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Chapter 1 Introduction

1.1 What is "tofu"?

Tofu, also called soybean curd, is manufactured by adding coagulant to soymilk extracted from ground soaked soybean. Tofu first appeared in China and is ancient soybean food with a history of 2000 years. Tofu has been popularly accepted by rice-eating people in Korea, China, and South-eastern Asian regions including Japan, and in addition, in Europe and the USA. It is considered as a healthy food.

It was known that the inventor of tofu is Liu An, king of Huai Nan (178-122BC) of the Han dynasty. The technique to extract and solidify water soluble proteins contained in soybeans is very unique and unable to be found in other vegetable foods. The manufacture method and utilization method differ in various Eastern Asian countries, regions or times. The material for tofu production is soybean.

1.2 Physicochemical property of soybean

Soybean is called meat of the field from ancient time. Lipid and protein are the two major components in soybean. For lipid, there are many polyunsaturated fatty acids in soybean, which makes soybean oil one of the excellent vegetable oils. Here, we mainly discuss soy protein, which has rich amino acids with a good balance. The protein content of soybean is about 40% (dry base) and 90% of it is storage protein. The storage protein mainly consists of β-conglycinin (7S globulin) and glycinin (11S globulin). The amino acid profile, basic structure and structure of the two main proteins concern much with the physicochemical functions, including emulsification, foaming, gelation and water and fat binding abilities. β-conglycinin is a glycoprotein and a trimer with a molecular of 150-200 kDa. Major subunits are α' (72 kDa), α(68kDa) and β (52kDa). Besides these, there is another minor subunit called γ (~52kDa). There are cysteine residue in both α' and α subunit while not in β one. β-conglycinin trimers cause association and/or dissociation depending on the pH and ionic strength of the solution. Glycinin is a hexamer and each subunit consists of acidic and basic polypeptides, which are linked together with a disulfide bond. In glycinin, there are five subunits called as A1aB1b, A2B1a, A1bB2, A5A4B3 and A3B4, which are classified into group I (the former 2), II (the latter 3) by the extent of the homology. Glycinin hexamers dissociate to their constituent polypeptides, subunits, and half molecules, depending upon pH, ionic strength and heating temperature. For tofu production, protein takes great influence on the gelation of tofu gel.

1.3 Physiological function of soybean storage proteins and minor components

For the nutrition evaluation, in many cases, rat is used. However, the nutrition need of human being is not always similar with that of rat. For this reason, the quality of soybean proteins has actually been undervalued until recently. It is now concluded that the quality of soybean proteins is comparable to that of animal proteins sources like milk and beef. Recent studies also indicate that the lower amount of methionine in soybean proteins compared with casein may contribute to the selective retardation of tumors (rat was used) (Hawrylewicz, et al., 1995). The physiological functions of soybean storage proteins and minor components were shown in Table 1-1. With the economic development of economy in the world, chronic diseases, such as osteoporosis, cardiovascular diseases are becoming more and more popular to us. Food custom involves much with this kind of disease. Soy protein represents a safe, viable and practical nonpharmacological approach to lowering cholesterol. The most interesting thing about the physiological function of soybean is that many minor components that were considered as antinutrient factor, now are thought to have preventing effect on cancer. These components include isoflavones, saponins, trypsin inhibitors, phytic acid, etc. Among these, isoflavones (mainly genistein and daidzein) are becoming one of the hotspots of functional food research because soybean is the only significant dietary source of these compounds. Although soybean has so many physiological functions, its absorbent efficiency is not good for human. In the production of tofu, heat treatment is done and this process can greatly improve the nutrition value of soybean, especially improve the absorbance efficiency. From this point, tofu is considered as a very good protein resource.

1.4 Production of tofu

Tofu is manufactured by adding coagulant to soymilk extracted from ground soaked soybean. Generally speaking, tofu can be divided into momen (pressed type) tofu and kinogoshi (no press type) tofu. The process procedures of the two types are shown in Fig.1-1. The main differences between the 2 methods are: (1) in momen tofu production way, tofu is broken down one time and then pressed, while kinugoshi tofu is produced without [breaking down] (Fig.1-1) process and before the addition of coagulant, soymilk is cooled; (2) the solid content of soymilk is different, soymilk for monen tofu production has a lower solid content than kinugoshi one.

In China, most of hand-made tofu is produced by momen-tofu production method while kinugoshi production method is adopted in industrial production. For coagulants using in tofu production, there are 3 major materials: bittern (residue during salt production from sea water, major component: magnesium chloride); gypsum (major component: calcium sulfate); glucono-delta-lactone (GDL). In this study, tofu produced by these 3 types of coagulants are called as Mg-tofu, Ca-tofu and GDL-tofu. In China, gypsum is mainly used in the south part and bittern in the north part.

Table 1-1 Physiologically functional substances in soybeans (Hasler, 1998)

Components in soybeans	Physiological functions
Soybean storage proteins	Reduction of serum cholesterol, prevention of cardiovaslular diseases, reduction of body fat, and promotion of serum insulin
Peptide from storage proteins	Antioxidant activities, inhibition of angiotensin-converting enzymes, and promoting action of phagosytosis
Isoflavenes	Anticarcinngenetic activities, prevention of cardiovaslular diseases, prevention of osteoporosis, antioxidant activities, and alleviation of menopausal sympotoms
Saponins	Anticarcinogenic activities, hypocholesterolemic effects, inhibition of platelet aggregation, HIV preventing effects (DDMP saponin), and antioxidant activities
Phytosterol	Anticarcinogenic activities
Phytic acid	Anticarcinogenic activities
Lectin (Hemaglutinin)	Activation of lymphocytes (T cell) and aggregating action of tumor cells
Nicotianamine	Inhibitor of angiotensin-converting enzyme
Protease inhibitors	Anticarcinogenic activities

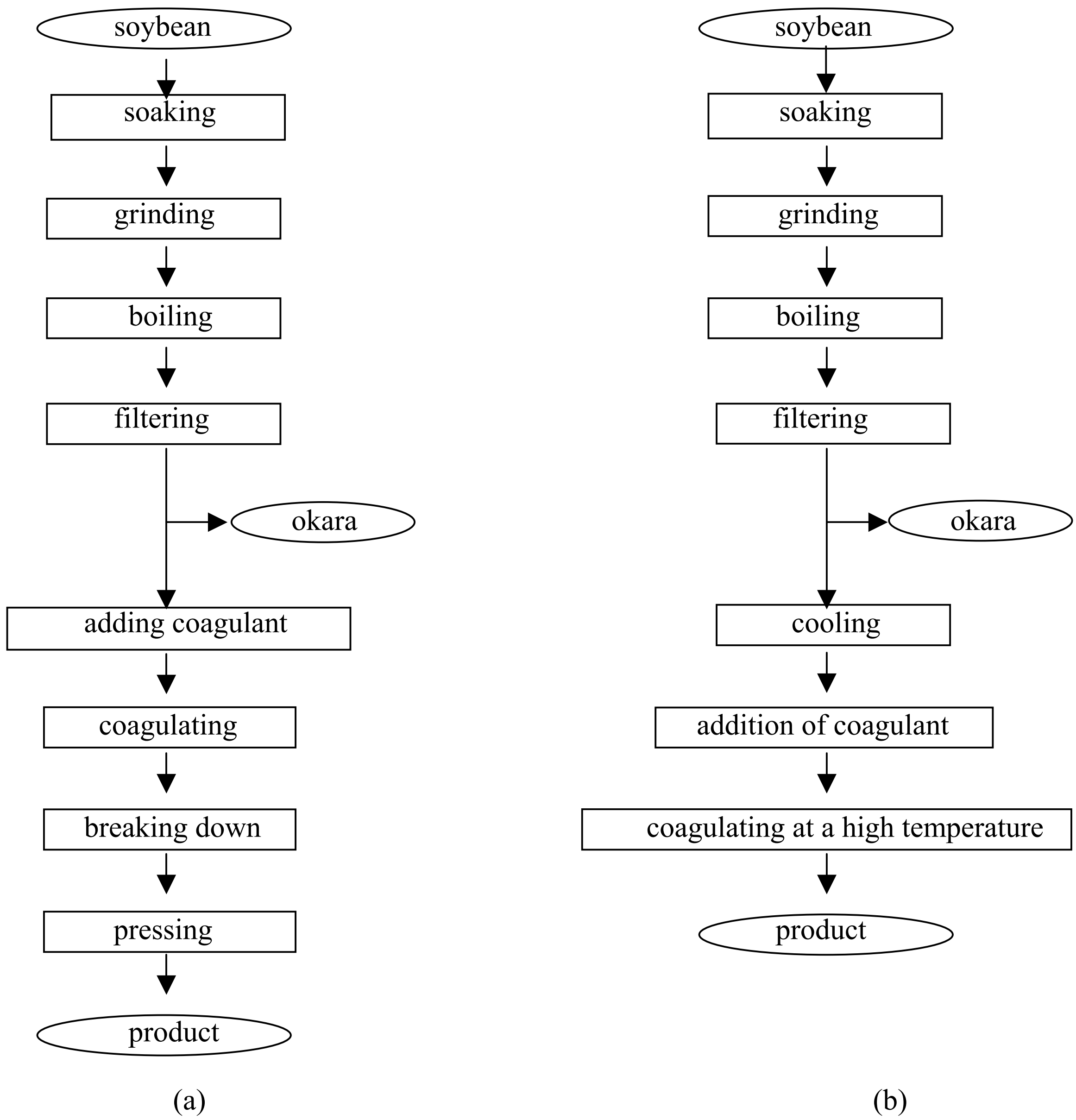


Fig.1-1 Production procedures of momen tofu (a) and kinugoshi tofu (b)

1.5 Previous researches on tofu production and gelation mechanism of soy protein and soymilk

Tofu production has been studied for several decades. Watanabe (1964), Saio (1979), Hashizume (1975) and many other researchers in Japan systematically studied the production conditions of tofu. They researched for the effects of materials, production conditions on the texture of momen tofu, kinugoshi tofu, filled kinugoshi tofu and also studied the mechanization of tofu production and summarized them in a book of soybean processing (Watanabe et al., 1987, 1997).

Production of good quality tofu involves many factors (such as quality of soybean, water soaking conditions, grinding conditions, heating, concentration and components of soymilk, coagulation conditions, etc.) (Watanabe, 1997). Among them, concentration and components of soymilk and coagulation conditions (type and concentrations of coagulants, the ratio of protein/coagulant) are very important factors that affect the quality of tofu. These factors can be summarized as following:

(1) Control of material soybeans

Including the variety of soybean, storage conditions and time, soybean components (water content, protein content, ratio of 11S protein/ 7S protein, etc). Generally speaking, a higher protein content fresh soybean will cause good texture and high yield of tofu and a higher 11S/7S will induce hard tofu.

(2) Water soaking

The soaking time greatly varies to materials and temperature. Soaking time is determined by the water absorbing ratio. It is desirable to minimize microbial and enzymatic effects during soaking. In common cases, one night or 12 hrs is used.

(3) Grinding

Grinding conditions (such as water : bean ratio) have great effects on the extraction ratio, and are factors that govern the quality of tofu. Beddows and Wong (1987a) found that the 10:1 water : bean ratio gave the best result.

(4) Heating

The heating conditions take influence on the denaturation of protein. This preheating treatment is necessary for the coagulation of tofu after addition of coagulant in the later processing. Too short heating time will cause the difficulty of coagulation of good quality tofu. Overheating causes protein supposed to be eluted in soymilk to become insoluble, resulting in a great decrease in the yield of tofu. In actual practice, 100℃, 3 min is used, i.e., boiling the soymilk for 3 min.

(5) Cooling conditions for kinugoshi tofu

During kinugoshi tofu production, cooling of heated soymilk is necessary. Cooling will slow the reaction between coagulant and protein and a slow reaction will help the production of tofu with good quality. Cooling below 10℃ is often used.

(6) Coagulation (solidification) conditions

Soymilk concentration, components of soymilk, type and concentrations of coagulants, the ratio of protein/coagulant, coagulation temperature will affect the quality of tofu. Beddows and Wong studied the heating process (1987b) and coagulant concentration, mixing and filtration pressure (1987c) for kinugoshi tofu production. They found that the optimal coagulation temperature was 75-80 ℃. Different soymilk concentration will have a different optimal coagulant concentration to produce good quality tofu.

A good gel formation is very important for tofu production. High concentration of soy protein or soymilk forms gel at high temperature. If the concentration becomes lower, after heating at an elevated temperature, the addition of coagulant can help to convert preheated soymilk into gel. The mechanism of gelation has been studied for more than a decade, but conclusions are still difficult to be drawn. It was suggested that the network structure of tofu gel may be formed via hydrogen bonding, hydrophobic associations, ionic interactions and electrostatic cross-links, and also through some sulphydryl-disulphide linkages of unfolded polypeptides (Catsimpoolas et al., 1970; Utsumi & Kinsella, 1985). The network formation of tofu gel mainly involves unfolding of soy protein by heating, aggregate by the addition of coagulant and the formation of gel.

The gelation mechanisms of different coagulants are a little different. Saio (1979) concluded that the mechanism of tofu gel formation as: the unfolding of hydrophobic regions of protein by heat treatment; formation of S-S bond between cysteines in protein; aggregation of protein and gel formation.

Kohyama et al. (1995) suggested that the mechanism of Ca-tofu and GDL-tofu as following after investigating the dynamic viscoelasticity of tofu: the gel formation consists of two steps: protein denaturation and hydrophobic coagulation. At first, the hydrophobic regions of the protein molecules in the native state are located inside and are exposed to the outside by heat denaturation. Since the denatured soybean protein is negatively charged, the formation of protons induced by GDL or calcium ions neutralizes the net charge of the protein in the second step. As a result, the hydrophobic interaction of the neutralized protein molecules becomes more predominant and induces the aggregation.

