PHYSICOCHEMICAL CHARACTERISTICS OF SOFT TOFU FORMULATED FROM SELECTED SOYBEAN VARIETIES[1]

K.A. KHATIB, F.M. ARAMOUNI[3], T.J. HERALD and J.E. BOYER[2]

Department of Animal Sciences and Industry
Kansas State University
Manhattan, KS 66506

[2]Department of Statistics
Kansas State University
Manhattan, KS 66506

ABSTRACT

Currently, hundreds of soybean varieties with a wide range of quality attributes are available. The majority of these soybean varieties have not been evaluated for their quality in soymilk and tofu. In this research, tofu was prepared using the hot ground method by coagulating soymilk from four soybean varieties (Macon, Saturn, OHIOFG1 and OHIOFG2) with calcium sulfate dihydrate. Soybean variety significantly affected yield, held water, color parameters, viscosity, storage modulus, and textural properties of tofu cakes. Soymilk with higher pH, viscosity, and protein produced tofu with higher protein, water held, and yield. Varietal differences should be considered in selecting soybeans for tofu production. Macon soybeans produced the best tofu with regards to quality attributes.

INTRODUCTION

Soybean is one of the most consumed proteinaceous plants in the world. Sales of tofu and soy food products have been growing on an average about 12.5% every year since 1990 (Berry 1998) and are expected to increase with the recent FDA ruling that approved soy protein health claims (Anon. 1999). Soybean's inherent nutritional properties and health benefits claimed include reduction in cholesterol levels (Carroll and Kurowska 1995; Potter *et al.* 1995), mitigation of symptoms of menopause, osteoporosis (Clark 1997), and reduction in risk of heart diseases, and cancer (Kurowska 1997).

The most recognized soy food in the west is tofu. Tofu, a traditional oriental soybean food, can be defined as the gel-like precipitate obtained by the addition of calcium or magnesium salt or acids to heated soymilk, followed by molding and pressing to form a desirable texture. Several factors affect the composition of soybeans and subsequent tofu quality. Many of these factors are not fully understood leading to conflicting results among soy studies (Skurray et al. 1980; Lim et al. 1990).

Lim et al. (1990) noted that tofu texture should be coherent, smooth, and firm in order to be accepted by western consumers. Soybean varieties high in protein content produce tofu high in yield and firm texture (Schaefer and Love 1992; Wang and Chang 1995). Conversely, Murphy et al. (1997) reported that varieties high in protein content did not correlate to tofu yield. In addition, Murphy et al. (1997) found a negative correlation between protein content and tofu firmness.

Most recently, Cai and Chang (1999) found that firmness, yield, and sensory quality of tofu were dependant on the processing method to make tofu. Soybean constituents other than proteins may also affect physical and chemical properties of tofu. A study of physical and chemical characteristic of soy variety is essential to determine their potential for use in food products. Soybean varieties that produce high protein tofu usually are preferred by manufacturers. There are hundreds of soybean varieties with a wide diversity of quality attributes currently available in the market (Khatib 1999). The majority of these varieties have not been tested to determine the quality of soymilk, tofu, or other soy products such as soy sauce. Further studies are needed to elucidate varietal differences. The objectives of this study were to compare the effect of soybean variety on chemical and physical characteristics of soymilk and tofu formulated from four soybean varieties grown in the same location under the same environmental conditions.

MATERIALS AND METHODS

Materials

Four food grade soybean varieties (Glycine max var. OHIOFG1, OHIOFG2, Macon, and Saturn; 1998 crop) supplied by the Kansas State Soybean Breeding Program were used in the study. The varieties were grown in the same location and under the same environmental conditions. All beans were stored in a cool dry storage room until used for making soymilk and tofu.

