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Rheological and textural characteristics of black soybean touhua (soft soybean curd) prepared with glucono- δ -lactone

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ABSTRACT

Viscoelastic studies on the mixture of black soymilk and glucono- δ -lactone (GDL) have been done to analyse the gelation process of touhua. The isothermal gelation curves of touhua were well predicted by firstorder reaction kinetics. The saturated storage modulus (G'_{sat}) of touhua was affected by the solids content, coagulation temperature and GDL concentration. The G'_{sat} value was proportional to the 1.68th power of solids content. The rate constant of gelation increased with increasing coagulation temperature and GDL concentration, but decreased with increasing solids content of soymilk. The gelation time decreased with increasing coagulation temperature and GDL concentration, and increased with increasing solids content. The hardness and adhesiveness values of packed touhua, measuring by textural profile analysis, increased with increasing solids content, GDL concentration and coagulation temperature. The storage modulus of touhua, during gelation, correlated positively with the textural characteristics of the packed touhua.

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1. Introduction

Soybean is nutritionally attractive in having a high protein content of good quality. Black soybean is one variety of soybeans (Glycineax (L.) Merrix) and has a black seed coat. Isoflavone contents of black soybean ([Kim et al., 2007](#page-6-0)) and tofu [\(Shih, Yang, &](#page-6-0) [Kuo, 2002\)](#page-6-0) are higher than those of yellow soybean and tofu. Soybean isoflavone helps to prevent osteoporosis, breast cancer and cardiovascular diseases [\(Song, Paik, & Joung, 2008](#page-6-0)), and few people are allergic to soy proteins ([Wilson, Blaschek, & de Mejia, 2005\)](#page-6-0). Soybean is extensively utilised for food processing. Touhua, a soft soybean curd, is a traditionally popular dessert in Taiwan and is a soy protein gel-like product. Production of touhua is similar to that of packed tofu. Touhua is usually produced from a low solids content cooked soymilk with coagulants, such as glucono- δ -lactone (GDL) or calcium sulphate. High-quality touhua has a fine, smooth, even and elastic texture, as well as good flavour.

Both touhua and tofu are gel products relating to the aggregation and gelation of soy proteins. Factors affecting the quality of soy protein gels include soybean varieties ([Shih et al., 2002](#page-6-0)), the water/bean ratio during grinding or solids content of soymilk [\(Bed](#page-6-0)[dows & Wang, 1987a; Kohyama & Nishinari, 1993; Kohyama, Yosh-](#page-6-0) [idaa, & Nishinari, 1992](#page-6-0)), kinds and concentrations of coagulants ([Kohyama, Sano, & Doi, 1995; Prabhakaran, Perera, & Valiyaveettil,](#page-6-0) [2006; Shih, Hou, & Chang, 1997](#page-6-0)), coagulation temperature ([Beddows & Wang, 1987b; Shih & Shiau, 2003; Wang & Hesseltine,](#page-6-0) [1982\)](#page-6-0), and pressure ([Saowapark, Apichartsrangkoon, & Bell,](#page-6-0) [2008](#page-6-0)).

GDL is a GRAS substance and is a weak acid, which converts to gluconic acid in water and slowly dissociates into hydrogen ions with time. As the pH of soymilk is lowered to the isoelectric point of soy protein, coagulation of soy protein occurs; then a protein gel forms under proper conditions. The higher the concentration of GDL used, the lower is the pH value of the tofu prepared ([Kohyama et al., 1992](#page-6-0)). Calcium sulphate has very low solubility in cold water and needs to mix with hot soymilk to produce touhua. GDL is easily soluble in water and its solution can be used in cold soymilk. Therefore, GDL is widely used as a coagulant in packed touhua and tofu products and calcium sulphate is used in traditional touhua and Momen tofu [\(Kohyama](#page-6-0) [et al., 1992](#page-6-0)).