Ono et al. (1991) found that protein particles in soymilk could be fractionated by differential centrifugation. Large size protein (LSP, in diameter >100 nm) constituted 40% of the total protein in raw soymilk. LSP decreased with heating, and particles of 100-40 nm in diameter (MSP) increased. They suggested that the changes of content of medium and large particles played an important role in the gelation of tofu gel.

Nagano et al. (1994a) investigated the heat-induced gelation of β-conglycinin, one of the most important storage proteins in soybean, by dynamic viscoelastic measurements as a function of pH. They found that β-conglycinin undergoes denaturation with increasing protonation of its carboxyl groups, resulting in an increase in the amount of exposed β-strands. The exposed β-strands then intermolecularly bond to form gel networks. This process is promoted with decreasing pH, and as a result, rigid gels form at acidic pH values. This research may help to elucidate the gel formation resulting from addition of GDL.

1.6 Texture evaluation of tofu

For foodstuff, texture is very important. The term "*texture*" was originally taken to refer to the structure, feel, and appearance of fabrics. It was not until 1660s that it started to be used to describe "the constitution, structure or substance of *anything* with regard to its constituents, formative elements" (Oxford English Dictionary, 1989). Various attempts to define food texture have culminated in some international agreement with the development of the international standard ISO 5492 (International Organization for Standard [1992]), which deals with the vocabulary used for sensory evaluation. This defines texture as "All the mechanical, geometrical and surface attributes of a product perceptible by means of mechanical, tactile and, where appropriate, visual and auditory receptors". Texture evaluation of soy products is very important for quality control. Sensory method is the most effective way for the evaluation. But for the culture differences, age, sex and experience, even the same soy product, thoroughly different results could be obtained. For an instance, the beany flavor (or off-flavor) is a very good smell for many eastern people who often drinks soymilk, but almost all the western people do not like it and consider it as a strange flavor. An objective evaluation method is necessary. Matsumoto and Matsumoto (1977) reported that for food like tofu, the physical factors are involved much more with the taste than chemical ones. Texture is significantly related to the quality of tofu.

For tofu can be considered as a kind of uniform food, it is easier to be evaluated by objective methods using equipment. Tofu is a kind of viscoelastic foodstuff. Static viscoelastic tests, such as stress-strain, stress relaxation and creep tests and dynamic viscoelastic test are usually used as objective evaluation methods for texture evaluation of viscoelasctic foodstuff. In many cases, break stress obtained from the stress-strain test is a major index (Kohyama et al., 1995; Hou et al., 1997; Hou and Chang, 1998; Saio, 1979). Dynamic viscoelastic test can measure the changes of storage and loss modulus of tofu gel and the changes of these parameters during the gel formation can be measured. These changes can reflect the gel formation and help to elucidate the gel formation mechanism. The dynamic viscoelasticity of 7S, 11S storage proteins (Kohyama et al., 1992; Kohyama and Nishinari, 1993; Nagano et al., 1994a, b, c) were investigated. The difference between the gelation properties of 7S and 11S proteins were approved by dynamic viscoelastic approach.

Many factors take influence on the texture of tofu. High quality soybean is one of the important factors. In the last two decades, the various soybean mutant genes that control the production of enzymes, allergenic proteins, storage proteins, etc. have been identified in the world germplasm. Using these mutants, the commercially available soybean cultivars without having undesirable substances or with the beneficially modified component of storage proteins have been bred. For examples, the lipoxygenase-free, β-conglycinin-free lines have been acquired by breeding. Processing conditions, such as soaking temperature, time, type and concentration of coagulant, coagulation temperature also have great influence on the texture of tofu.

1.7 Present situation of tofu production in China

Nowadays in China, the problem of protein intake inadequacy still exists comparing with developed countries like Japan and USA (Table 1-2). In 1996, the protein intake was only 69.7 g/person/day, which is much lower than that in Japan (95.0 g/(person·day)) and in America (112.5 g/(person·day)). Animal-resource proteins have good quality, but it is difficult to increase the output of them in a short time for their high cost. From the view of cost, vegetable origin proteins arouse people’s attention. Among them, in particular soy protein is one of the most important proteins.

A project called “soybean project” started in 1990s in China to promote the production and utilization of soybean. There are many varieties of soy products in China, such as tofu, soy sauce, douchi, tofu sheet (doufupi), fuzhu, etc.. From ancient China, Chinese has utilized soybean as a foodstuff. Tofu was invented by Chinese and then transferred to Japan and Korea.

During the long period of soybean utilization, people acquired many precious experiences. However, nowadays, researches on soybean processing and utilization, especially the industrialization are still laggard comparing with Japan and some other countries.

Table 1-2 Protein intake in 1996 of different countries (g/(person·day)) (from FAO (1996))

China	Japan	USA
69.7 g	95.0 g	112.5 g

Note: Goal of person's daily protein intake in 2010 is 77 g/day (in 2000, it was 70.5 g/day) (From "the Tenth-five years plan of China Food Industry (2001-2005))

Because of the palatability of Chinese and the cooking way of dishes, Chinese prefer hard tofu, while in Japan, soft tofu is preferred. In tofu production, most of the factories are family-style small factories (handmade type). Although such kind of factories can provide hard tofu suitable for Chinese consumers, the shelf life of the products is very short, in summer, even only half a day, which takes great influence on the distribution. There are also some other demerits of this handmade way: uniform quality of products is difficult to be obtained; high labor density, etc.. In 1980's, some industrial production lines were imported from abroad. Most of the production lines are taking GDL as coagulant. The production way of tofu is filled kinugoshi way (Fig.1-1 (b)). The products from these lines have a relatively long shelf life and can be well distributed. However, tofu coagulated only by GDL has an acidic taste, which is not preferred by consumers. The texture of such kind of tofu is soft and is not suitable for most of the Chinese dishes. Lack for the basic information on industrial hard tofu production limits the good running of these industrial production lines. To improve the hard tofu production, improvement of tofu texture is final goal. Thus, improvement of tofu texture should be considered.

1.8 Objectives of this study

From the present situation of hard tofu production in China, it was shown that improvement of hard tofu production in China is necessary. The objective of this study is to obtain useful information for improvement of tofu texture in China.

To realize this goal, firstly, a proper texture evaluation method for tofu should be determined. Then, effects of coagulants on the texture of tofu can be investigated by using this method. At the same time, because the objective of this study is the improvement of tofu production in China, investigation of the texture of tofu in China is necessary. Okara is the residue of tofu industry. The addition of okara to soymilk for tofu production will take influence on the texture of tofu. Investigation of okara addition on the texture of tofu is expected. This study will mainly focus on above respects.

Chapter 2 Determination of proper texture evaluation method for tofu and effects of coagulants on the texture of tofu

2.1 Introduction

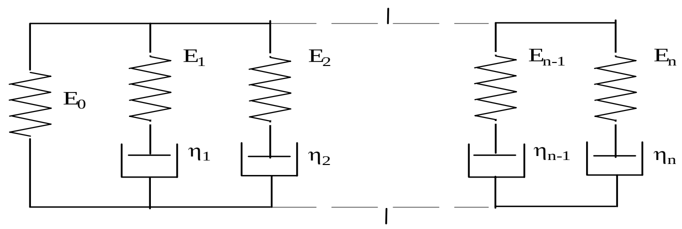
To improve the production of tofu, proper texture evaluation method is always necessary for such kind of hard tofu. Both chemical and physical factors are involved with the taste of foodstuffs. Matsumoto and Matsumoto (1977) reported that for food like tofu, the physical factors are involved much more with the taste than chemical ones. Texture is significantly related to the quality of tofu. Saio (1979) reported the relationship between texture and fine structure of tofu. To evaluate the texture of tofu, different methods were tried. Break stress obtained from stress-strain behavior is an important index being used by many researchers (Kohyama et al., 1995; Hou et al., 1997; Hou and Chang, 1998; Saio, 1979). Stress relaxation and creep tests are also used for food materials.

In stress relaxation tests, a food sample is compressed at a controlled crosshead speed to a desired strain. This strain is maintained as a constant, while the accompanying stress decays with time to provide the characteristic relaxation behavior. An empirical evaluation method for stress relaxation (Peleg, 1979; Peleg, 1980; Lee et al., 1983) was tried for different solid food materials. Stress relaxation curves were analyzed by this empirical method. Lee et al. (1983) used this method to evaluate some commercial compressed tofu. They found that tofu’s deformability mode, like that of sponges, is regulated by both the solid matrix properties and internal hydrostatic pressure.

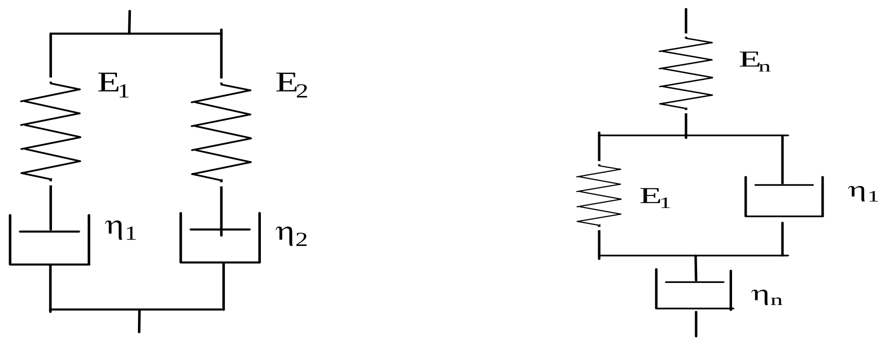
Results of viscoelastic tests like stress relaxation can also be analyzed by other methods. If being analyzed by different models like Maxwell model and Vigot model (Fig. 2-1 (a) (b)), parameters obtained from this method can include both viscous and elastic parts (Mohsenin, 1986; Kawabata, 1989).

Using this analysis method, Isozaki et al. (1976) found that the stress relaxation behavior of hydrogels of agar could be fit with a 6-element model. The stress relaxation curves of 20% (w/v) soybean gel were considered to be fit for a 6-element model (Kobayashi et al., 1981). One of the authors examined the stress relaxation characteristics of commercial momen and kinugoshi tofu in Japan by a 7-element model and compared the parameters with results by Peleg’s empirical method (Taneya et al., 1993). Stress relaxation tests were applied to many foodstuff including soy protein gels (Kuwabatake and Nakahama, 1975; Akabane et al., 1981; Kobayashi et al., 1981), agar gel (Isozaki et al., 1976) and other materials.

(a) Maxwell model (b) Vigot model



(c) generalized Maxwell model



(d) 4-element Maxwell model (e) 4-element Burgers model

Fig.2-1 Characteristics of Maxwell model (a), Vigot model (b), generalized Maxwell model (c), 4-element Maxwell model (d) and 4-element Burgers model (e), E, elastic modulus of spring model, η, viscous modulus of dashpot model

Creep test is another test method for understanding viscoelastic property of foodstuff. In a creep test, a load is kept as a constant and the strain will increase with time, which causes the creep behavior. Analysis of the creep behavior by models can also give both elastic and viscous parameters. The creep properties of agar gel (Isozaki et al., 1976), 20% soybean gel, egg white gel (Kuwahata and Nakahama, 1975), whey protein gel (Katsuta et al., 1990), procine serum-myosin gel (Ni and Hayakawa, 2001) were investigated and fit by different models. Kuwahata and Nakahama (1975) investigated the creep behavior of 20% soybean gel and compared it with egg white gel and 1.5% agar gel. They reported that the creep behavior of the three types of materials could be fit by a 4-element model. In this chapter, the creep properties of different tofu were also investigated and analyzed by viscoelastic models.

For the texture of tofu, soybean takes a great influence. Variety (Skurry et al., 1980; Johnson and Wilson, 1984), storage period (Hou and Chang, 1998), components of soybean (Murphy et al., 1997) can partly determine the final texture of tofu. Skurry et al. (1980) studied the effect of different variety (15 varieties) of soybean on the texture of tofu and found that a slight variation in the texture of tofu was due to the different contents of 7S and 11S proteins in various soybeans. Hou and Chang (1998) reported the yield and quality of soft tofu as affected by soybean physical damage and storage and they found yield of tofu decreased significantly beyond 30 days of storage. Sensory evaluation showed significant off-flavor was produced in tofu made from soybeans stored for 45 days or longer at 85% RH and 30℃. In this study, to reduce the influences of soybean and to simplify the experiments, soy protein isolate (SPI) was used for preparation of soymilk.

There are different ways of producing tofu and different types of coagulants. Gypsum (major component: $CaSO_4$), bittern (major component: $MgCl_2$), and GDL or their mixtures are major coagulants used in industrial practice. In China, especially in the north, many consumers prefer Mg-tofu while Ca-tofu is very common in the south. It is thus necessary to understand more about tofu texture coagulated by these 3 kinds of coagulants (Mg-tofu, Ca-tofu, GDL-tofu). Scanning electron microscopy (SEM) observations of tofu can help clarify the relationship between the microstructure and texture. SEM observations of Mg-tofu were conducted in this work. The texture of Mg-tofu, Ca-tofu and GDL-tofu was investigated and compared with each other.

2.2 Materials and Methods

2.2.1 Materials

Tofu produced in laboratory was used. The materials used are described as following, respectively.