Preparation of Soymilk

Soymilk was prepared using the method described by Lim et al. (1990) with modifications to produce smooth soft textured tofu with high bulk weight

(Fig. 1). Approximately 150 g of soybeans were soaked in distilled water for 16 h at 24±2C. The hydrated beans were drained, rinsed, and blended with 400 mL boiling distilled water in a commercial Waring blender (John Oster MFG. Co., Milwaukee, WI) for 2 min on high speed. Another 400 mL of boiling distilled water was added and mixed at the same speed for 2 min. The resultant slurry was strained through four layers of cheesecloth to separate soymilk from the insoluble materials called okara. The final volume of soymilk was dependent on the amount of water added to dilute the soymilk to 11% solids (Ono *et al.* 1993). Twenty mL of boiling water was held back for dispersion of the coagulant calcium sulfate dihydrate ($CaSO_4.2H_2O$) (United States Gypsum, Terra Alba, UT).

Preparation of Tofu Cake

A total of 400 mL fresh soymilk was heated at 95C for 12 min with constant stirring using a magnetic hot plate (Fisher Scientific, Pittsburgh, PA). The soymilk was immediately removed from the heat source and cooled to 85C. A 15 mL fresh suspension of 0.04M $CaSO_4.2H_2O$ was poured into the hot soymilk. Curds were immediately formed. The curds were left for 20 min at 24±2C to ensure complete gel formation before transferring to perforated Teflon molds (10.0 cm i.d.) to form tofu cakes. The molds were lined with one layer of cheesecloth. A mass of 3, 5, or 7 kg was placed on top of the tofu cake mold (4 × 9 cm) (length × diameter) which gave an applied force of ~8.6 g/cm^2 for 10 min, ~14.3 g/cm^2 for 10 min, and ~20.0 g/cm^2 for 15 min, respectively. The force was applied for different times to produce approximately 350 g tofu cakes as adapted from Zhang and Chang (1995). The weight of pressed tofu and volume of collected whey were recorded. Tofu cakes were transferred onto Styrofoam plates, covered with clear polyethylene films (First Brand, Danbury, CT), sealed, and stored at 4C overnight before testing.

Moisture Content

Whole tofu cakes were homogenized in a Waring blender. Approximately 7 g of tofu was weighed and dried at 104C for 18 h using a conventional oven (Franklin Product Corp., Chicago, IL). To reduce moisture absorption, the samples were cooled at room temperature in a desiccator containing calcium chloride and phosphorous pentoxide until dried weights were taken. Moisture content was calculated using the following equation:

$$\% \text{ Mositure} = \frac{(\text{Wet Weight} - \text{Dry Weight})}{\text{Wet Weight}} \times 100 \qquad (1)$$
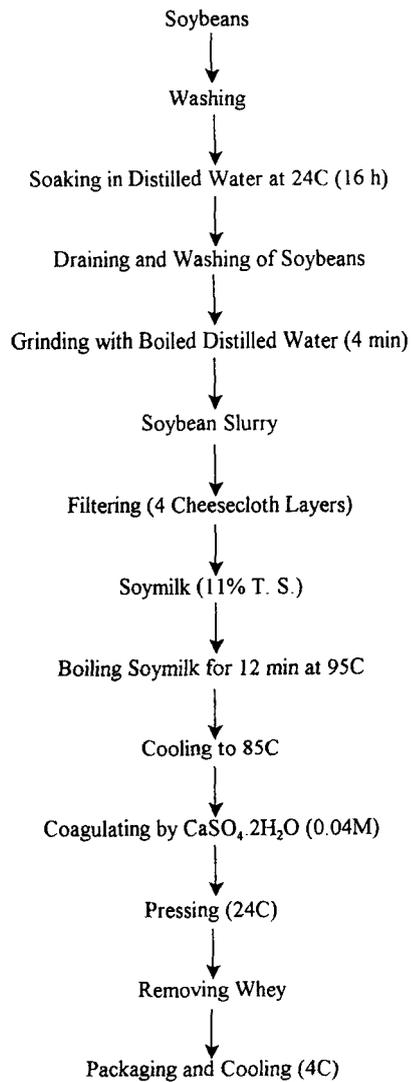
Soybeans

↓

Washing

↓

Soaking in Distilled Water at 24C (16 h)

↓

Draining and Washing of Soybeans

↓

Grinding with Boiled Distilled Water (4 min)

↓

Soybean Slurry

↓

Filtering (4 Cheesecloth Layers)

↓

Soymilk (11% T. S.)