[Kohyama and Nishinari \(1993\)](#page-6-0) found that the gelation times of 7S and 11S proteins became shorter with increasing concentration of GDL. The aggregation rates of soy proteins with GDL were in the order, 11S > 2S > 7S [\(Tay, Xu, & Perera, 2005\)](#page-6-0). [Kohyama et al.](#page-6-0) [\(1995\)](#page-6-0) found that the rate of gelation for soy protein isolated by GDL was slower than that by calcium sulphate, and suggested that the addition of GDL or calcium ion induced gelation by promoting

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protein aggregation via hydrophobic interaction. Optimum processing conditions for making packed touhua, according to the result of a RSM study, were 3.67% solids content, 0.0225 N GDL and 71 °C coagulation temperature [\(Shih & Shiau, 2003](#page-6-0)).

Heating results in the denaturation of soy proteins. The denatured proteins are easy to aggregate and they form soy gels, with or without coagulants. In the absence of coagulants, soy proteins at a high concentration could form heat-induced gels. [Bikbov, Grin](#page-6-0)[berg, Antonov, Tolstoguzov, and Schmandke \(1979\)](#page-6-0) reported that, within a 10–20% solids concentration range, the relation between storage modulus (G') and protein concentration (C) was G' \propto C $^{4.67}.$ [Renkema and van Vilet \(2004\)](#page-6-0) indicated that the critical concentrations of purified glycinin and β -conglycinin for gelation differed according to pH and ionic strength.

Coagulation temperature affects the rate of gelation and the quality of soy gel products. The gelation rate of 11S protein in the presence of GDL increased with coagulation temperature, increasing from 50 to 90 °C [\(Kohyama et al., 1992](#page-6-0)). The hardness value of packed touhua and the amount of exuded water increased with coagulation temperature, increasing from 65 to 85 °C [\(Shih &](#page-6-0) [Shiau, 2003\)](#page-6-0). At a higher temperature, a more rapid aggregation and precipitation of soy protein may occur and, consequently, this reduces the retention of water. [Beddows and Wang \(1987b\)](#page-6-0) reported that the optimum coagulation temperature was 75-80 °C for making silken tofu.

Dynamic viscoelasticity measurement has been used to study the gelation process of protein gels, such as GDL-induced soy protein ([Kohyama et al., 1992; Kohyama et al., 1995](#page-6-0)), heat-induced soy protein ([Ahmed, Ramaswamy, & Alli, 2006; Bikbov et al.,](#page-6-0) [1979; Renkema & van Vilet, 2004](#page-6-0)), heat-induced whey protein ([Aquilera & Rojas, 1997](#page-6-0)) and enzyme-induced casein [\(Tokita, Hiki](#page-6-0)[chi, Niki, & Arima, 1982](#page-6-0)). However, data on the change of viscoelasiticity of soymilk during touhua preparation and the effect of coagulant concentration on the textural quality of touhua are rare so far. The purposes of this study were to investigate the gelation process of black soybean touhua with GDL by dynamic rheometry and to observe the correlations between the dynamic rheological properties and textural parameters, such as the hardness and adhesiveness, of touhua.

2. Materials and methods

2.1. Materials

Black soybeans (Tainan No. 5), having a black seed coat and yellow cotyledon, were obtained in a single batch from the local Tainan Farmers' Association. The proximate composition of the soybean was 8.92% moisture, 38.6% crude protein, 17.5% crude fat, and 5.34% ash (as is basis). GDL was purchased from Sigma–Aldrich Co. (St. Louis, MO, USA). Silicone defoamer KM 72S, foodgrade anti-foaming agent, was purchased from Shin-Etsu Chemical Co. Ltd. (Gunma, Japan).

2.2. Preparation of soymilk powder

Black soybeans (100 g) were washed and soaked at 4° C in 500 ml of distilled water for 18 h. After being rinsed, soaked beans were weighed (about 200 g) and subsequently blended with 800 ml of distilled water using an Osterizer blender at low and high speeds for 1 min. The slurry was filtered through muslin cloth and 100-mesh screen to obtain raw soymilk. To the soymilk, distilled water was added to make the volume up to 1000 ml, and two drops of anti-foaming agent were also added. The soymilk was heated and allowed to boil for 10 min. After cooling, the soymilk was frozen and lyophilised in a freeze-dryer (Stoppering Tray

Dryer System, Labconco Co., Kansas City, MO, USA) for preparing soymilk powders.

