Four methods of determining optimum coagulation levels in tofu manufacture were compared. Full-fat soyflakes were used to produce 5% solids soymilk. Each soymilk batch was coagulated with calcium sulfate dihydrate (0.000–0.0450 N). Electrical conductance of the coagulating batches was recorded at 72°C and 21°C. Coagulated batches were pressed to make tofu and whey volume, pH, transmittance (400 nm) and tofu composition were measured. Whey transmittance and conductance correlated with coagulant concentration (r = 0.87). Tofu yield, conductivity, and absorbance data were also related. Measuring the conductance of the coagulating soymilk was faster than obtaining pH and spectrophotometric values. Conductivity and transmittance could be used to optimize tofu coagulation.

**Key Words:** conductivity, coagulation, soymilk, tofu

**INTRODUCTION**

Tofu manufacturers use a variety of tofu grade soybeans, identity preserved, blended, and flaked, for soymilk and tofu. Work has been reported on several variables affecting the quality and yield of tofu such as cultivar, geographic location, crop year, and storage conditions of beans (Wang et al., 1983; Thomas et al., 1989; Lim et al., 1990; Murphy et al., 1997). Some important manufacturing variables are the soak and grind, water to soybean ratio for soymilk (Watanabe et al., 1964; Beddows and Wong, 1987a); solids in the soymilk (Johnson and Wilson, 1984); and time and temperature of heating the soymilk (Saio et al., 1979). Also important are the temperature and extent of stirring during coagulation (Wolf and Tamura, 1969; Wang and Hesseltine, 1982; Beddows and Wong, 1987b) and type and concentration of coagulant (Appu Rao and Narasiga Rao, 1975; Shurtleff and Aoyagi, 1979; Skurray et al., 1980; Wang, 1984; Johnson, 1984; de Man et al., 1986; Beddows and Wong, 1987c). The amount of coagulant added in the manufacture of tofu is one of the critical control points, which helps determine the product’s texture, taste, flavor and yield (Wilson, 1995). Past laboratory and pilot-scale tofu research has focused on determining and standardizing optimum levels of coagulant to be used for tofu processes (Johnson and Wilson, 1984).

Shurtleff and Aoyagi (1979) indicated that good soymilk coagulation occurs when the curd has separated and has moved away from the edges of the coagulation vessel. Existing methods to determine optimum coagulant concentration such as light transmittance (%T) of whey, whey volume and tofu yield (Sun and Breene 1991) are measured after the coagulation is complete. The transparency method also requires the whey to be free of coagulated particles for reproducibility. The time required to cool the samples for measurement allows the soymilk to cool below the critical coagulation temperature. This would also limit the measure of pH to indicate the optimum amount of coagulant during processing. The whey volume, tofu yield and composition could be used for prescreening. However, they cannot serve as indicators to adjust the batch being coagulated. Rapid methods for measuring the optimum coagulant concentration during tofu manufacture have not been reported.

Our objective was to compare existing methods for measuring the optimum coagulant concentration during tofu manufacture with a rapid method based on the electrical conductivity of the coagulating soymilk batch.

**MATERIALS & METHODS**

**Raw material**

XLRB soybean flakes were provided by Mycal Company of America (Jefferson, IA). All chemicals used in the analytical work were reagent grade (Fisher Scientific, Fair Lawn, NJ). Food-grade calcium sulfate dihydrate (CaSO₄ · 2H₂O) was used as the coagulant in tofu production (Allied Custom Gypsum, Lindsay, OK).

**Soymilk production**

Soymilk (85g ± 0.01g) and 1.2 L cold tap water were used in a prototype Hot Soymilk Machine (commercially available TOBE Products, RI), to produce 1.1 L of 5 °Brix solid soymilk. The water was heated to 85°C with an electric heating element while the soyflakes soaked in the heating water (encapsulated in a 60-mesh screen cage with a blender blade). The motor was switched on (water 85°C) three times for 30 sec each (with 10 sec between blending) for a total of 110 sec. The blended product was heated to 95°C and held at >95°C for 7 min to provide 80% inactivation of trypsin inhibitors and to lower the microbial load (Bai, 1997). The hot soymilk was filtered through a 100-mesh nylon filter sack (Kawanishi Shoko Co. LTD, Los Angeles, CA) and hand-pressed to remove large particles. The soymilk (1 L) was poured into a polypropylene Nalgene beaker to prevent possible ground loop currents (recommended by instrument manufacturer), which could cause errors during conductivity measurements.