↓

Boiling Soymilk for 12 min at 95C

↓

Cooling to 85C

↓

Coagulating by $CaSO_4.2H_2O$ (0.04M)

↓

Pressing (24C)

↓

Removing Whey

↓

Packaging and Cooling (4C)

FIG. 1. FLOW DIAGRAM FOR SOFT TOFU MANUFACTURE

Total Soluble Solids and pH Measurements

Total soluble solids of soymilk were determined using a modification of a method described by Johnson and Wilson (1984). Samples were taken immediately after collection and measured. Approximately 0.5 mL of suspension was taken at room temperature and loaded in a Reichert Jung Mark II Abbe Tabletop Refractometer (Cambridge Industries Inc., Buffalo, NY). Percent soluble solids was read from the digital display.

The pH of soymilk was measured using a Fisher Accumet 25-pH meter with automatic temperature compensation fitted with Fisher Universal glass pH electrodes (Fisher Scientific).

Texture Profile Analysis (TPA)

The TPA of tofu cake was performed with a TA.XT2 Texture Analyzer (Stable Micro System, Scarsdale, NY). TPA parameters measured were adhesiveness, chewiness, cohesiveness, firmness, gumminess, and springiness. The TPA mode is a set of two-bite cycle testing that simulates chewing. The whole tofu cake was taken immediately from 4C storage and placed on the textural analyzer stage for testing. One tofu cake was used for each TPA test.

The limits of the texture parameters set up were determined in preliminary experiments using an acquisition rate at 200 pps (points per second), force threshold at 20 g, test-speed at 5.0 mm/s, pre test speed at 3.0 mm/s, post test speed at 5.0 mm/s, deformation (distance) of 75%, and trigger force at 5 g. All measurements were tested and recorded as force (g) and strain (cm). The analyses were conducted using a 1.27 cm diameter spherical stainless steel probe.

Dynamic Rheological Testing

Small amplitude dynamic oscillation measurements of the fundamental dynamic parameters storage modulus (G') and loss modulus (G") were determined on tofu gels using a Bohlin VOR computer controlled Rheometer System (Bohlin Instruments, Cranbury, NJ). Tofu samples were cut to approximately 2.0 × 2.0 × 0.2 cm (length × width × thickness). A strain sweep was used to determine the region in which G' was independent of strain. 110 g cm torque bar and 30 mm diameter parallel plates were used during measurements in the frequency interval between 0.1 to 10 Hz at a strain of 0.0443. Refrigerated tofu gels (4C) were placed immediately between VOR parallel plates which was tempered at 4C for 5 min to equilibrate the temperature of both plates and gel. The gap between the upper and lower plates was set at 2 mm. The measurements were taken at 4C to simulate commercial storage of tofu. Temperature was controlled automatically using a circulating bath to

provide precise temperature control. For statistical analysis, G' and G" were analyzed at 1 Hz.

Steady Shear Testing

Apparent viscosity (Pas) of soymilk was measured using the Bohlin VOR Rheometer. Approximately 15 mL of soymilk was placed in a concentric cylinder (25 mm i.d. and 27.5 mm o.d.). The limits of the steady shear parameters were determined within a shear rate range of 1.16 to 91.9 s^{-1} at a constant delay (relaxation) time of 5.0 s and integration time of 5.0 s. A 4.1 g cm torque bar was used. The apparent viscosity was measured at 4C to simulate commercial storage of soymilk. Temperature was controlled automatically with a circulating bath to provide precise temperature control. For statistical analysis, apparent viscosities were compared at 9.2 s^{-1}.