2.3. Isothermal gelation process

Gelation of soymilk was observed by using of a controlled stress rheometer (Carri-Med CSL-100, TA Instrumental Ltd., Surrey, England) operated with a double-cylinder geometry. Dynamic rheological measurements were performed at a constant strain of 0.1%, which was within the linear region, and at a frequency of 1 Hz.

Soymilk solutions (19 ml), with different solids contents, were prepared from the soymilk powder (0.4–1.4 g) with distilled water. The soymilk solutions were heated at different temperatures (55– 80 °C) for 10 min. Then, 1 ml of freshly prepared GDL solution was mixed into each soymilk solution. The mixture solutions with final concentrations of 0.01–0.05 N GDL and 2–7% solids contents were stirred for 10 s, and 3.5 ml of the mixture solutions were immediately injected into the gap between the double-cylinders of the rheometer, which was pre-heated to the same temperature. A taylor-made lid covered the top of the double-cylinder geometry to minimise water evaporation during measurements. The sample solution was subjected to sinusoidal shear oscillations. The G' , loss modulus (G") and loss tangent (tan δ) were recorded as a function of time (90 min in total). Zero time was taken as the time when GDL was added to the soymilk. The observed data were fitted to an empirical formula proposed by [Kohyama et al. \(1992\)](#page-6-0)

$$
G(t) = G'_{sat}(1 - e^{-k(t - t_0)})
$$
\n(1)

where $G(t)$ is the G' at time t, G'_{sat} is the saturated G', k is the rate constant of gelation, and t_0 , gelation time, is defined as the time when the G' begins to rise from the baseline. Values of G'_{sat} and k were estimated by a nonlinear regression method from the curve fitting the exponential equation i.e. $y = a(1-e^{-bx})$, where y is $G(t)$, x is time (t–t₀), and a and b are constants.

2.4. Non-isothermal gelation process

Soymilk solution (19 ml) was heated at a set temperature (60– 80 °C) for 10 min. A freshly prepared GDL solution (1 ml) was added to the soymilk. Dynamic rheological properties of the mixture with 4% solids content and 0.02 N GDL were measured from the set temperature to 5 $\mathrm{^{\circ}C}$ at a descending rate of 1 $\mathrm{^{\circ}C/m}$ in. When the non-isothermal gelation process was finished, the frequency sweep of the gelled sample at 5° C was immediately measured from 0.1 to 10 Hz at 0.1% strain.

2.5. Preparation of packed touhua

Packed touhua was prepared according to the procedures proposed by [Shih and Shiau \(2003\)](#page-6-0) with some modifications. Black soybeans (400 g) and distilled water were used to prepare 3200 ml of the cooked soymilk, with an average 7.8% solids content. The mother soymilk was cooled to room temperature and was diluted to different concentrations with distilled water if necessary. In each experiment, the cooked soymilk was freshly prepared, daily. The Brix of soymilk in each batch was monitored by digital refractometer (Pocket PAL-1, Atago Co. Ltd., Japan).

Cool soymilk (195 ml) was heated up to the set temperatures (60-80 °C), 5 ml of a freshly prepared GDL solution was added, and the mixture was stirred for 5 s at 200 rpm by using a mixer (Variomag Multipoint HP 15, Germany). The mixture solutions with 0.01–0.05 N GDL were transferred to plastic cups (92 mm in height, 90 and 55 mm in upper and lower diameters), and were stood at room temperature for cooling and gelation. The touhua samples were sealed and stored in a refrigerator $(5^{\circ}C)$ overnight.