The soy protein isolate (SPI, 90%/dry base (84.6%/wet base) protein content (moisture, 6%; fat, 0.5%; carbohydrate, 3%; ash, 5%), FujiproE) (Fuji Oil Ltd., Osaka) was used to prepare soymilk. Analysis-grade Glucono-delta-lactone (GDL) , food-grade $CaSO_4 \cdot 2H_2O$ and $MgCl_2 \cdot 6H_2O$ were purchased from Wako Pure Chemicals Industries Ltd. (Osaka). They were used without further purification.

2.2.2 Production of tofu in laboratory

Tofu production method in the laboratory was based on the industrially used kinugoshi (silken) tofu production method in Japan (Watanabe, 1997). Procedures were shown in Fig. 2-2. SPI was dissolved in distilled water to prepare for 5, 6, 7, 8, and 9% (w/v) protein soymilk, respectively. Solid content is about 1.11 times of the protein concentration. Soymilk was heated to 100℃ (boiling) and kept for 3 minutes, then was cooled to below 10℃ (for Mg-tofu, below 5℃). $MgCl_2$ solution, GDL solution or $CaSO_4$ suspension was added as coagulants. The final concentrations of $MgCl_2$ used were: 15mM, 20mM, 25mM and 30 mM and that for GDL-tofu and Ca-tofu was adjusted to 30 mM. The soymilk mixture was poured into a steel tank where glass molds were put in advance to shape the columnar tofu samples. The soymilk was then heated to 75℃ by a water bath and kept for more than 40 minutes to let the soymilk coagulate. Samples were stored at room temperature for 60 min before viscoelastic tests.

2.2.3 Measurement of viscosity of soymilk

Viscosity is a very important index of soymilk. Viscosity of soymilk with different concentrations was measured to understand the influence of concentration on viscosity by Viscometer LV2000 (Cannon Instrument Company, PA, USA). The rotation speed was 30 rpm for all the cases. The experiments were repeated for at least 3 times.

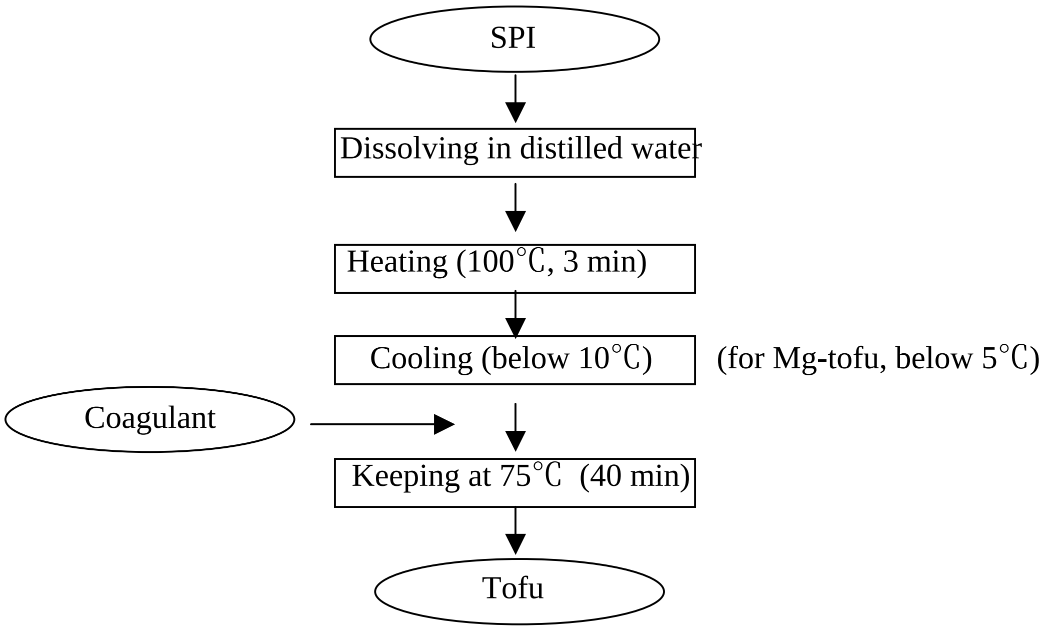


Fig. 2-2 Process procedures of tofu (silken tofu production method) in laboratory

2.2.4 Stress-strain, stress relaxation and creep tests of tofu

Stress-strain, stress relaxation and creep tests of tofu were done by Texture Analyzer (TA-XT2I, Stable Micro Systems, UK) with 5 kg load cell. The samples were 30 mm in height, 36 mm in diameter. A 70 mm in diameter flat plunger was used and the compression speed for the stress-strain test was 1 mm/s. Texture analyzer and the appearance of test were shown in Fig. 2-3. After the linear range was determined by stress-strain profiles, the relaxation and creep tests were done. Strain for stress relaxation was 5% while below 10% for creep test. The stress relaxation and creep periods were both 10 min for all samples.

All the parameter settings, operation of the instrument and saving of data obtained from the measurements were accomplished through a PC with Texture Expert software version 1.0. Experiments were repeated at least 3 times.

2.2.5 Analysis of results of stress-strain, stress relaxation and creep tests

2.2.5.1 Analysis of stress-strain test

Stress and strain data were recorded by the software with the Texture Analyzer. The apparent elasticity $E(t)$ was calculated as $\sigma(t)/\varepsilon(t)$, where $\sigma(t)$ is the stress and $\varepsilon(t)$ is the strain, which reflects the time-dependent changes of elasticity. Two types of break curves (brittle break and ductile break) were observed (Fig. 2-4). Brittle break has a clear break point, while break point of ductile break is not much clear and the decrease of stress is not significant after break (Okada, 2001). The peak point of the stress-strain curve was regarded as the break point, and the magnitude of the stress peak at this point was defined as break stress. All the experiments were done at least three times. When significance was indicated among the samples, data from 2 groups were compared by using the “paired t-test” in Sigmaplot® (Jandel Corporation, San Rafael, CA) at the 5% probability level.

2.2.5.2 Analysis of stress relaxation test by Peleg’s empirical method

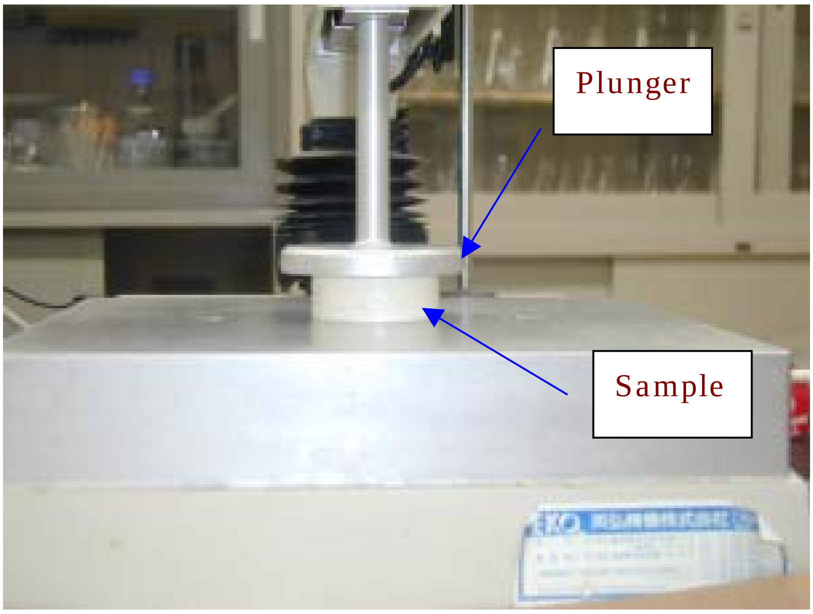
Stress relaxation data were analyzed by Peleg’s empirical method described as following:

Firstly, a parameter called decaying number $Y(t)$ was calculated as

$$Y(t) = \frac{F_0 - F(t)}{F_0} \qquad (1)$$

Where F_0 is the force recorded initially and $F(t)$ is the force recorded after t min at relaxation. Since during relaxation the deformation is maintained constant (and with very few exceptions the cross-sectional area of the specimen is also constant), the parameter $Y(t)$

(a) Texture analyzer



(b) Appearance of test

Fig.2-2 Photographs of texture analyzer (a) and the appearance of test (b)

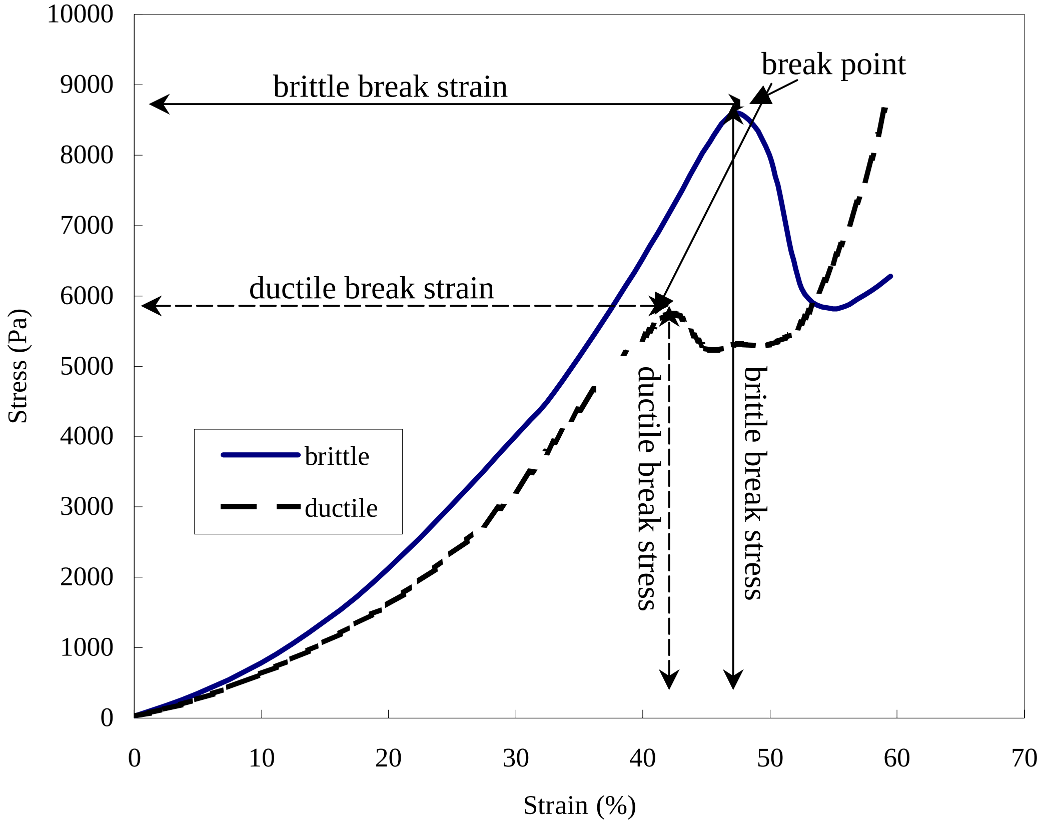


Fig. 2-4 Typical stress-strain curves of tofu (brittle break and ductile break)

represents the decay of force, stress or modulus in the same way. Being a dimensionless ratio it is also independent of the absolute stress level.

After the function *Y(t)* vs *t* was plotted, it was suggested that the relation can be expressed as following mathematical form:

$$Y(t) = \frac{abt}{1+bt} \qquad (2)$$

Here *a* and *b* are constants.

The meaning of *a* in the present context is to what level the stresses decay during relaxation. If $a = 0$ the stress does not relax at all (i.e. in an ideal elastic solid) and if $a = 1.0$, the stress level eventually reaches zero (e.g. in liquids). For $0<a<1$, a represents the asymptotic residual values of $Y(\infty)$. The constant *b* is the representative of the “rate” at which the stress relaxes

(1/b is the time necessary to reach the level of a/2). If $b = 0$, the stress does not relax at all (e.g. an ideal elastic solid). When viscoelastic solids are concerned the lower the value of b the slower the stress relaxation. In terms of the relaxation curve itself *a* higher b value expresses a steeper descent of the relaxation curve toward the residual value.

When plotted in the form as following, above formula will give a straight line.

$$\frac{t}{Y(t)} = \frac{1}{ab} + \frac{t}{a} \qquad (3)$$

Then values of a and b can be obtained.

2.2.5.3 Analysis of stress relaxation test by Maxwell and Vigot models

Stress relaxation curves were tried to fit with different models. Mohsenin (1986) summarized the rheological evaluation theory of physical properties of plant and animal materials in detail. The Maxwell model (Fig. 2-1 (a)), serialized by a Spring model and a Dashpot model, and Vigot model (Fig.2-1 (b)), paralleled with a Dashpot model and a Spring model, were used as primary units. The generalized Maxwell model (Fig. 2-1 (c)) can be expressed as equation 4, which consists of a single Spring model and Maxwell model numbered from 1 to n and the detailed derivation was shown by Mohsenin (1986).

$$E(t) = E_0 + \sum_{k=1}^{n} E_k e^{-\frac{t}{\tau_k}} \qquad (4)$$

Where t is time (s), $E(t)$ is apparent elasticity (Pa), E_0 is stress elasticity of the spring model (Pa), E_k is stress elasticity of Maxwell model number k (Pa), η_k is viscosity of Maxwell model number K (Pa・s), and $\tau_k = \eta_k/E_k$, is relaxation time of Maxwell model number k(s).