**Laboratory-scale tofu manufacture**

Tofu manufacturing procedures were followed as described by Johnson (1984) and Kaan (1987), with the following modifications: a 5 °Brix solid soymilk (1 L) was cooled to 77°C and coagulated using pre-suspended CaSO₄ · 2H₂O in 25 ml of 55°C water. The amount of CaSO₄ · 2H₂O used at each concentration was calculated using the following formula: Amount of CaSO₄ · 2H₂O (g) = N × Tv × M, where N = normality of calcium sulfate dihydrate, Tv = total volume (L) of soymilk to be coagulated and M = half the molar weight of calcium sulfate dihydrate (86.0g).

A Hamilton Beach hand blender (Proctor-Silex Inc., Washington, NC, Model # 59700) was used to blend the soymilk/coagulant mix for 3 sec. The batch was allowed to stand for 2 min and then gently broken and stirred with a spoon, to ensure uniform mixing of coagulant with soymilk, followed by a 5 min holding. The coagulum was poured into a press box lined with a single layer of cheesecloth and incrementally pressed for 8 min (1 kg for 3 min, 2 kg for 2 min, and 3 kg for 3 min). The press box was constructed of 1 mm-thick stainless steel mesh with 6 mm dia holes, which allowed the whey to flow out. The outer dimensions of the press box were 13 cm × 10 cm × 8 cm.
**Tofu yield and proximate analysis**

Yield was expressed as wet weight of tofu obtained from 85 g raw soy flakes. Samples were analyzed for moisture using AOAC (1990) method sec. 925.10. Fat was determined using AOAC (1983), sec. 30–25, the Goldfisch method, and crude protein analysis was performed using the Kjeldahl method, AOAC (1990), sec. 954.01, 955.04 (c) with the following modification: Kjeltab TCT was used instead of mercuric oxide.

**Whey analysis**

*By volume:* Whey was collected from the pressed tofu and the volume measured at 45°C with a 1 L graduated cylinder. *By pH:* A Corning pH meter, model # 340 (Corning, NY) with a 3 in 1 combination pH electrode was used to determine pH of the whey at 21°C. *By transmittance:* Whey collected at each coagulant concentration was tested for % transmittance (%T) at 400 nm (Watanabe et al., 1964; Johnson, 1984) using a Spectronic Model 20 D+ (Milton Roy Spectronic Instrument, Inc., Rochester, NY). *By conductivity:* A YSI model 35 conductance meter (Yellow Springs Inst. Co., Inc., Yellow Spring, OH), with a YSI # 3417 conductance probe (K = 1.0 mho/cm) was used at a 20-millimho setting to determine electrical conductivity of whey at 72°C to measure conductance during coagulation and at 21°C to compare with other methods tested at that temperature.

**Textural properties of tofu**

The Texture Profile Analysis (TPA) parameters of hardness and fracturability were determined on a 1 cm-cube sample using a Universal Testing Machine (Model 1122, Instron Co., Canton, MA) equipped with a compression head (Bourne, 1978). The sample was compressed from 1 to 0.2 cm (80% deformation). Crosshead and chart speeds were 100 mm/min. Six replicate samples were tested for each batch of tofu.

**Statistical analysis**

A randomized block design (Cochran and Cox, 1957) with 5 replications at each coagulant concentration was used. The data were analyzed using the General Linear Models procedure and differences among treatment means were evaluated by least significant differences (LSD). Significance of differences was defined at P < 0.05 (Statistical Analysis System, version 6.03, SAS Institute, Inc., 1985).

**RESULTS & DISCUSSIONS**

**Methods to determine optimum coagulation**

The whey volumes from each soymilk batch (1 L) coagulated at different coagulant concentrations (0.00–0.045 N) were compared (Fig. 1). Whey from the batches with low concentration of CaSO₄·2H₂O were cloudy and/or contained small fragile curd fragments which, when undisturbed, would settle to the bottom of the beaker. At an optimum coagulation range of 0.018–0.020 N, the amount of whey being pressed out of the tofu was lowest and the tofu yield was highest (r = 0.93, Fig. 2). Using whey volume to indicate optimum coagulation was slow because of the time required to press the tofu. Also, this method did not allow for rapid batch adjustment.

A sample of whey was collected and cooled to 21°C to measure pH, %T and conductivity. The pH of the whey decreased with increases in coagulant concentration (Fig. 3). The rate of pH decrease doubled from 0.015 to 0.020 N CaSO₄·2H₂O. Kroll (1984), reported that Ca²⁺ and H⁺ competed for the same binding sites on protein molecules and that, between pH 3 and 7, a small change in pH resulted in a large change in amount of Ca²⁺ bound. The increase of H⁺ would cause the decrease in pH. The change was slower after most of the protein bodies had coagulated. Since the yield of tofu was highest at 0.018–0.020 N CaSO₄·2H₂O concentration (Fig. 2), and the pH decreasing rate sharply slowed at 0.020 N concentration, the optimum coagulation level based on pH measurement was between 0.018 and 0.020 N CaSO₄·2H₂O. Using pH to indicate optimum coagulant was slow, because of the time required to cool an adequate volume of whey (temperature sensitivity of electrodes). The pH electrodes must be resistant to leakage and breakage, to prevent contaminating the soymilk. Batch adjustment could be made with difficulty, but chances of tofu yield loss increase due to prolonged heating of the coagulating batch.