2.6. Texture of packed touhua

Packed touhua sample was removed from the refrigerator; the texture of the touhua was immediately measured by using of a Texture Analyzer (Model TA-XT2i, Stable Micro Systems, Surrey, UK) fitted with a P10 adapter (10 mm in diameter) moving at a rate of 2 mm/s, and the penetration depth into touhua samples was 20 mm. The hardness (g) and adhesiveness (g *s) of packed touhua were recorded from the maximum force in the graph curve and from the area under the X-axis, respectively. The hardness and adhesiveness values were means of 27 measurements performed on 9 sample replicates.

2.7. pH of packed touhua

After the texture analysis, the pH value of the touhua sample was directly measured by using of a pH metre (InLab427, Mettler Toledo, Switzerland). The pH value was the mean of measurements on 9 sample replicates.

3. Results and discussion

3.1. Analysis of gelation curve

During gelation, the G' value of soymilk began to rise after a certain time (gelation time, t_0) and gradually reached a limiting value. The G' values determined during gelation were well fitted $(R^2 > 0.98)$ to the empirical formula (Eq. 1). In other words, the gelation curves of touhua fitted first-order reaction kinetics. Therefore, both G'_{sat} and k values can be estimated by the regression method described above. Similar results were found for the gelation process of soy proteins and tofu [\(Kohyama et al., 1992; Kohy](#page-6-0)[ama & Nishinari, 1993; Kohyama et al., 1995\)](#page-6-0).

3.2. Effect of solids content of soymilk

Touhua is commonly prepared with 2–5% soymilk and has a lower solids content than has tofu. Fig. 1 shows the effects of solids content on the values of t_0 , G'_{sat} , and k for the gelation of touhua prepared with 0.02 N GDL at 70° C. Within 2-7% solids content, the t_0 value linearly increased, but the k value linearly decreased, with increasing solids content of soymilk (Fig. 1A). This agrees with a previous report that t_0 (latent time) became longer with increasing 11S protein concentration (2–7%) at a fixed GDL concentration ([Kohyama et al., 1992](#page-6-0)). However, different results were found for heat-induced gelation or gelation of soy protein with a high concentration. [Ahmed et al. \(2006\)](#page-6-0) reported that the rate of heat-induced gelation for soy protein isolate increased with increasing protein concentration (10–20%). Furthermore, [Yoshimura, Shibata,](#page-6-0) [Eto, and Nishinari \(2004\)](#page-6-0) found that t_0 became shorter with increasing concentration of soymilk powder (11–17%) in the presence of GDL.

The pH values of packed touhua with 2–7% solid contents were 4.43, 4.68, 4.95, 5.12, 5.33 and 5.40, respectively. The pH value of touhua became higher with increasing solids content of soymilk containing 0.02 N GDL. This indicates that soy proteins possess a pH-buffering capacity and higher protein concentration inhibits the tendency of the system to go to a lower pH. Therefore, the increase of t_0 and the decrease of k values observed in this study could be related to the pH of the system (mixture of soymilk and GDL).

The $G_{\rm sat}'$ value of touhua was dependent on the solids content of soymilk (Fig. 1B); this indicates that a more rigid gel was formed from soymilk with a higher solids content. The shear modulus

Fig. 1. Effects of soilds content on the gelation time (t_0) , the saturated storage modulus (G'_{sat}), and the rate constant (k) of gelation of touhua prepared with 0.02 N GDL at 70° C.

(G) of polymer gels is often described by an exponential function of polymer concentration (C_p) , such as, $G = m * C_p^n$, where m and n are constants. In many cases, the exponent (n) was 2, i.e. square power law ([Kohyama et al., 1995; Ross-Murphy, 2004\)](#page-6-0). This power law equation will be practically useful for estimating the soybeanto-water ratio in touhua and tofu processing. The exponents for 7S and 11S protein gels with 0.4% GDL were 2.43 and 3.40, respectively, as the protein concentrations ranged from 2% to 6% ([Kohy](#page-6-0)[ama et al., 1992; Kohyama & Nishinari, 1993](#page-6-0)). [Bikbov et al.](#page-6-0) [\(1979\)](#page-6-0) found that the exponent for 10–20% soy protein gels (formed by heating in the absence of coagulant) was 4.67. The G'_{sat} value in this study was found to be proportional to the 1.68th power of the solids content of soymilk $(R^2 = 0.973,$ Fig. 1B). The small value of the exponent might have resulted from the low protein content or constituents of soymilk more complicated than soy proteins.