A ‘least squares’ non-linear algorithm in Sigmaplot®(Jandel Corporation, San Rafael, CA) was used to fit experimental data to Equation 4. The best fit was determined on the basis of the standard error and coefficient of variation of the parameters, the value of the regression coefficient, and by comparison of the residual value between the plots for the experimental and fitted data. The appropriate number of Maxwell elements was determined on the basis of the ‘dependence’ criterion for the non-linear algorithm in Sigmaplot (Jandel Corporation, 1995). After the model was determined, the parameters of the model were calculated with iterations from different initial values by the Sigmaplot software. A similar non-linear regression procedure was applied by Ojijo et al. (2000) for the analysis of stress relaxation data of soybean cotyledons.

2.2.5.4 Analysis of creep property

After creep curves of different tofu were recorded, they were fit by Maxwell and Vigot models or their combinations. The algorithm used for creep model fit was similar to that introduced for stress relaxation in *2.2.5.3*. The best fit was determined on the basis of the standard error and coefficient of variation of the parameters, the value of the regression coefficient, and by comparison of the residual value between the plots for the experimental and fitted data. The appropriate type of model was determined on the basis of the 'dependence' criterion for the non-linear algorithm in Sigmaplot (Jandel Corporation, 1995). After the model was determined, the parameters of the model were calculated with iterations from different initial values by the Sigmaplot software.

2.2.6 Scanning Electron Microscope (SEM) observation

SEM observation can help to clarify the relationship between microstructure and texture. SEM observation of Ca-tofu and GDL-tofu was done by many researchers (Saio, 1979; Takahashi, 1995). To understand the microstructure of Mg-tofu, SEM observation was conducted. For the pretreatment, method used by Takahashi (1995) and Lee et al. (2002) was utilized. A rectangular gel specimen was cut (1×1×2mm) and fixed in solutions of 2% glutaraldehyde and 1% osmium acid. The sample was then dehydrated by immersion in a series of ethanol mixtures of 50%, 70%, 90%, and 100% and finally immersed in isoamyl acetate. After dehydration, critical point drying was done in liquid CO_2 using a critical point dryer HCP-2 (Hitachi Koki Co. Ltd., Tokyo, Japan). The dried sample was carefully fractured into small pieces to reveal the internal microstructure. The fragments were mounted on an aluminum SEM stub with a small droplet of graphite paste and coated with platinum. All samples were then examined and photographed using a JSM-5600LV scanning electron microscope (JEOL Ltd., Tokyo, Japan).

2.3 Results and Discussion

2.3.1 Viscosity of soymilk with different concentrations

Viscosity is an important index for a liquid. The viscosity property of soymilk involves with the component. Thus, the change of viscosity could reflect the component changes at some extent. The viscosity of soymilk increased with increasing the concentration (Fig.2-5).

Since SPI was used in our study, protein was the major component of the solid content. A higher concentration means more protein content. Circle et al. (1964) reported that the increase of soymilk viscosity had an exponential relationship with the increase of soymilk concentration and the viscosity of soymilk highly concerned with the gelation. Fig. 2-5 showed a similar trend. On the other hand, in industrial practice, a too high viscosity will cause difficulties in transportation in pipes. Thus, the viscosity of soymilk should be controlled at a proper range.

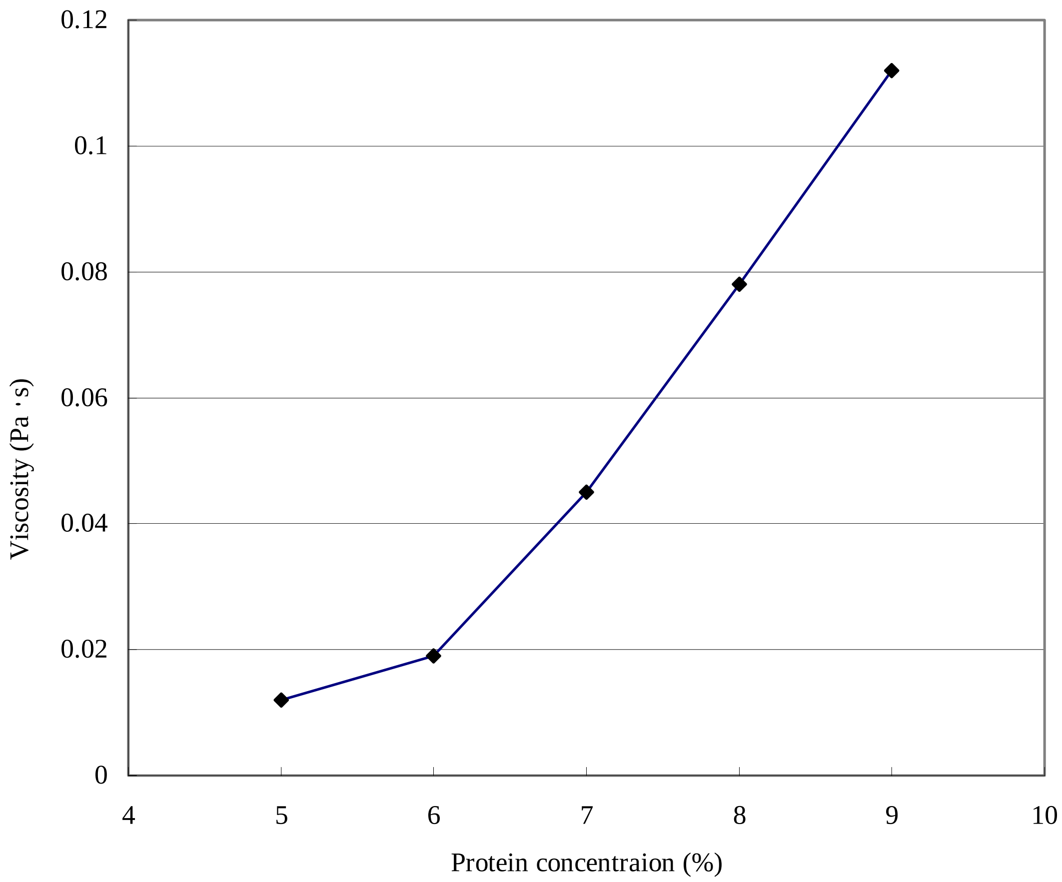


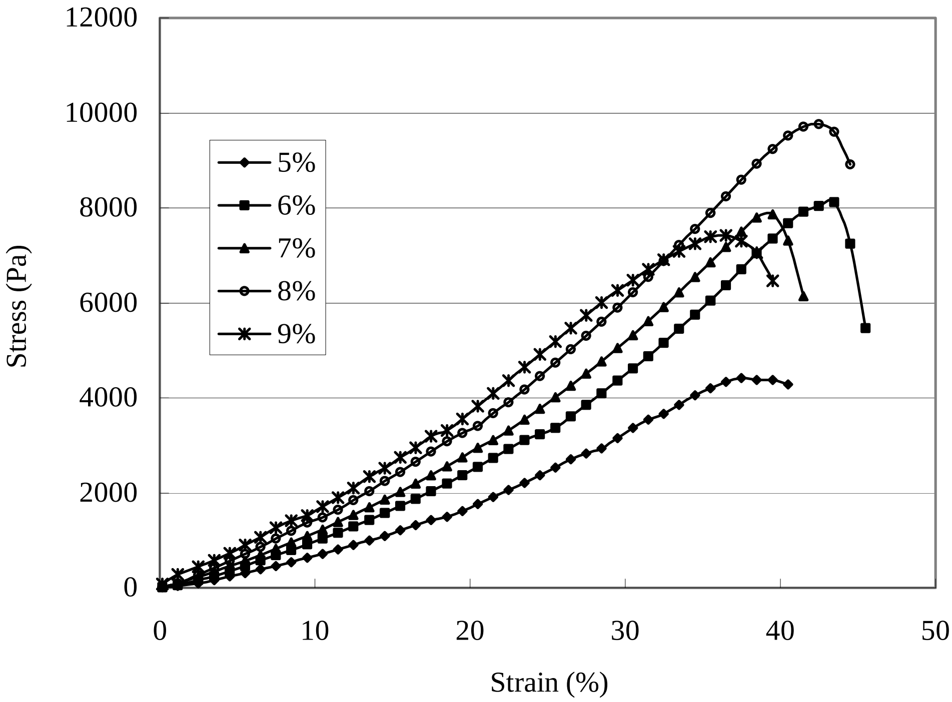
Fig.2-5 Effect of protein concentration on the viscosity of soymilk

2.3.2 Stress-strain behavior of tofu with different coagulants

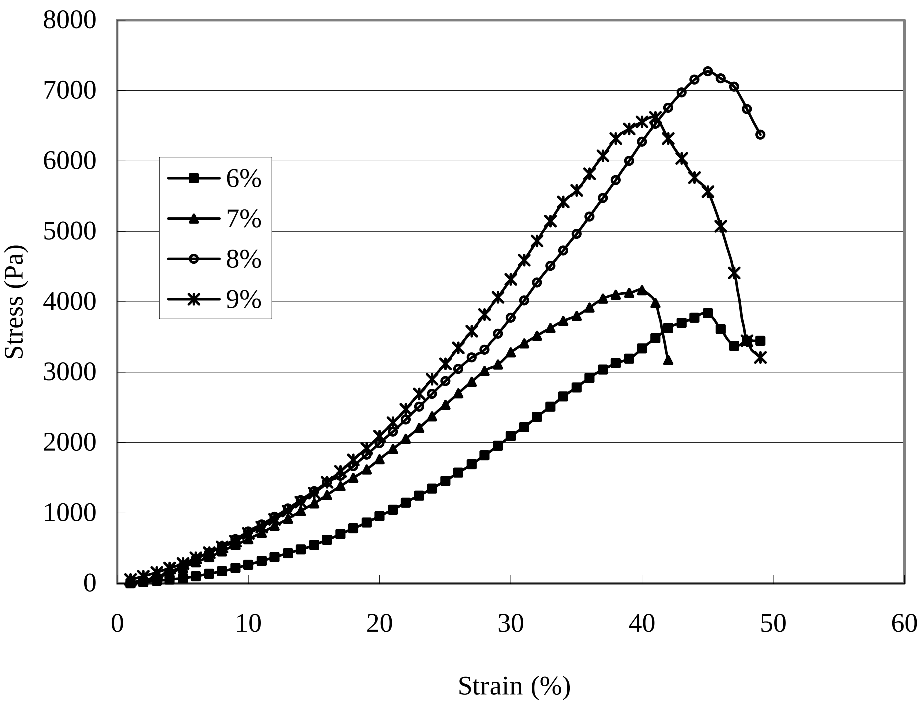
Based on the stress-strain curves, strain below 10% is similarly considered as a linear area and the Young's modulus could be known from the slope of the curve during this range. For both GDL-tofu and Ca-tofu, with the increase of soymilk concentration, Young's modulus increased (Fig. 2-6). For Ca-tofu, a good gel was not obtained for 5% soymilk and the stress-strain behavior was not shown. Except for the 9% soymilk, the break stress increased for both GDL-tofu and Ca-tofu (Fig. 2-6; Table 2-1). That is to say, increasing the soymilk concentration can obtain a harder tofu. This result corresponded to the previous report (Saio, 1979). The total amount of protein increased with increasing the soymilk content. For a high protein amount, the density of the network became higher and this resulted in a higher break stress. It was noted that the break stress (peak point of the stress-strain curve) value was not always consistent with the Young's modulus (Fig. 2-6). Break stress of 9% GDL-tofu and Ca-tofu decreased a little while Young's modulus increased. The contents of GDL and $CaSO_4$ were constant at 30 mM for all cases and the coagulant / protein ratio will decrease with increasing the protein amount. The break stress decrease for 9% GDL-tofu and Ca-tofu may be due to the decreasing coagulant / protein ratio. It might be that 30 mM GDL or $CaSO_4$ was not enough for 9% protein. Kohyama and Nishinari (1992) reported the effect of measurement temperature on the stress-strain properties of tofu and found that break stress was not consistent with the elastic modulus, which is in agreement with the results of this study. It also suggested that only break stress could not reflect the overall profile of the texture and some other complementary method would be necessary.

Table 2-1 Break stresses of Ca-tofu and GDL-tofu produced in the laboratory

Soymilk concentration (%)	Break stress (Pa)	
	GDL	$CaSO_4$
6%	6960	2960
7%	7950	5040
8%	8900	6960
9%	7430	6630



(a) GDL-tofu



(b) Ca-tofu

Fig. 2-6 Stress-strain behaviors of GDL-tofu (a) and Ca-tofu (b)

When at the same soymilk concentration, break stress of GDL-tofu was higher than that of Ca-tofu. This was also reported by other researcher (Saio, 1979). Because of the acidic taste of GDL-tofu, it is not preferred by many consumers (Cheng et al., 1999). But it is suggested that as a coagulant, GDL could provide a higher break stress. Thus, for improving the hardness of tofu, the mixing of GDL coagulant will be an alternative way. These results also indicated that concentration of soymilk and type of coagulant had a great influence on the texture of tofu gel.

The break stresses of Mg-tofu with different concentrations of soymilk and coagulants are provided in Table 2-2. Tofu with differing protein contents had lower stress at a relatively lower $MgCl_2$ concentration (15mM), suggesting that there was not enough coagulant.

Table 2-2 Break stresses (Pa) of Mg-tofu with different protein and coagulant concentrations[a]

Coagulant (mM)	15	20	25	30
6% protein	$2000^{a,(A)}$	$6910^{a,(B)}$	$5750^{a,(B)}$	$6160^{a,(B)}$
7% protein	$4800^{b,(A)}$	$9500^{b,(C)}$	$10700^{b,(D)}$	$7480^{b,(B)}$
8% protein	$4390^{b,(A)}$	$11300^{c,(B)}$	$14200^{c,(C)}$	$11000^{c,(B)}$
9% protein	$4660^{b,(A)}$	$12200^{c,(B)}$	$12400^{c,(B)}$	$12500^{c,(B)}$

[a]Expressed as means of 3 replicate. a-c: Means with different superscript within the same column are significantly ($p<0.05$) different. (A-D): Means with different superscript within the same row are significantly ($p<0.05$) different.