Protein Content

Prior to protein analysis, tofu and soymilk samples were homogenized using a blender. Nitrogen contents of tofu and soymilk samples (1 to 2 g) were determined using LECO Nitrogen Analyzer model FP 2000 (LECO Corp., St. Joseph, MI) (Combustion Method number 968.06. AOAC 1996). The sample nitrogen was converted to crude protein by multiplying with a conversion factor of 6.25 (AOAC 1996). Ethylene diamine tetra acetic acid was used as standard to check the accuracy of the measurements.

Held Water

The held water properties of tofu gels were measured using a modification of the net test (Kocher and Foegeding 1997). A cylindrical gel sample was placed inside a cell (0.5 mm Ultra Durapore Membrane) that contained a filter and was centrifuged at 9000 rpm for 12 min. The amount of drained water was recorded. Total weight of water in the samples was determined by Eq. (1), and the total weight of protein in the samples was determined with the LECO Nitrogen Analyzer. The held water was calculated by the following equation:

$$\text{Held Water} = \frac{(\text{Total Weight of Water in Sample} - \text{Weight of Water Released})}{\text{Total Weight of Protein in Sample}} \quad (2)$$

Tofu Yield

Tofu yield was expressed as kg tofu obtained from one kg soybeans (dry basis). Yield was calculated using the following equation:

$$\text{Yield} = \frac{\text{Weight of Tofu (kg) (wet wt basis)}}{\text{Weight of Soybeans (kg) (dry wt basis)}} \qquad (3)$$

Water Activity (Aw)

A representative sample (3-5 g) was taken for determination of water activity. The tofu samples were measured at 4C using an Aqualab Model Decagon CX-2 Water Activity Meter (Decagon Devices, Pullman, WA). The Aw meter uses the chilled mirror mechanism to determine dewpoint temperature of air equilibrated with the food sample.

Color

The color of tofu was measured using a Hunter D-54 Reflectance Lab Ultra Scan Sphere Spectrophotometer (Hunter Associate Laboratories, Reston, VA). The colorimeter used L (lightness), a (redness), and b (yellowness) scales and illuminant C, which is the average daylight, at an observe angle of $10°$ to measure the color of the tofu surface (Hunter 1987). The colorimeter was calibrated using black and white standards. The white color reflectance standards were $X = 83.4$; $Y = 88$; $Z = 93.9$, and $D65/10°$.

Statistical Analysis

Three separate batches of tofu were processed from the soybeans (randomized block design). Three subsamples from each batch were taken at 1-day intervals to measure chemical and physical properties. Data were analyzed for differences among soybean varieties using analysis of variance in the SAS program, version 6.12 (SAS 1996). Analysis of variance and least significant difference were used to ascertain significant effects at the 5% level for a specific response variable.

RESULTS AND DISCUSSION

Protein Content of Soybeans

Macon and OHIOFG2 were significantly higher in protein content than OHIOFG1 and Saturn (Table 1). There is no information available in the literature about the ranges of high protein content of soybeans to be compared with the varieties studied here. Therefore, such variation could be related to the differences in soybean genotypes.

TABLE 1.
CHEMICAL AND PHYSICAL PROPERTIES OF SOYBEANS, SOYMILK AND TOFU
OBTAINED FROM FOUR SOYBEAN VARIETIES

	Soybean Varieties				
	OHIOFG1	OHIOFG2	Macon	Saturn	SEM
Soybeans					
Protein[1]	46.30^b	50.29^a	51.27^a	45.13^b	1.20
Soymilk					
Protein[1]	45.01^b	49.52^a	50.06^a	44.60^b	1.18
pH	6.67^b	6.69^{ab}	6.71^a	6.54^c	0.03
Tofu					
Yield[2]	4.24^c	4.28^b	4.62^a	3.94^d	0.11
L	88.31^{ab}	89.34^a	86.35^c	87.94^b	0.34
b	9.50^b	8.52^a	7.61^c	8.59^a	0.32
a	4.39^a	3.27^c	4.06^b	4.14^b	0.20
Protein[1]	41.69^{ab}	43.11^{ab}	47.40^a	38.41^b	1.52
Held Water[3]	6.78^b	7.14^b	8.08^a	6.32^b	0.31
Whey Volume (mL)	43.1^b	49.2^b	52.1^b	61.3^a	3.1