3.3. Effect of GDL concentration

As mentioned, GDL is a weak acid and is widely used as a coagulant for preparing packed touhua and tofu products. As the pH of soymilk is lowered by the addition of GDL to the isoelectric point of soy proteins (around 4.6), the protein molecules become aggregated and coagulated. Consequently, a network of gel is formed under proper conditions. The pH values of packed touhua with 0.01– 0.05 N GDL were 5.50, 4.95, 4.69, 4.48 and 4.42, respectively. This indicates that the higher the concentration of GDL, the lower is

Fig. 2. Effects of GDL concentration on the gelation time (t_0) , the saturated storage modulus (G'_{sat}), and the rate constant (k) of gelation of touhua prepared with 4% solids content at 70 °C.

the pH value of touhua. A previous report ([Shih & Shiau, 2003\)](#page-6-0) indicated that black soybean touhua made by a high concentration of GDL (>0.0375 N) had low sensory scores, due to its tartness.

The t_0 value showed a negatively linear relationship (R^2 = 0.964, Fig. 2A), whereas the $G_{\rm sat}'$ value showed a positively linear relationship (R^2 = 0.929, Fig. 2A), to the concentration of GDL in a doublelog plot. The slope of the regression line for GDL concentration vs. G'_{sat} value was 1.01, which was lower than that (1.68) of the solids content vs. G'_{sat} value [\(Fig. 1B](#page-2-0)). This result indicates that G'_{sat} was less sensitive to the GDL concentration than to the solids content of soymilk.

The k value showed a linear relationship to the concentration of GDL in a semilog plot of k value against GDL concentration $(R^2 = 0.983,$ Fig. 2B), and the correlation coefficient between values of k and pH was -0.982 (p < 0.01). More acid was generated in a given time at a higher concentration of GDL, which further resulted in lower t_0 and higher k values. [Kohyama and Nishinari \(1993\)](#page-6-0) found that the gelation times of 7S and 11S soy proteins became shorter with increasing concentration of GDL. [Yoshimura et al.](#page-6-0) [\(2004\)](#page-6-0) also observed a shorter gelation time and a higher gelation rate with the increase of GDL concentration from 0.2 to 0.5%. Therefore, pH value of the system is an important rate-determining factor in the gelation of touhua, using GDL as a coagulant.

3.4. Effect of coagulation temperature

As indicated, soy gels formed at 2–7% solids content of soymilk with 0.01–0.05 N GDL solution. Furthermore, [Shih and Shiau](#page-6-0) [\(2003\)](#page-6-0) concluded that the optimum processing condition for making packed touhua was 3.67% solids content, 0.0225 N GDL and 71 °C. Therefore, 4% soymilk solids content and 0.02 N GDL concentration were chosen for further studies. Temperature is one of the important factors affecting the rheological and textural properties of touhua and tofu products. Fig. 3A shows the plots of t_0 and G'_{sat} against temperature. The results indicate that t_0 value decreased with increasing temperature of coagulation. In other words, the higher temperature of coagulation originating, the shorter was the time of gelation. This agrees with the observation of [Kohyama](#page-6-0) [et al. \(1992\)](#page-6-0) that it took a long time for the gelation of 11S soy protein to commence at a low temperature. Contrary to the tendency of t_0 , the G'_{sat} value increased with increasing coagulation temper-ature and reached a maximum value at 75 °C. [Yoshimura et al.](#page-6-0) [\(2004\)](#page-6-0) found a maximum G' value at 70 °C for dispersion of pow-dered soybean (40-90 °C). However, [Kohyama et al. \(1995\)](#page-6-0) reported that the G'_{sat} of 5% soy protein isolate gels decreased with increasing coagulation temperature, ranging from 50 to 90 $^{\circ}$ C in the presence of 20 mM GDL.