Increasing the concentration of coagulant (20, 25, 30mM) resulted in much greater break stress than that at 15 mM. The 30 mM Mg-tofu with 7% and 8% protein had a significantly lower break stress than the 25 mM Mg-tofu. The shapes of the stress-strain curves occasionally differed. The stress-strain curves of 20 mM Mg-tofu with 7% protein demonstrated a clear break point (brittle break), while the break point of 30 mM Mg-tofu was unclear and seemed to plateau (ductile break) (Fig. 2-7). The ductile break suggested that a good network had not formed in the 30mM Mg-tofu (7% protein). This indicates that 30 mM may be too high for 7% protein. Some other types of tofu in addition to 30 mM 7% protein tofu exhibited ductile breaks, such as 25 mM, 6% protein tofu and 30 mM, 6% tofu . The 20 mM tofu displayed a brittle break at all protein concentrations. Increasing the protein content of 20 mM tofu resulted in a significant increase in break stress. The range of the optimal concentration of $MgCl_2$ differed among various protein concentrations. For example, the shapes of the stress-strain curves and values of the break stresses indicated that 15 mM $MgCl_2$ was somewhat low for a 6% protein concentration while 25mM $MgCl_2$ was somewhat high; on the other hand, 25 mM appeared to be the optimal concentration for an 8% protein concentration. Increasing the $MgCl_2$ concentration from 25 mM to 30 mM at 8% protein resulted in a significant break stress decrease (Table 2-2). Suitable combinations of coagulants and protein concentrations can be summarized as 20 mM, 6-9%; 25 mM, 7-9%; 30 mM, 8-9% (Table 2-3). $MgCl_2$ exhibited a narrow optimal concentration range for tofu production. The optimal ranges for $CaSO_4$ and GDL are much wider (Watanabe, 1997). The narrow optimal addition range will cause handling difficulties in industrial Mg-tofu production. The reaction between $MgCl_2$ and the soy proteins was very rapid in this study, compared with $CaSO_4$ and GDL. The viscosity of soymilk increased suddenly after the addition of $MgCl_2$ (data not shown). This is why $MgCl_2$ is considered a quick-acting coagulant (Watanabe, 1997). The aggregate becomes too large to form a good gel when the reaction is too rapid. Uniform distribution of coagulant for a rapid reaction between the coagulant and proteins is also difficult in industrial production. There are few reports about $MgCl_2$, one of the major coagulants used in industrial practice. This may be due to its narrow optimal concentration range and the rapid reaction with protein, which make the process difficult. Liu and Chang (2003) studied a rapid titration method for predicting optimal coagulant concentration for filled tofu. They found that the optimal concentration of coagulant differed with soymilk concentration. This is in agreement with the results of this study.

Break stresses of Mg-tofu (20 mM), Ca-tofu (30 mM) and GDL-tofu (30 mM) were summarized in Fig. 2-8 to compare these 3 types of tofu. Generally speaking, Ca-tofu showed a lower break stress than the other two when at the same protein concentration.

Table 2-3 Combination of $MgCl_2$ and protein concentration for Mg-tofu production

$MgCl_2$	6% protein	7% protein	8% protein	9% protein
15 mM	×	×	×	×
20 mM	○	○	○	○
25 mM	×	○	○	○
30 mM	×	×	○	○

Note: ○ – suitable ; × - not suitable

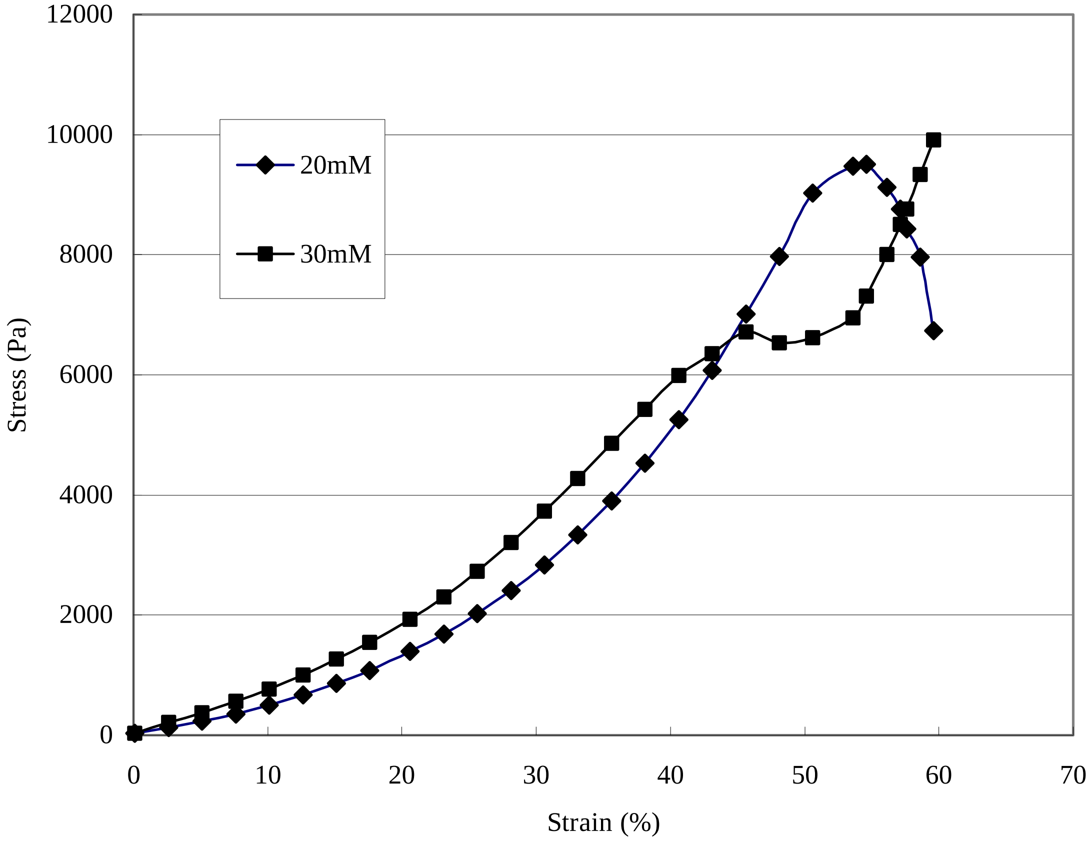


Fig.2-7 Stress-strain curves of 7% protein Mg-tofu with different coagulant concentrations

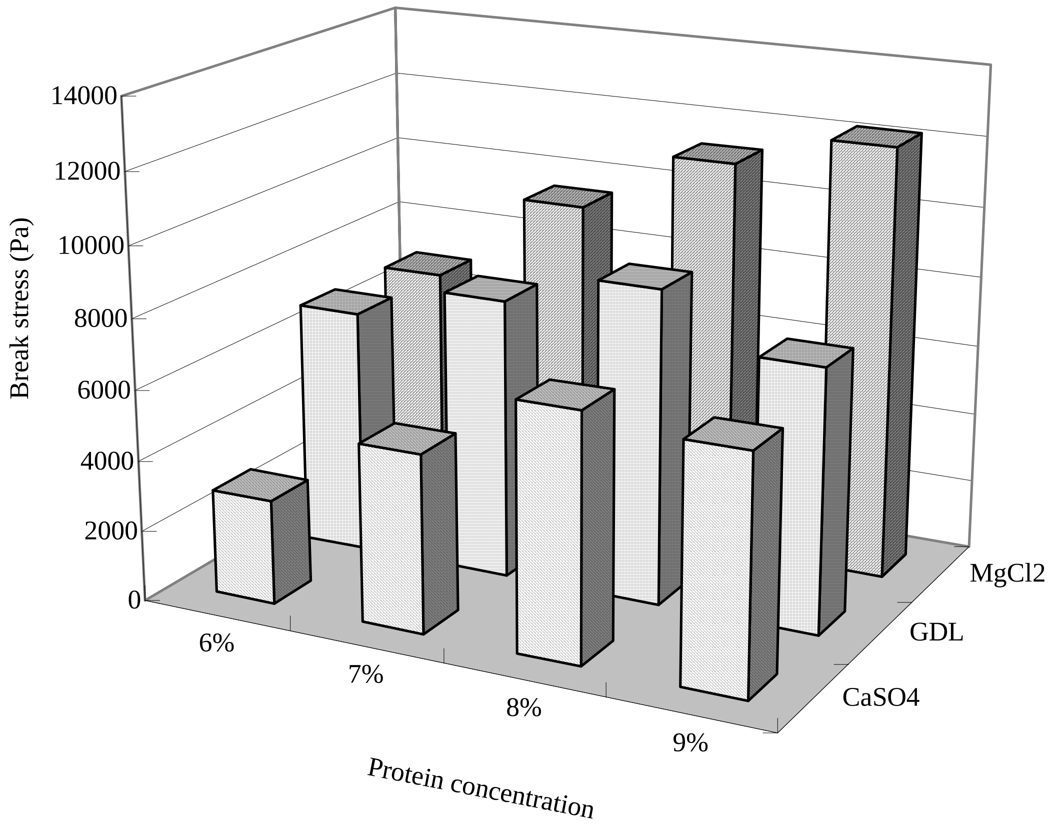


Fig. 2-8 Break stresses of Mg-tofu (20 mM), Ca-tofu (30 mM) and GDL-tofu (30 mM)

2.3.3 Stress relaxation behavior of tofu with different coagulants

2.3.2.1 Results of stress relaxation tests analyzed by Peleg's empirical method

Calculated and plotted the data based on Equation (1) and (3) in this chapter, results of *a*, *b* values at different protein concentration were shown in Table 2-4. It was shown both for

Table 2-4 Regression results of stress relaxation of Ca-tofu and GDL-tofu by Peleg's empirical method

	a		b	
coagulant	GDL	$CaSO_4$	GDL	$CaSO_4$
6% protein	0.956	0.864	0.034	0.048
7% protein	0.879	0.742	0.033	0.044
8% protein	0.821	0.697	0.031	0.043

Ca-tofu and GDL-tofu, values of *a* and *b* decreased with increasing soymilk concentration. As described earlier, the lower the value of *a*, the material showed more elastic property and the lower the value of b the slower the stress relaxation. For the decrease of values of *a* and *b* with the increasing of soymilk concentration, it was suggested that tofu showed more elastic property and the stress relaxation became slower with increasing soymilk concentration. Lee et al. (1983) investigated the characteristics of four types commercial tofu using this empirical method. They compared the results of compressed and uncompressed tofu. However, the parameters determined by this method seemed insufficient to establish that in previously compressed tofu some matrix reinforcement may also occur. In this work, all the tofu were uncompressed type and when using the same coagulant, the changes of *a* and *b* values can reflect the changes of texture at some extent. However, when comparing Ca-tofu with GDL-tofu, although it was shown that break stress of GDL-tofu was higher than Ca-tofu when at the same soymilk concentration (Table 2-1), GDL-tofu showed a higher *a* and *b* values when the stress relaxation curve was analyzed by Peleg's empirical method.

Theoretically, *a* and *b* values of GDL-tofu should be lower than Ca-tofu, but the results were opposite. It seemed that using this empirical method was not suitable to evaluate tofu with different coagulants. Further, although *a* and *b* values can show the trend of elastic property and stress relaxation, they cannot reflect the quantity changes of elastic and viscous index. As tofu is a viscoelastic material, understanding of both elastic and viscous changes of tofu is expected. For these reasons, it can be concluded that the employment of this empirical method is not a suitable method for the texture evaluation of tofu. Analysis of stress relaxation property by viscoelastic models is tried to evaluate the texture of tofu.