[abcd] Different letters following means in a row indicate significant differences among varieties ($p < 0.05$).
[1] Expressed on % dry weight basis   L represents lightness
[2] (kg tofu/kg beans)   a represents redness
[3] g H_2O/g protein   b represents yellowness
SEM represents standard error of the mean

pH, Total Solids, and Protein Content of Soymilk

The total solids in all the soymilk samples were standardized to ~11%. Thus, moisture content of the soymilk was approximately ~89% for all the varieties. Therefore, there was no significant differences noticed among varieties in soymilk total solids nor moisture content. Soymilk prepared from Macon and OHIOFG2 contained the highest protein contents while OHIOFG1 and Saturn exhibited the lowest protein contents and were not significantly different from each other (Table 1). For all soybean varieties, there was approximately a 98% recovery rate in protein during the preparation of soymilk from the beans. The recovery rate would be an indication of the efficiency of the protein extraction and protein quality of the different varieties. This indicated that soybean varieties were different in the protein recovered because of the differences in solubility and extractability.

Soymilk made from Macon exhibited a significantly higher pH than that made from OHIOFG1 and Saturn (Table 1). This may be attributed to lower protein contents that may affect buffering capacity in Saturn. Lim *et al.* (1990) concurred with the observation that differences in pH may be due to variations in the ionic strength of proteins present in the soymilk.

Yield

Significant differences in tofu yield occurred among soybean varieties (Table 1). The Macon variety was ~15% higher than Saturn. This may be related to soybean protein contents that were high in Macon and low in Saturn or the effectiveness of extractability (Table 1). Macon exhibited approximately 12% higher protein content in the bean versus Saturn. Lim *et al.* (1990) and Shen *et al.* (1991) found a significant relationship between tofu yield and total solids (including proteins) of soymilk. Higher pH soymilk will expose more protein hydrophobic regions. When the hydrophobic interaction is more predominant, aggregation could be induced, and yield increased (Kohyama *et al.* 1995). The calcium and/or hydrogen ions may neutralize the negatively charged and heat-denatured soy protein molecules and lead to zero net charge of the protein, subsequently inducing aggregation. Although total solids of soymilk was standardized among varieties, the quality and content of protein for each variety was different which may have lead to differences in yield.

Color

Color differences were detected among tofu varieties (Table 1). Macon had the lowest L-value, which indicated a significant darker color than the other varieties. OHIOFG2 had the highest L-value. The b-value showed that Macon tofu was significantly more yellow than the other tofu samples. The a-value

showed OHIOFG1 tofu was significantly more red than the other varieties. Such significant differences may affect the values of saturation index and hue angle which mean different intensity in the colors of soybeans from different varieties, probably because of seed coat hilum (Wang *et al.* 1983). Cober *et al.* (1998) reported that different hilum colors of soybean seeds was due to the distribution of pigments in the seed coat, which could migrate to soymilk components upon grinding. Although the differences were relatively small in numbers, they did affect the color properties of tofu. Visual observation indicated that seed coats of Macon had a dark brown hilum, Saturn had a yellow hilum, and OHIOFG2 had a slightly white hilum. Usually, tofu producers prefer soybeans with a light hilum color, because the product is a white or pale yellow curd (Wang 1981).

Protein Content

Tofu prepared with Saturn had a significantly lower protein content compared with the other varieties (Table 1). By comparing the protein content of the soymilk to that of tofu, the recovery rates of protein were 86% for Saturn, 91% for OHIOFG1, 88% for OHIOFG2, and 94% for Macon. This indicates that soybean varieties were different in the protein recovered because of the differences in coagulability.