Fig. 3B shows the plots of log k value against the inverse value of absolute temperature (T^{-1}) for cooked and uncooked soymilks. Results showed that linear correlations between log k and T^{-1} values were found for both the cooked and uncooked samples $(R^2 = 0.980$ and 0.995, respectively) in the temperature range tested. This fitted the following Arrhenius equation,

$K = A * \exp(-Ea/RT)$

where Ea is activation energy (kJ/mol), R is gas constant $(8.314$ J/ $mol*K$), and A is a proportional constant. The gelation Ea values for the cooked and uncooked soymilks were 43.4 and 27.2 kJ/mol, respectively. [Kohyama et al. \(1995\)](#page-6-0) reported that the Ea values of 5% soy protein isolate containing 0.02 M and 0.03 M GDL were 50.1 and 14.9 kJ/mol, respectively. The Ea values of this study were

Fig. 3. (A): Temperature-dependencies of the gelation time (t_0) and the saturated storage modulus (G_{sat}') of touhua prepared with 4% solids content and 0.02 N GDL. (B): Arrhenius plots for gelation of touhua prepared by uncooked or cooked soymilks with 4% solids content and 0.02 N GDL.

Table 1

Effects of coagulation temperature on the gelation time (t_0), saturated storage modulus ($G_{\rm sat}$) and rate constant (k) of gelation of uncooked soymilk and soymilk cooked at 100 °C for 10 min.

Coagulation temperature $(^{\circ}C)$	Uncooked soymilk				Cooked soymilk			
	t_0 (min)	G'_{sat} (Pa)	$k (s^{-1})$	D ² \mathbf{v}	t_0 (min)	$G'_{\text{sat}}(Pa)$	$k (s^{-1})$	R^2
60	10.90	41.3	4.03×10^{-4}	0.998	8.19	83	4.08×10^{-4}	0.999
70	5.47	43.8	5.55×10^{-4}	0.996	4.12	120	6.03 x 10^{-4}	0.996
80	2.76	56.2	7.03×10^{-4}	0.985	1.85	120	9.12×10^{-4}	0.988

lower than those of insolubilization of 11S globulin (381 kJ/mol) ([Watanabe, 1988\)](#page-6-0), heat denaturation of 7S globulin (113 kJ/mol) ([Iwabuchi, Watanabe, & Yamauchi, 1991](#page-6-0)), and heat denaturation of soy protein isolate (74.0 kJ/mol) [\(Ahmed et al., 2006\)](#page-6-0). However, an activation energy of 42 kJ/mol was found for the major gelation mechanism of rennet being clotted from casein by enzyme instead of by heat [\(Tokita et al., 1982](#page-6-0)). The lower Ea demonstrates that the gelation process of touhua in the presence of GDL is not highly dependent on temperature.

Table 1 shows the effects of coagulation temperature on the values of t_0 , G'_{sat} and k of touhua gels made from uncooked and cooked soymilks with 4% solids content and 0.02 N GDL. The gelation of cooked soymilk showed higher values of k and G_{sat}' and a lower value of t_0 than those for the gelation of uncooked soymilk treated at the same coagulation temperature. This indicates that the formation of gel commenced earlier, and gel was much stronger in the gelation of cooked soymilk. Heating resulted in the denaturation of soy proteins, whilst denatured proteins tended to coagulate and to form a rigid and intact gel. [Kohyama et al. \(1995\)](#page-6-0) suggested that the gelation of tofu was a two-step process, with first, protein denaturation by heat and, second, hydrophobic coagulation promoted by protons from GDL or by calcium ions. The addition of GDL or calcium sulphate induced gelation by promoting protein aggregation via hydrophobic interactions.