2.3.2.2 Results of stress relaxation tests analyzed by Maxwell and Vigot models

Stress relaxation histories of GDL-tofu made from different concentration soymilks are

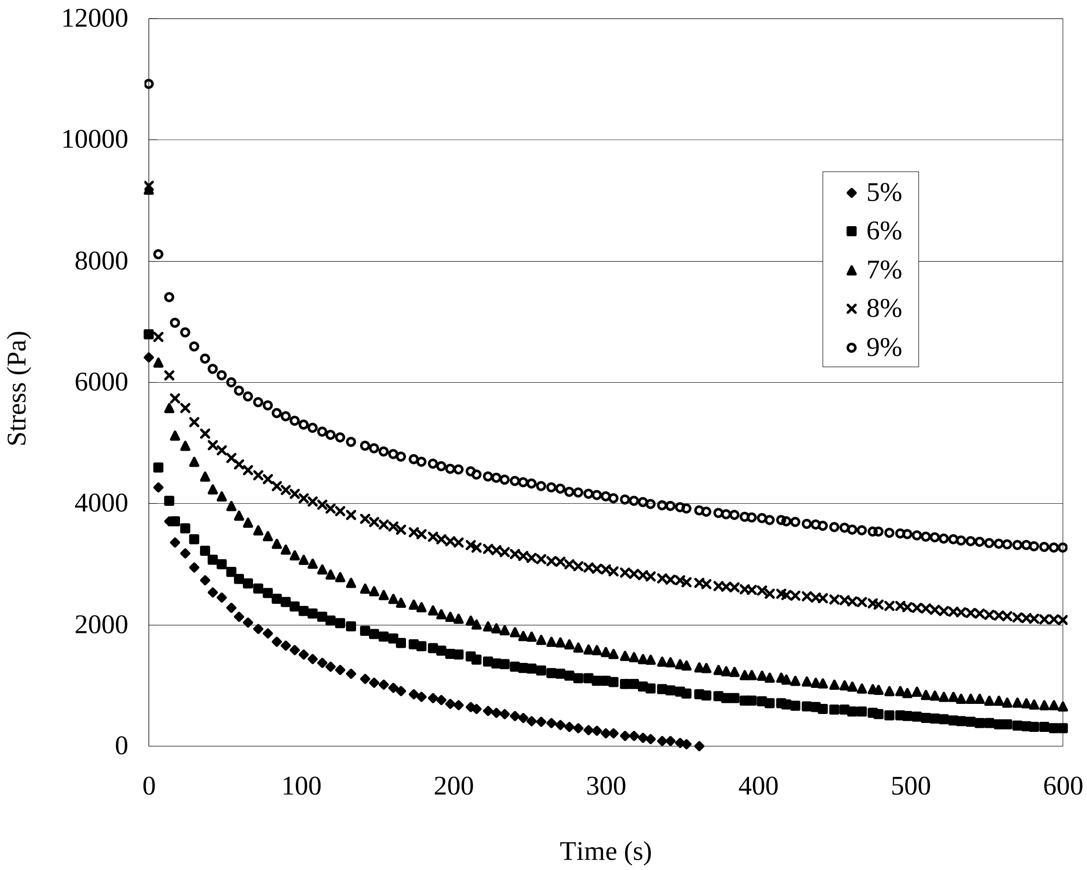


Fig.2-9 Stress relaxation histories of GDL-tofu with different protein concentrations

shown in Fig. 2-9. With the increase of soymilk concentration, stress relaxation became slower. It did not reach to equivalent stress during 600 s for all cases (even for 1200 s, it did not reach to equivalent stress). It was implied that E_0 does not exist for the generalized

Maxwell model (Fig.2-1 (c)). A similar pattern was obtained for the samples of Ca-tofu. Thus, a model with 2 or 3 Maxwell elements (4 or 6-element model) could be considered. In common cases, about 90% content of tofu is water. When it is subjected to stress relaxation test, water will play an important role, which makes tofu show a viscous behavior at some extent as well as the elastic behavior that was mainly influenced by network formed by proteins. In this study, the solid content of soymilk is about 1.1 times of the protein concentration and water content can be easily calculated from the protein concentration. After calculating by a 'least squares' non-linear algorithm in Sigmaplot®, it was found the 4-element Maxwell model (Fig. 2-1(d)) could fit for the stress relaxation behaviors of tofu. The minimum objective function obtained by the algorithm was ensured to be a global convergence by repeating the iterations using different initial model parameters values (Van Boekel, 1996). Data for all the cases could be adequately fitted by the 4-element model. Burger's model is commonly used for the evaluation of static viscoelasticity (Senoh et al., 2000). In this study, fitting of the experimental data with a best fitting model is considered and the four-element Maxwell model was used. The relationship between apparent elasticity and viscous and elastic parameters can be expressed as following (Equation 5) (Kawabata, 1989):

$$E(t) = E_1 e^{-\frac{t}{\tau_1}} + E_2 e^{-\frac{t}{\tau_2}} \qquad (5)$$

Where *E(t)* is apparent elasticity, *t* is time (s), E_1, E_2 are elasticity of 2 Maxwell models, and $\tau_i = \eta_i/E_i$(i=1,2), is stress relaxation time(s).

Comparison between experimental data and regression data by the 4-element Maxwell model was shown in Fig.2-10. Related coefficient R^2 was 0.996. For analysis by models, when a simpler model could fit the data well, the simpler one will be adopted for it is easier for calculation and number of parameters will be less. In this study, for 4-element model showed a high enough related coefficient, although 6-element Maxwell model could also fit the experimental data very well (R^2=0.999), the 4-element Maxwell model was used. After the model was determined, parameters for models were calculated and given by the 'least squares' non-linear algorithm in Sigmaplot®(Jandel Corporation, San Rafael, CA). The best fit was determined on the basis of the standard error and coefficient of variation of the parameters, the value of the regression coefficient, and by comparison of the residual value between the plots for the experimental and fitted data. Results were showed in Tables 2-5, 2-6. Both for the GDL-tofu and Ca-tofu, when the soymilk concentration increased, elastic parameters E_1 and E_2 had a tendency to increase, but did not change very much, while viscous parts η_1 and η_2 increased significantly (Tables 2-5, 2-6). Similarly, relaxation times τ_1, τ_2 also increased with the soymilk concentration (Tables 2-5, 2-6), which suggested the tendency that the relaxation became slower. It correlated with the stress relaxation histories (Fig.2-9). These results indicated that tofu structure became firmer as the soymilk concentration increased. All of these changes of parameters are consistent with the stress-strain behaviors and are more detailed. Furukawa and Ohta (1982) reported that relaxation time of soy protein gel was dependent on the network formation, while the modulus of elasticity was hardly affected. Shimoyamada et al. (1999) also showed that the gel strength of the freeze-gel formed from soymilk was related to the viscosity of the soymilk before freezing. The viscosity of soymilk increased with increasing protein concentration (Fig.2-5). In our study, it also seemed that the viscous parameters were more involved with the hardness of tofu than elastic parameters.

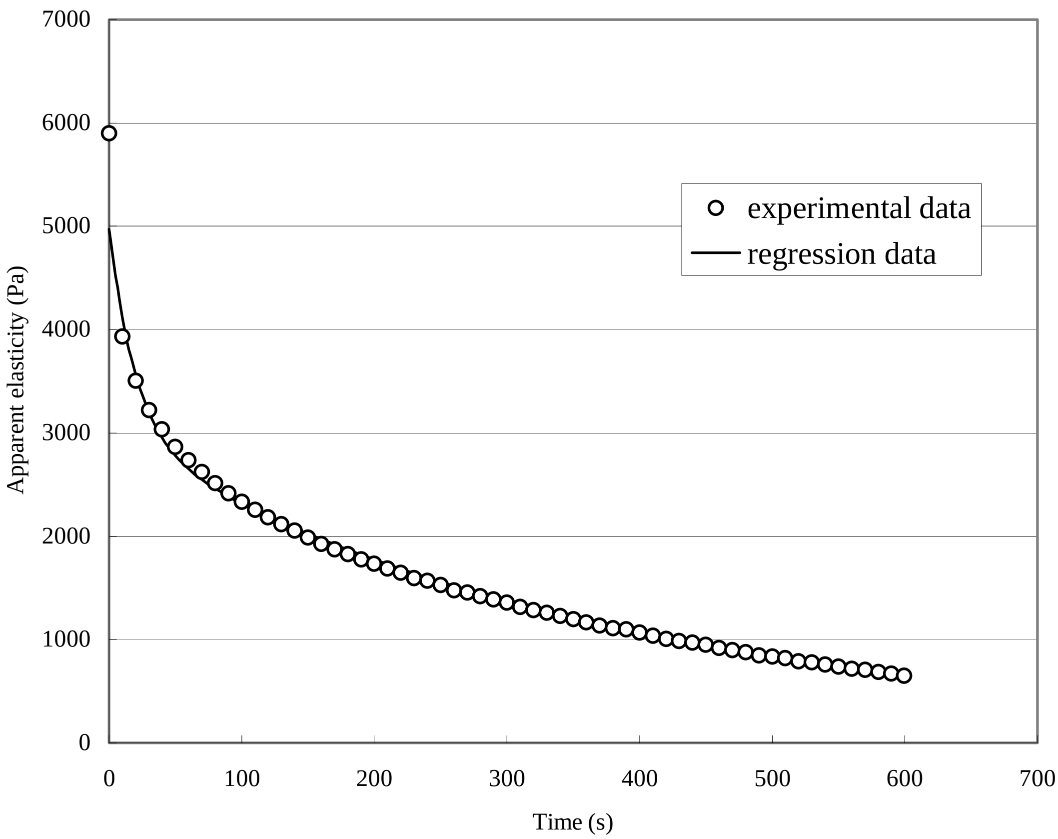


Fig.2-10 Comparison between experimental data and regression data by 4-element Maxwell model of stress relaxation of tofu

Although break stress of GDL-tofu was much higher than Ca-tofu at the same soymilk concentration, stress relaxation results showed that elastic parameters did not change too much, while the viscous parameters and the relaxation times of GDL-tofu were smaller (Tables 2-5, 2-6). This might be because GDL-tofu was more fragile and less cohesive than Ca-tofu (Saio, 1979). The network of GDL-tofu consisted of flocculent aggregates, and that of Ca-tofu showed a spongy structure with tight frames (Saio, 1979). It was suggested that the network of tofu gel may be formed via hydrogen bonding, hydrophobic associations, ionic interactions and electrostatic cross-links, and also through some sulphydryl-disulphide linkages of unfolded polypeptides (Catsimpools et al., 1970; Utsumi and Kinsella, 1985). Compared with Ca-tofu, GDL–tofu was harder to penetrate but had a lower inner hardness and was more fragile and less cohesive than Ca-tofu. The Ca-tofu has a Ca-bridge, which may also have a role (Saio, 1979). It was indicated that both large (stress-strain) and small deformation (stress relaxation) behaviors depended greatly on the soymilk property and the type of coagulant.

Table 2-5 Parameters of 4-element Maxwell model for stress relaxation of GDL-tofu

Soymilk concentration	5%	6%	7%	8%	9%
E_1 (10^3 Pa)	3.8	3.4	4.2	4.5	5.6
E_2 (10^3 Pa)	2.1	2.5	3.3	3.1	3.4
η_1 (10^6 Pa•s)	0.43	0.88	1.3	3.2	5.8
η_2 (10^4 Pa•s)	1.4	3.1	7.3	8.7	10.2
τ_1(s)	115	255	309	713	1026
τ_2(s)	7	13	22	28	30

Table 2-6 Parameters of 4-element Maxwell model for stress relaxation of Ca-tofu

Soymilk concentration	6%	7%	8%	9%
E_1 (10^3 Pa)	3.2	4.2	4.5	4.0
E_2 (10^3 Pa)	2.0	3.3	3.1	2.2
η_1 (10^6 Pa•s)	1.1	1.8	3.5	4.1
η_2 (10^4 Pa•s)	3.4	5.3	5.7	7.0
τ_1 (s)	341	502	810	1021
τ_2 (s)	17	19	30	32

The results of the stress-strain properties of Mg-tofu indicated that only 20mM $MgCl_2$ exhibited a brittle break at all protein concentrations. Thus, the stress relaxation test results of 20mM tofu with different protein concentrations are reported here. The stress relaxation curves of 20 mM Mg-tofu with different protein contents are depicted in Fig. 2-11. The apparent elasticity decreased with time. It did not reach an equivalent stress even after 600 s. It was revealed that the four-element Maxwell model (Fig. 2-1(d)) fits the stress relaxation behavior of Mg-tofu. It was shown previously that the stress-relaxation curve of Ca-tofu and GDL-tofu could also be fit by the same 4-element Maxwell model.

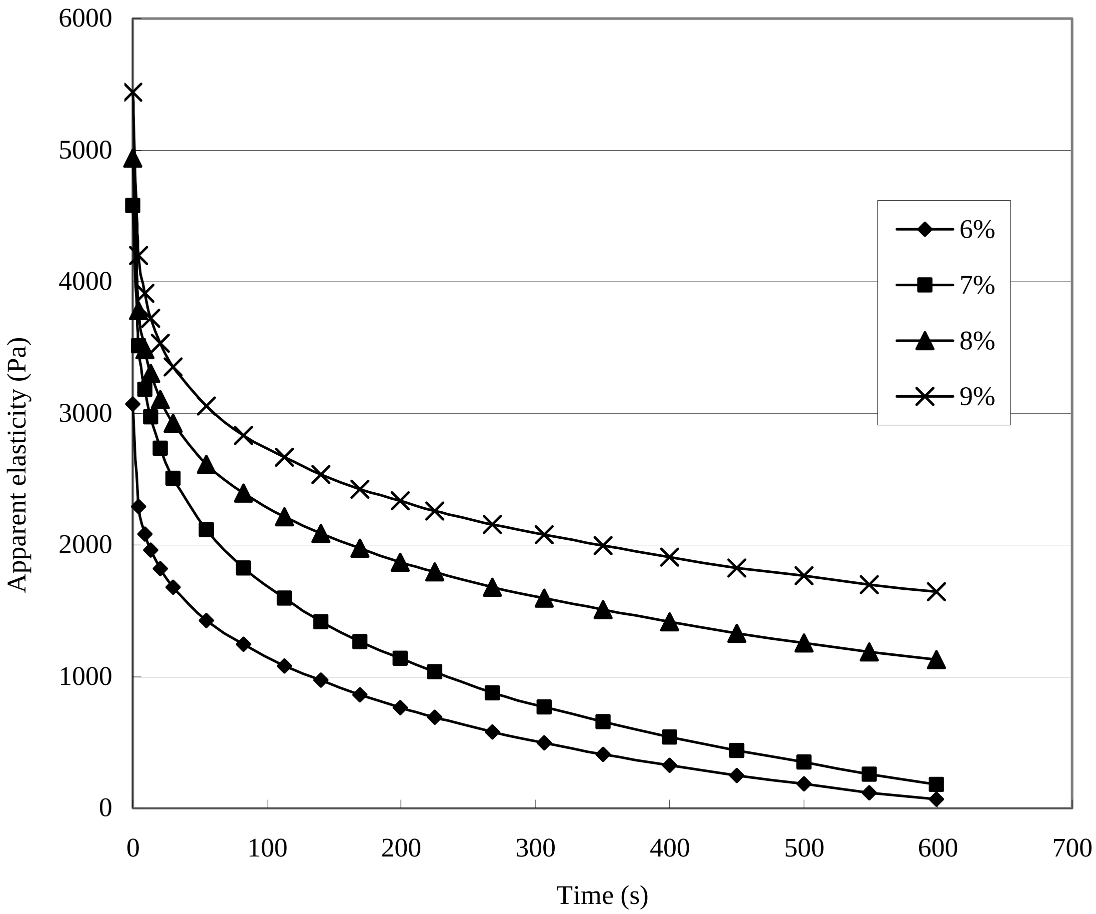


Fig. 2-11 Stress relaxation curves of 20 mM Mg-tofu with different protein

The parameters were calculated after determining the models. The parameters of the 20 mM Mg-tofu with different protein concentrations are provided in Table 2-7. All the parameters tended to increase as the protein content increased from 6% to 9%, in particular η and the relaxation times. Relaxation times τ_1 and τ_2 increased from 227s to 1024s and from

