leq 0.05$. For abbreviation see Table 1.

naturally contains approximately 12 mg calcium per 100 mL, whereas bovine milk contains 120 mg calcium per 100 mL. Our data indicate that the calcium content of soymilk could be increased to at least 28 mg calcium per 100 mL, which more than doubles its natural content, without adversely affecting stability. This is not possible with traditional soymilk.

Tofu texture profiles. Effects of HTC processing conditions and calcium chloride concentration on tofu texture profiles (including hardness, fracturability, and adhesiveness) were also studied to predict the suitability of HTC-processed soymilk for tofu manufacturing. When 0.05% calcium chloride was added, too little tofu resulted to perform texture analyses. Therefore, texture data for this calcium chloride concentration could not be obtained.

Hardness was defined as the force encountered by the analyzer probe as it traveled to the maximum distance when penetrating the curd. For all soymilks, tofu hardness values initially increased to a maximum and then declined with increasing calcium concentration; 0.2% calcium chloride gave the hardest tofu (Table 4).

The effects of HTC processing on tofu texture were also extremely significant. HTC processing greatly reduced the hardness of tofu, and the higher the processing temperature, the softer the resulting tofu.

Fracturability was defined as the force encountered by the probe when it first punctured the sample surface. The higher the fracturability value, the less fragile the texture. The effects of the HTC processing condition and calcium chloride concentration followed the same trend as for hardness (Table 5). HTC-processed soymilks, especially those processed at higher temperatures, resulted in very fragile tofu compared with traditionally prepared soymilk.

Adhesiveness is defined as the negative force encountered by the probe when it withdraws from the penetrated curd. Adhesiveness was measured as negative peak area (mm^2) from the texture profile. Tofu adhesiveness increased with increasing calcium chloride concentration. Use of HTC-processed soymilk resulted in tofu that was more adhesive, and the higher the processing temperature, the more adhesive the tofu (Table 6).

HTC-processed soymilks, especially those processed at high temperatures (132, 154, and 162°C), gave tofu with inferior texture characteristics, namely, very soft, fragile, and adhesive tofu.

Yields and recoveries of dry matter and protein for tofu from HTC soymilk processed under different conditions followed a common pattern with increasing calcium chloride concentration, namely, initial increases to maximal values followed by decreases. HTC-processed soymilks, especially those processed at a high temperature (162°C), gave lower tofu yields and lower recoveries of dry matter and protein in tofu. HTC-processed soymilks, especially those processed at high temperature (132, 154, and 162°C), resulted in tofu with textures that were very soft, fragile, and adhesive compared with that of traditional tofu. These unique characteristics may be useful in preparing other foods such as snack dips. The high calcium salt tolerance of HTC-processed soymilk might also prove beneficial in calcium fortification of soy-based dairy analogs.

TABLE 4
Effects of HTC Processing on Hardness (g) of Tofu[a]

Processing conditions		$CaCl_2$ concentration (%)		
Temperature (°C)	Time	0.10	0.20	0.30
HTC				
100	20 s	10.3^{e}	16.2^{c}	13.9^{d}
132	26 s	$4.4^{h,i}$	$9.7^{e,f}$	$7.9^{f,g}$
154	31 s	$2.7^{j,i}$	7.2^{g}	$5.9^{g,h}$
162	35 s	2.2^{j}	4.9^{h}	5.0^{h}
Traditional				
100	5 min	24.6^{b}	30.0^{a}	18.2^{c}

[a]Means with common superscripts are not significantly different at $P \le 0.05$. For abbreviation see Table 1.

TABLE 5
Effects of HTC Processing on Fracturability (g) of Tofu[a]

Processing conditions		$CaCl_2$ concentration (%)		
Temperature (°C)	Time	0.10	0.20	0.30
HTC				
100	20 s	11.5^{e}	18.7^{c}	16.3^{d}
132	26 s	$4.6^{h,i}$	12.6^{e}	9.3^{f}
154	31 s	$2.3^{k,j}$	7.2^{g}	5.2^{h}
162	35 s	$1.0^{k,l}$	$4.5^{h,i}$	$2.8^{i,j}$
Traditional				
100	5 min	22.1^{b}	30.0^{a}	20.2^{c}

[a]Means with common superscripts are not significantly different at $P \le 0.05$. For abbreviation see Table 1.

TABLE 6
Effects of HTC Processing on Adhesiveness (mm) of Tofu[a]

Processing conditions		$CaCl_2$ concentration (%)		
Temperature (°C)	Time	0.10	0.20	0.30
HT				
100	20 s	$21.4^{j,i}$	$30.5^{g,h,i}$	$48.8^{d,e,f}$
132	26 s	$37.6^{f,g,h}$	$40.7^{e,f,g}$	$54.0^{c,d,e}$
154	31 s	$52.9^{d,e}$	$48.9^{d,e,f}$	$57.0^{c,d}$
162	35 s	$68.2^{c,d}$	94.7^{b}	120.1^{a}
Traditional				
100	5 min	15.3^{j}	$24.4^{h,i,j}$	$33.6^{g,h,i}$

[a]Means with common superscripts are not significantly different at $P \le 0.05$. For abbreviation see Table 1.

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