3.5. Effect of non-isothermal gelation process

Commercially, packed touhua is produced by a non-isothermal process. Hence dynamic rheological properties of soymilk with 4% solids and 0.02 N GDL were also determined under non-isothermal conditions. The G' value of soymilk during non-isothermal gelation increased with the decrease of temperature from the starting temperatures (60–80 °C)–5 °C at a descending rate of 1 °C/min (Fig. 4A). Furthermore, the G' value decreased with decrease of starting temperature. The G' values showed linear correlations $(R^2 > 0.989)$ with the log values of temperature during measurement, for all treatments. Compared to the results shown in [Fig. 3](#page-3-0)A, both the G' (at the final temperature of 5 °C, Fig. 4A) and G'_{sat} (at isothermal temperature, [Fig. 3A](#page-3-0)) values of touhua (within 60–75 °C) had a high correlation coefficient ($r = 0.991$). However, the same is not true for the touhua prepared at 80 \degree C. This could be because the gelation was too fast, so coarse and uneven gel was formed at 80 °C, and water vapourization occurred during isothermal gelation after a long heating time (90 min).

Frequency sweep (0.1–10 Hz) tests were performed on the touhua gels, immediately after the temperature was lowered to 5 $^{\circ}$ C, by using the same dynamic rheometer. Both G' and G'' values of touhua gels increased as the oscillation frequency increased (Fig. 4B and C). The tan δ of touhua gels ranged from 0.14 to 0.25. [Clark and Ross-Murphy \(1987\)](#page-6-0) reported that systems with tan δ values of about 0.1 belonged to the so-called "weak" gels or colloidal dispersions. Furthermore, in weak gel systems, the G and G'' values were dependent on the frequency. Compared to weak gels, typical ''true gels" show a small frequency-dependence of G' and G" at a certain frequency ([Clark & Ross-Murphy, 1987\)](#page-6-0).

Fig. 4. Storage modulus (G') and loss modulus (G'') of soymilk with 4% solids content and 0.02 N GDL during the non-isothermal gelation. (A): G during gelation from the indicated temperatures to 5 °C with 1 °C/min cooling rate. (B) and (C): Frequency sweeps of G' and G'' of touhua at 5 °C.

Accordingly, the touhua gel made by isothermal or non-isothermal gelation processes in this study is considered as a weak gel.

The relationships between G' , as well as G'' , values and oscillation frequency (F) were well predicted by power law models, which are $G' = a \times F^{b}$ ($R^{2} > 0.99$, [Fig. 4](#page-4-0)B) and $G'' = c \times F^{d}$ ($R^{2} > 0.97$, [Fig. 4C](#page-4-0)). The b values were 0.133, 0.119, 0.114, 0.109 and 0.112 in ascending order of coagulating temperatures from 60 to 80 $^{\circ}$ C, respectively. Moreover, the d values were 0.203, 0.172, 0.150, 0.162 and 0.160 in the same ascending order, respectively. These results indicate that the touhua gels made at low coagulation temperatures were weaker than those made at high temperatures, and that the moduli of touhua gels made at low coagulation temperatures were more dependent on frequency than were those of gels made at high coagulation temperatures.

3.6. Textural characteristics of packed touhua

Fig. 5 shows the textural characteristics of packed touhua gels prepared by various processing conditions. Within 2–7% solids contents, both the hardness and adhesiveness of touhua gels were linearly increased with increasing solids content in double-log plots (R^2 > 0.98, Fig. 5A), which is similar to that of the G'_{sat} value vs. solid content [\(Fig. 1B](#page-2-0)). The slopes of the regression lines were 1.36 and 2.45 for the hardness and the adhesiveness, respectively. This indicates that the adhesiveness value was more sensitive to the solids content than was the hardness value. A similar result was reported by [Shih and Shiau \(2003\)](#page-6-0), i.e. the hardness of packed touhua gels increased with increasing solids content (2.8–5.6%) of soymilk. The hardness [\(Shen, de Man, Buzzell, & de Man, 1991\)](#page-6-0) and breaking stress [\(Guo & Ono, 2005](#page-6-0)) of tofu increased with protein content in the soymilk. Therefore, the increase of the solids or protein contents reinforced the interactions amongst protein molecules during gelation, forming a stronger network of touhua gels.