10s to 34s. The stress-relaxation parameters obtained correlated well with the strength and the degree of cross-linking of the network (Tang et al., 1998). A longer relaxation time for a foodstuff indicates slower stress relaxation and a firmer material structure (Mohsenin, 1986). This suggests that a firmer tofu structure is produced by increasing the protein content of soymilk. While elastic moduli E_1 and E_2 also tended to increase, this increase was not substantial, particularly among the 7%, 8%, and 9% samples. The increase in relaxation times in this study may also reflect the structure of the tofu gel becoming firmer with the increased protein content. Stress relaxation tests for 25 mM Mg-tofu (7, 8, 9% proteins) were also conducted (Table 2-7). The stress relaxation of 6% protein tofu was not performed due to its ductile break. The parameters of 25 mM Mg-tofu exhibited a trend similar to that of 20 mM Mg-tofu, i.e., the relaxation times increased with an increase in the protein concentration. A comparison with the parameters of stress relaxation from 20 mM and 25 mM tofu indicated that the elastic parameters E_1 and E_2 of 25 mM Mg-tofu were greater than those of 20 mM Mg-tofu. There were no significant differences between the viscous parameters. Changes in the amount of coagulant appeared to primarily influence the elastic parameters of stress relaxation.

Table 2-7 Parameters of 4-element Maxwell model for stress relaxation property of 20 mM and 25 mM Mg-tofu

Coagulant	20 mM $MgCl_2$				25 mM $MgCl_2$		
Soymilk concentration (%)	6	7	8	9	7	8	9
E_1 (10^3 Pa)	1.8	2.5	2.5	2.9	3.1	3.4	3.7
E_2 (10^3 Pa)	0.9	1.5	1.5	1.6	2.1	2.2	2.5
η_1 (10^6 Pa・s)	0.41	0.64	1.8	2.9	0.75	1.7	2.3
η_2 (10^4Pa・s)	0.93	2.3	4.5	5.4	3.1	5.7	6.9
τ_1 (s)	227	253	707	1024	240	509	616
τ_2 (s)	10	15	30	34	14	26	27

2.3.4 Creep behavior of tofu with different coagulants

For texture evaluation, both large and small deformations can be used. Large deformation refers to compression test, while small deformation concludes stress relaxation, creep and dynamic viscoelastic tests. Creep property is also very important for tofu. Compliance (the opposite of elasticity) was used as an index for creep property in this study. A typical creep curve is shown in Fig. 2-12. The compliance increased with time. It increased quickly at the initial stage and the increase became slower at the latter stage. After unloading, a constant deformation was left, which showed that tofu was a viscoelastic material. At the start point, the sudden increase reflected the existence of constant elasticity. From the characteristics of the creep curve, a 4-element model could be considered. It was found that a 4-element Burgers model (Mohsenin, 1986) could fit the curve. The model is shown in Fig. 2-1 (e). The relationship between compliance and the viscoelastic parameters could be expressed as the following (Kawabata, 1982):

$$J(t) = \frac{1}{E_n} + \frac{1}{E_1}(1 - e^{-\frac{t}{\tau_{ret1}}}) + \frac{t}{\eta_n} \qquad (6)$$

Where, $J(t)=1/E(t)$ is compliance (Pa^{-1}); E_n is constant elasticity (Pa); E_1 is elastic parameter of the Vigot model (Pa); η_n is constant viscosity; τ_{ret1} is retardation time (s), = η_1/E_1 (η_1, viscous parameter of the Vigot model).

Creep behaviors of Ca-tofu produced from 6-9% soymilk are shown in Fig. 2-13. A higher concentration tofu showed lower compliance, which meant a smaller deformation. When at the same measure condition, a smaller deformation reflected the firmer structure of material. Based on the property of creep behavior, it was indicated that a higher protein concentration resulted in a firmer structure of tofu, which is consistent with the stress-true strain results. Soy protein played an important role in the gel formation. For the high protein amount, the density of the network became higher and this resulted in higher break stress.

Creep parameters of the 4-elelement Burgers model of Ca-tofu and GDL-tofu were obtained (Tables 2-8, 2-9). It was shown that constant viscosity η_n increased with increasing the concentration of protein, but not always the same for E_n, especially for GDL-tofu. For both Ca-tofu and GDL-tofu, the retardation time (τ_{ret1}) deceased with increasing the protein concentration. The changes of both η_n and τ_{ret1} reflected the formation of a firmer structure with the increase of protein concentration. Same as stress relaxation parameters in Tables 2-5, 2-6, creep parameters obtained from the models could reflect both the elastic and viscous changes of the gel, which could help to get more information for hard tofu production in

China because hard tofu which has an elastic structure would be expected. Furthermore, although both large deformation properties (stress-strain test) and small deformation properties (stress relaxation, creep, dynamic viscoelastic tests) could reflect the texture of tofu, they are not always consistent (Kuwahata and Nakahama, 1975). To understand the overall properties of tofu, both large and small deformation tests are expected.

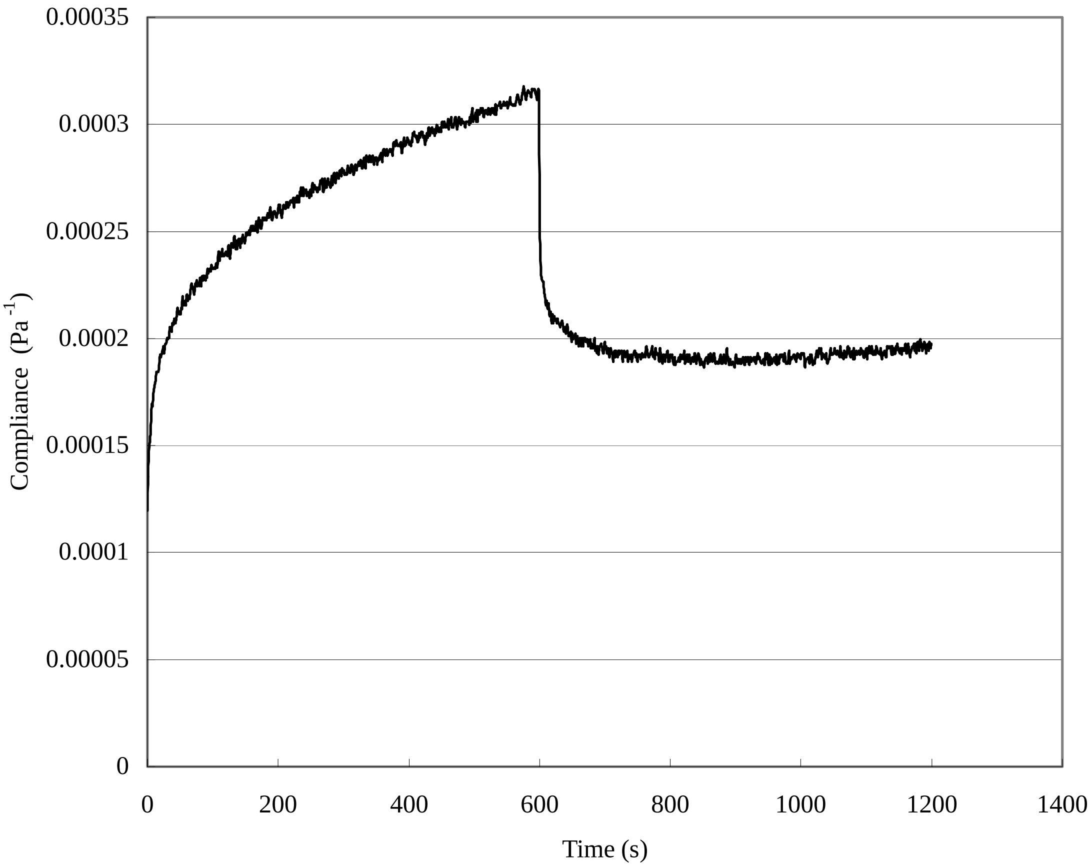


Fig. 2-12 Typical creep curve of tofu

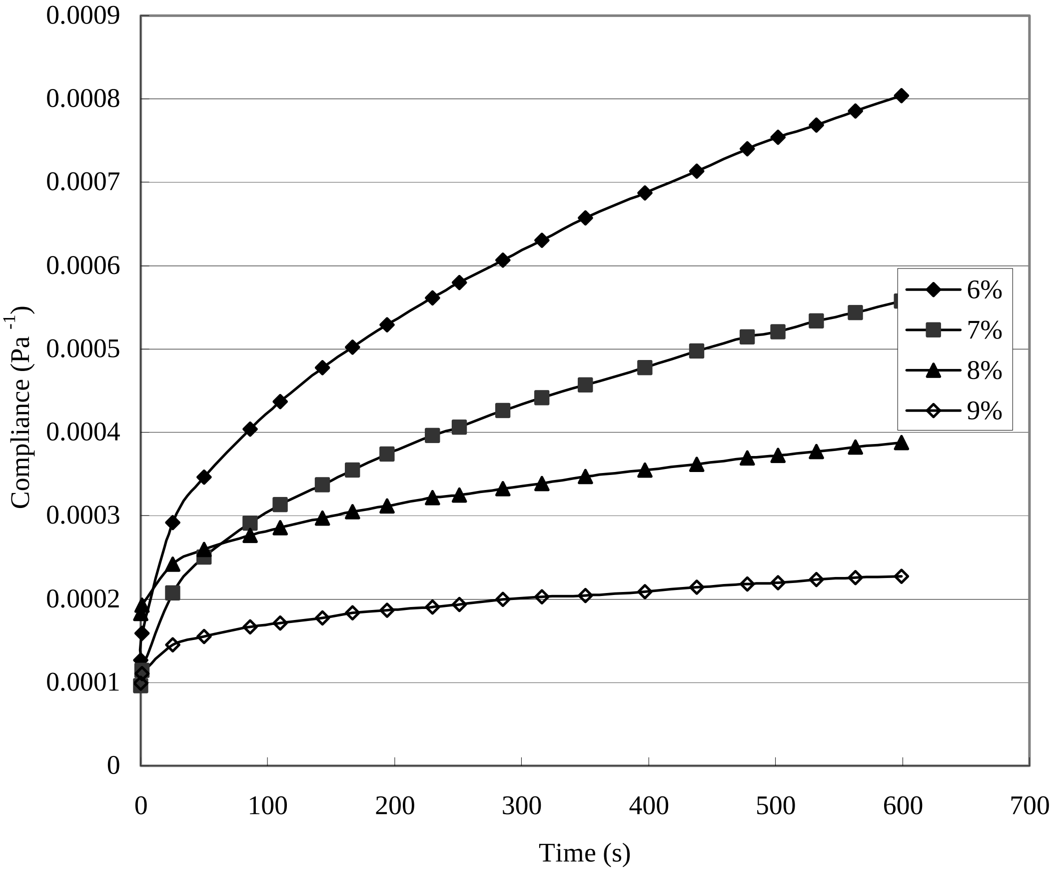


Fig.2-13 Creep properties of Ca-tofu with different protein concentrations

Table 2-8 Parameters of 4-element Burgers model for creep of Ca-tofu

soymilk concentration.	6%	7%	8%	9%
E_n (10^3 Pa)	5.7	4.4	7.7	4.5
E_1 (10^3 Pa)	5.6	7.6	7.4	8.5
η_1 (10^6 Pa•s)	0.38	0.26	0.10	0.16
η_n (10^6 Pa•s)	1.9	2.2	4.5	4.3
τ_{ret1} (s)	68	34	13	19

Table 2-9 Parameters of 4-element Burgers model for creep of GDL-tofu

Soymilk concentration	5%	6%	7%	8%	9%
E_n (10^3 Pa)	5.0	8.3	8.7	5.2	6.1
E_1 (10^3 Pa)	6.4	9.4	14.8	17.7	17.2
η_1 (10^6 Pa•s)	0.79	0.70	1.0	0.87	0.72
η_n (10^6 Pa•s)	1.5	2.6	3.8	6.9	8.9
τ_{ret1} (s)	124	75	68	50	42