Shurtleff and Aoyagi (1979) indicated optimum coagulant concentration was the minimum coagulant needed to produce maximum whey transmittance. The transparency of the whey was measured at 400 nm (Fig. 4) with increasing CaSO₄·2H₂O concentrations. Percent transmittance of the whey increased 4 fold from 0.015 to 0.018 N. The whey transmittance changes between 0.018–0.040 N were not significant (P > 0.05) when compared with tofu yield. Using both tofu yield and % T, the optimum coagulation concentration was 0.018–0.020 N CaSO₄·2H₂O. Johnson (1984) and Wilson (1995) reported optimum clarity of whey transmittance, with three cultivars of soybeans, depended on coagulant concentrations and the percent solids of soy milk, 0.018, 0.019, and 0.035N for soy milk concentrations of 4, 5, and 8% solids, respectively (Fig. 5). Using whey transmittance to determine optimum coagulant concentration was slow because the sample from the coagulating batch had to be filtered and cooled. Batch adjustment could only be made with difficulty, but chances of tofu yield loss increased due to prolonged heating of the coagulating batch.

Electrical conductivity of whey at 72°C and at 21°C (Fig. 6), showed
Soymilk Coagulation Conductivity...

Fig. 3—Effect of coagulant concentration on whey pH. Points with same letter not significantly different (P>0.05).

Fig. 4—Effect of coagulant concentration on whey transmittance. Points with same letter not significantly different (P>0.05).

Fig. 5—Effect of coagulation concentration on whey transmittance at 4, 5 and 8% soymilk solids. Points with same letter not significantly different (P>0.05). (Used with permission, Wilson, 1995)

Fig. 6—Effect of coagulant concentration on whey conductivity R = 0.96. Points with same letter not significantly different (P>0.05).

Fig. 7—(a) Effect of coagulant concentration on tofu protein and moisture; (b) Effect of coagulant concentration on tofu fat and moisture. Points with same letter not significantly different (P>0.05).

a significant change from 0.015 to 0.018 N CaSO₄·2H₂O at both temperatures. The conductivity remained the same from 0.018 to 0.020 N. Beyond 0.020 N, the conductivity increased at a slower rate. To determine optimum coagulant concentration, conductivity results were compared with tofu yield data. Tofu yield was at maximum in the range 0.018–0.020 N (Fig. 2), and conductivity results also showed a sharp increase from 0.015–0.018 N followed by a plateau at 0.018–0.020 N concentration. Thus, the optimum coagulation concentration was 0.018–0.020 N CaSO₄·2H₂O. The plateau effect at 0.018–0.020 N was evident in all test methods.
Composition and yield of tofu influenced by CaSO$_4$·2H$_2$O concentration

Tofu batches were tested for proximate composition and textural characteristics. When the % protein in tofu was highest at 0.020 N CaSO$_4$·2H$_2$O, the %fat was lowest (Fig. 7a, b). Both results occurred when the coagulant concentration was at 0.020. From 0.020–0.025 N CaSO$_4$·2H$_2$O, the %fat (dry basis) sharply increased, then continued to increase at a slower rate (Fig. 7). Similarly the % protein (dry basis) dropped at the next concentration level (0.030 N). Therefore the optimum coagulation concentration based on proximate composition was 0.020 N CaSO$_4$·2H$_2$O. The tofu yield and composition could be used for prescreening; however, they could not be used to adjust the batch being coagulated.

Shurtleff and Aoyagi (1979), indicated that as the coagulant concentration increased, the bulk yield of tofu decreased and firmness increased. Our results showed that the firmness increased up to 0.025 N CaSO$_4$·2H$_2$O concentration. Further addition of CaSO$_4$·2H$_2$O decreased both fracturability and hardness of the tofu.

CONCLUSION

TRANSMITTANCE, WHEY VOLUME, AND pH COULD BE MEASURED to indicate the amount of coagulant needed for batch of soybeans or flakes prior to full scale processing. Measuring conductivity to determine optimum coagulation was fast and reproducible. The probe contained no chemical compounds that could leak like the pH probe and it could tolerate a high heat environment. Thus it could be used directly in the coagulation vessel or on-line in a continuous processing system.

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