The hardness and adhesiveness values of packed touhua gels were also linearly increased with increasing concentration of GDL in double-log plots ($R^2 > 0.90$, Fig. 5B). This indicates that the concentration of GDL used affected the textural properties of touhua gels made. [Beddows and Wang \(1987c\)](#page-6-0) concluded that the optimum calcium sulphate concentration for making silken tofu was 9.5 mM, and that tofu was soft at low concentrations of coagulant, but tofu was hard and rubbery when calcium sulphate concentration was above 10 mM. [Shih et al. \(1997\)](#page-6-0) reported that increasing coagulant concentration increased the elasticity and hardness of soft tofu.

The slopes of the regression lines shown in Fig. 5B were 0.63 and 1.12, respectively, which are close to the slope (1.01) of log t_0 vs. log GDL concentration [\(Fig. 2](#page-3-0)A). However, they were lower than those shown in Fig. 5A. These results indicate that the hardness and adhesiveness of packed touhua gels were less sensitive to the GDL concentration than to the solids content of soymilk.

Both the hardness and adhesiveness values of packed touhua gels increased linearly with increasing coagulation temperature from 60 to 80 °C (R^2 > 0.98, Fig. 5C). The slopes of the regression lines were 0.34 and 0.67 for the hardness and the adhesiveness, respectively. [Wang and Hesseltine \(1982\)](#page-6-0) indicated that the hardness of tofu increased with coagulation temperatures increasing from 60 to 80 °C. [Beddows and Wang \(1987b\)](#page-6-0) found that the optimum coagulation temperature was 75-80 °C for making silken tofu.

The G' values, at 5° C, of touhua gels prepared following the non-isothermal gelation treatment, were positively correlated with both the hardness and the adhesiveness of packed touhua ($P < 0.01$, r = 0.974 and 0.972, respectively). The same is true for the G'_{sat} values of touhua gels prepared following the isothermal gelation treatment and the hardness, as well as the adhesiveness, of packed touhua ($P < 0.01$, $r = 0.931$ and 0.970, respectively). The results demonstrate that the rheological measurements used in this study are sensitive enough to monitor the texture of packed touhua.

Fig. 5. Effects of process variables on the textural properties of packed touhua. The solids content of soymilk, GDL concentration and coagulation temperature are 4%, 0.02 N and 70 °C, respectively, unless otherwise stated in the Figure.

4. Conclusion

Gelation curves of touhua gels prepared following the isothermal heating were well predicted by first-order reaction kinetics $(R^2 > 0.98)$. The G'_{sat} values of touhua gels increased with the increases of the solids content of soymilk and the concentration of GDL. The G'_{sat} value was found to be proportional to the 1.68th power of soymilk solid content. The maximum G'_{sat} value was obtained at a 75 \degree C coagulation temperature. The k value increased with increasing coagulation temperature and GDL concentration, but decreased with increasing solids content. The activation energy of gelation was 43 kJ/mol and the t_0 became shorter with increasing coagulation temperature and GDL concentration. However, the

 t_0 increased with the increase of soymilk solid content. The denaturation of soy proteins resulted in a higher $G_{\rm sat}^\prime$ and a shorter t_0 than those of the natural soy proteins. Touhua gel is a weak gel and pH is an important rate-determing factor in the gelation of touhua using GDL as the coagulant. The hardness and the adhesiveness values of packed touhua increased with the increases of solids content, GDL concentration and coagulation temperature. The rheological properties (G' and G'_{sat}) of packed touhua were significantly and positively correlated with its textural characteristics (hardness and adhesiveness). Based on the results of in this study and the sensory evaluation in our previous study (Shih & Shiau, 2003), high-quality black soybean touhua can be prepared by using of $0.02 - 0.03$ N GDL, 3-4% solid content of soymilk, and 65-70 $^{\circ}$ C coagulation temperature.

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