Held Water and Whey Volume

Significant variations were found among varieties with regard to held water of tofu, which ranged from 6.3 (Saturn) to 8.1 (Macon) g H_2O/g protein (Table 1). Held water of Macon was significantly higher than the other varieties. Results showed that held water and tofu yield were directly related to pH and protein content of soymilk. Kinsella (1979) reported that protein-water and protein-protein interactions increase gel strength in tofu.

Tofu prepared with Saturn exhibited a significantly higher whey moisture volume compared with that prepared from the other varieties. This was expected, because Saturn produced tofu with the lowest protein content and held water.

Moisture content and Aw of tofu was not affected significantly by soybean variety. The moisture content difference between soymilk and tofu was ~1.3%, which indicated that the tofu gel was made with minimum loss of whey.

Rheology

Soymilks prepared from OHIOFG1 and Saturn had a significantly lower apparent viscosity than soymilks prepared from OHIOFG2 and Macon at 9.2 s^{-1} (Table 2). The increase in apparent viscosity could be related to different protein contents and their interactions with the coagulant. Also, protein content could

have an effect on viscosity. Usually a strong relationship exists between the ability of soy protein to absorb water and soymilk viscosity (Kinsella 1979).

TABLE 2.
RHEOLOGICAL AND TEXTURAL PROPERTIES OF SOYMILK AND TOFU OBTAINED FROM FOUR SOYBEAN VARIETIES

	Soybean Varieties				
Rheological	OHIOFG1	OHIOFG2	Macon	Saturn	SEM
Viscosity (mPas)	82^b	136^a	157^a	62^b	18
G' (Pa)	1293^b	1453^b	3127^a	1255^b	0.0
Textural					
Firmness (g)	75.78^b	86.25^a	86.40^a	69.45^b	3.40
Springiness	0.85^b	0.85^b	0.91^a	0.84^b	0.01
Cohesiveness (g)	0.42^b	0.42^b	0.45^a	0.41^b	0.01
Adhesiveness (g)	-110.92^b	-138.08^c	-184.79^d	-83.85^a	17.58
Chewiness (g)	28.63^b	32.21^a	32.88^a	24.51^c	1.57
Gumminess (g)	31.59^b	36.77^a	37.69^a	29.12^c	1.68

[abcd] Different letters following means in a row indicate significant differences among varieties ($p < 0.05$).
SEM represents standard error of the mean

Figure 2 shows that soymilk behaved as a pseudoplastic fluid. A yield value was noted for all soybean varieties when the shear rate of soymilk was plotted versus shear stress (rheogram is not shown). Macon's yield value was approximately 200% greater than that of Saturn.

Macon exhibited a significantly higher storage modulus (G') of tofu compared with the other varieties (Table 2). This may be related to tofu protein

content, which was significantly different among varieties. Macon was the only variety that showed high G'. There was a ~19% difference in protein content of tofu prepared with Macon and Saturn but a 60% difference in G'. This emphasizes the significant impact on the storage modulus. Storage moduli data confirmed that tofu prepared with Macon exhibited a more solid like property compared with the other tofu. The G' of Macon tofu was approximately 1.5 times greater than that of Saturn.
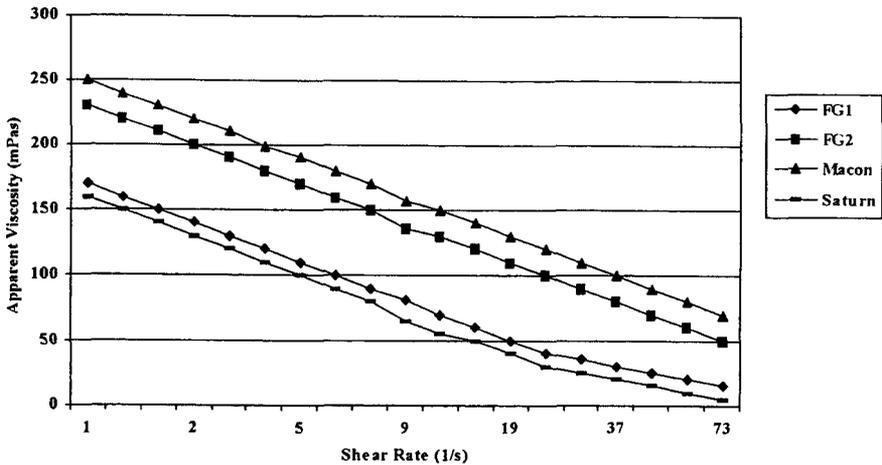

FIG. 2. REPRESENTATIVE RHEOGRAM OF THE APPARENT VISCOSITY (MPAS) OF SOY MILK PREPARED FROM FOUR SOYBEAN VARIETIES

The rheogram in Fig. 3 shows that G' is frequency dependent, but G" is less frequency dependent. No G'-G" crossover was observed. This behavior indicates a "physical gel" rather than a covalent or an entanglement gel mechanism (Stading and Hermanson 1990; Clark 1992). Usually, fundamental rheological testing has an advantage over empirical methods in that the results reveal structural characteristics of tofu.

Texture Profile Analysis

Significant differences were found for all tofu textural attributes (Table 2). Macon tofu was the most adhesive, chewy, cohesive firm, springy, and gummy, followed by OHIOFG2, OHIOFG1, and Saturn, respectively. Protein content of

tofu prepared with Macon and Saturn was found to significantly affect adhesiveness (55%), firmness (~20%), chewiness (26%), and gumminess (23%) and slightly significantly affecting springiness (8%) and cohesiveness (9%). Pant *et al.* (1993) found no differences for all textural properties except for springiness. In addition, the results disagreed with the findings of Skurray *et al.* (1980), who indicated that at a fixed calcium concentration, no differences should occur in firmness and cohesiveness of tofu.
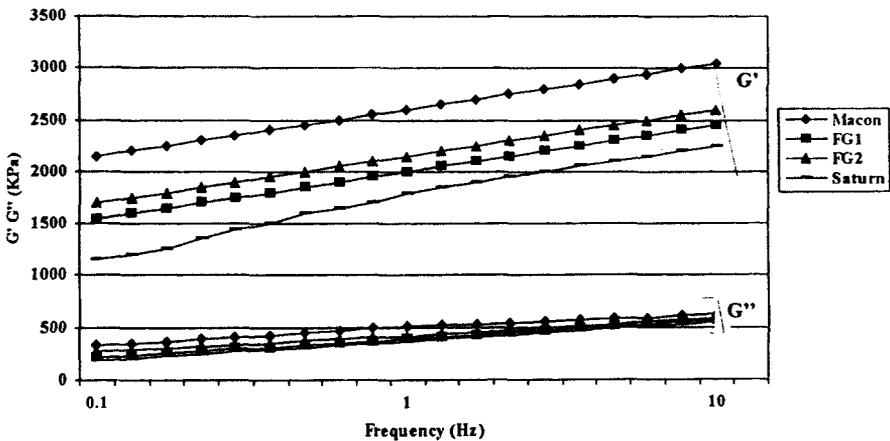

FIG. 3. REPRESENTATIVE RHEOGRAM OF THE STORAGE MODULUS (G') AND LOSS OF MODULUS (G'') OF TOFU PREPARED FROM FOUR SOYBEAN VARIETIES

CONCLUSION

Variation in soybean variety and protein content may lead to differences in tofu quality, which were evident for the chemical and physical properties measured. Among the varieties evaluated, Macon soybeans produced the best tofu with regards to quality attributes. This suggests that the variety effect should be considered before launching into a large-scale production of tofu. Therefore, it is vital that manufacturers work closely with growers to ensure consistent raw materials for tofu production.

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