2.3.5 Relationship between parameters from stress relaxation test and those from creep test

It was shown that curve of stress relaxation could be fit by a 4-element Maxwell model (Fig. 2-1(d)) and creep curves could be fit by another 4-element model (Fig. 2-1(e)). To distinguish parameters from these two 4-eleement models, they were shown in Fig.2-14 as a comparable style. Because tests were conducted to the same material – tofu, these two 4-element models should have a close relation. Theoretically, they can be converted with each other. Isozaki et al. (1976) analyzed the viscoelasticity of hydrogels of agar. They measured the stress relaxation and creep of agar gels as well as egg white and soybean gels. It was shown that stress relaxation and creep curves of all gels could be approximated by a 6-element Maxwell model (M-model) and 6-element Vigot model (V-model) in their study. They also determined the transformation formula for viscoelastic constants from the 6-element M-model and 6-element V-model. However, not all of the calculated constants and experiments results corresponded well. For 4-element M-model and V-model in this study (Fig. 2-14), the transformation formula was calculated and results were as following:

For 4-element M-model

$$\frac{\sigma}{\varepsilon} = (E_1 + E_2)p^2 + E_1 E_2 (\frac{1}{\eta_1} + \frac{1}{\eta_2}) / p^2 + (\frac{E_1}{\eta_1} + \frac{E_2}{\eta_2})p + \frac{E_1 E_2}{\eta_1 \eta_2}$$

For 4-element V-model

$$\frac{\sigma}{\varepsilon} = E' p^2 + \frac{E' E_1'}{\eta_1'} / p^2 + \frac{E'}{\eta_1'}(1 + \frac{E_1'}{E'} + \frac{\eta_1'}{\eta'})p + \frac{E' E_1'}{\eta' \eta_1'}$$

Here, $p = d/dt$, E_1, E_2, η_1 and η_2 are elastic and viscous parameters for M-model and E', E_1', η_1' and η' are parameters for 4-element V-model. After comparing these 2 formulas, following results were obtained.

$$E' = E_1 + E_2$$

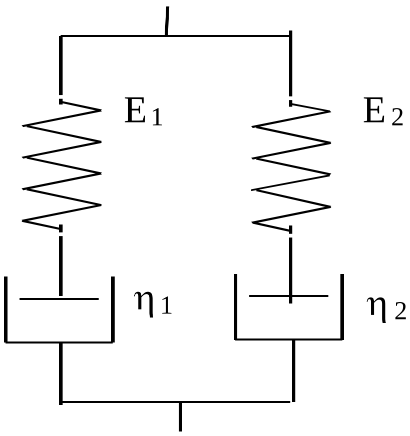
$$E_1' = \frac{E_1 E_2 (E_1 + E_2)(\eta_1 + \eta_2)^2}{(E_1 \eta_2 - E_2 \eta_1)^2}$$

$$\eta_1' = \frac{\eta_1 \eta_2 (\eta_1 + \eta_2)(E_1 + E_2)^2}{(E_1 \eta_2 - E_2 \eta_1)^2}$$

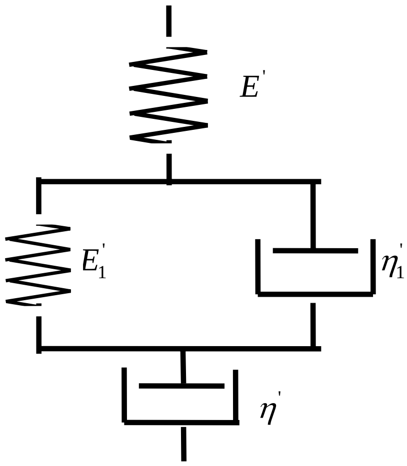
$$\eta' = \eta_1 + \eta_2$$

Parameters of stress relaxation and creep can be theoretically converted with each other. For example, after obtaining parameters from stress relaxation test, parameters of creep test can be calculated. However, when comparing the calculated data with experimental results (Tables 2-5, 2-6, 2-8, 2-9), they were not always consistent. In practice, for the equipment of creep test, it is very important to give an instant load and then keep it at a constant. It is difficult to realize it by the equipment in fact. This may be the main reason for the fact that calculated data is not always consistent with experimental data. For stress relaxation, it is easier for equipment to keep a constant strain and easier to be stably realized.

Stated as above, it was shown the stress relaxation and creep tests were effective for evaluating the texture of tofu and this method could reflect the changes of viscous and elastic parts, respectively. Furthermore, it is a kind of small deformation method and can be combined with large deformation method – stress-strain test. Based on this result, tofu evaluation in the rest of this study was done by using this method and mainly stress relaxation results will be reported.



(a) 4-element M-model



(b) 4-element V-model

Fig. 2-14 4-element Maxwell model for stress relaxation curves (a) and 4-element Vigot model (Burgers model) for creep curves of tofu (b)

In the viscoelastic tests, dynamic viscoelasitic test is also effective approach to elucidate the rheological properties of foodstuff. Nagano et al. (1994a, b, c) studied the dynamic viscoelastic properties of glycinin (11S) and β-conglycinin (7S) gel from soybeans. They found that their results support the idea that heat-induced gels are formed by cross-links with the intermolecular β-sheet structure in globular proteins. Kohyama et al. (1993) studied the gelation process of 7S and 11S soy proteins in the presence of GDL by using dynamic viscoelastic and compression tests. They found that the rate of gelation for 7S was much slower and the gelation time was longer than for 11S. The contents and ratio of 7S and 11S proteins played an important role in GDL-tofu. In this study, the same material was used, so mainly the content of protein took influence on the gelation of tofu.

2.3.6 Results of SEM observation

Electron microscopy has long been recognized as a valuable tool for relating the detailed structures of foods to properties such as texture. Saio (1979) discussed the relationship between texture and fine structure of tofu. SEM observations of Ca-tofu and GDL-tofu were conducted in this literature (Fig. 2-15). Based on the SEM observations of GDL and Ca-tofu, the author (Saio, 1979) found that the network of GDL-tofu consisted of flocculent aggregates, and that of Ca-tofu showed a spongy structure with tight flames.

Fig.2-15 SEM photograph of GDL-tofu (A) and Ca-tofu (B) (Saio, 1979). Tofu was frozen in liquid nitrogen and observed under a cryounit fitted to JSM 50-A

In this study, SEM observation of Mg-tofu was conducted. It was stated earlier that 30mM $MgCl_2$ appeared to be somewhat high for 6% Mg-tofu and might be more suitable for 8% protein Mg-tofu. SEM observations were performed to compare their structures. Figure 2-16 indicates that the pore size of 6% protein tofu (Fig. 2-16 (a)) was larger than that of 8% tofu (Fig. 2-16 (b)). The network formation of tofu gel involves denaturing of the protein, aggregation of the protein with a coagulant, and formation of a network (Kohyama et al., 1995; Utsumi & Kinsella, 1985). When at the same coagulant concentration, a higher ratio of coagulant/protein for 6% Mg-tofu than 8% caused a quicker reaction between the coagulant and soy proteins and resulted in a larger pore size in the network. This influences suitable network formation. The reaction of 8% protein with coagulant is slower than that with 6% protein. A slower reaction (8%) produces a denser network and results in a smaller pore size (Fig. 2-16). The break stress of 8% 30 mM Mg-tofu was greater than that at 6% (11000 Pa and 6160 Pa, see Table 2-2). The denser network and smaller pore size of 8% 30mM Mg-tofu may contribute to this effect.

A SEM observation of 25 mM $MgCl_2$ tofu with 8% protein was also conducted (Fig. 2-16 (c)). It revealed a similar structure to that of 30 mM $MgCl_2$, 8% protein tofu. Both of these types of tofu exhibited brittle break, which reflected suitable tofu gel formation. Consequently, their microstructures were similar in SEM observations.

2.4 Conclusions

In this chapter, texture evaluation methods of tofu were discussed and texture of Mg-tofu, Ca-tofu and GDL-tofu were investigated and compared. Following conclusions could be drawn:

(1) Viscosity of soymilk increased with increasing the concentration.

(2) Break stresses of Mg-tofu and GDL-tofu were higher than Ca-tofu when at the same protein concentration.

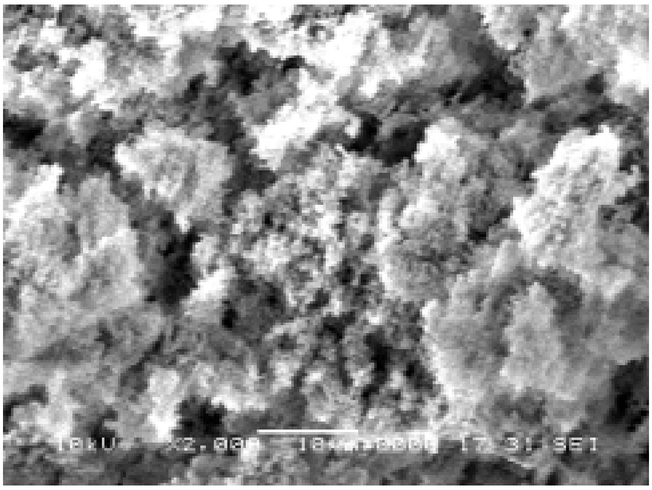
(3) Peleg’s empirical method for evaluating the stress relaxation behavior is thought to be not suitable for the texture evaluation of tofu. The results obtained from this method could not reflect both elastic and viscous changes of tofu and the comparison between tofu with different coagulants was difficult.

(4) Evaluation method by using Maxwell and Vigot models as primary models was effective for evaluation of tofu texture. Stress relaxation behavior of tofu was found to be fit for a 4-element Maxwell model. Creep property of tofu could be fit by a 4-element Burgers

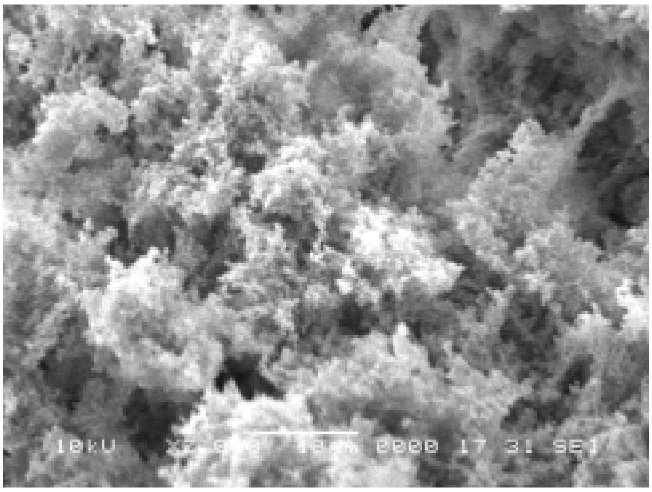
model. Parameters obtained from the model can reflect both elastic and viscous changes of tofu. For Mg-tofu, Ca-tofu and GDL-tofu, with increasing the protein concentration, viscous parameters, relaxation time of stress relaxation and constant viscous parameter η_n of creep behavior showed a consistent increase. It seemed viscous parameters obtained from small deformation test have a more consequent relationship with the break stress obtained from large deformation test.

(5) Parameters obtained from stress relaxation and creep tests can be theoretically converted with each other. However, when comparing the calculated data with experimental results, they were not always consistent.

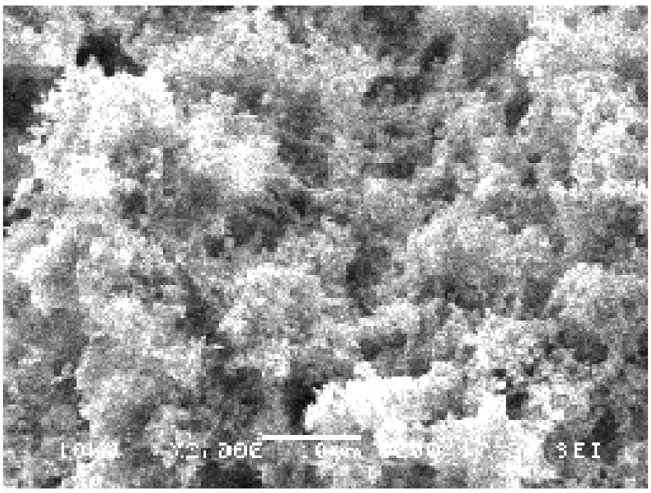
(6) The optimal concentration of $MgCl_2$ differed with different protein concentration. The suitable combinations of optimal concentrations of coagulant and protein were: 20 mM, 6-9%; 25 mM, 7-9%; 30 mM, 8-9%. $MgCl_2$ showed a narrow range of optimal concentration and it reacted rapidly with protein when added into soymilk comparing with $CaSO_4$ and GDL.



(a)



(b)



(c)

×2000 10 μm

Fig.2-16 SEM photograph (×2000) of Mg-tofu. 30mM $MgCl_2$, 6% protein (a); 30mM $MgCl_2$, 8% protein (b) and 25 mM $MgCl_2$, 8% protein (c)

Chapter 3 Texture of commercial tofu in local market of China

3.1 Introduction

In Chapter 2, an effective method for evaluation of texture of tofu was determined and effects of coagulants on the texture of tofu were investigated. To improve tofu production in China, it is necessary to understand the situation of tofu being distributed in local market in China. The analysis method of employing Maxwell model and Vigot model for stress relaxation was found to be effective for tofu texture evaluation in previous chapter. Thus, this method was also used for evaluation of the texture of tofu in local market in China. Evaluation method is tests of stress-strain and stress relaxation.

3.2 Materials and Methods

3.2.1 Materials

Three kinds of commercial tofu (not pressed GDL-coagulated (CMGDL-tofu), pressed gypsum ($CaSO_4$)-coagulated (CMCa-tofu) and bittern ($MgCl_2$)-coagulated (CMMg-tofu)) were purchased in local market in Beijing, China, and were tested in China.

3.2.2 Viscoelastic tests for commercial tofu in China

To understand the texture of commercial tofu in the local market in China, stress-strain and stress relaxation tests of three kinds of commercial tofu (CMGDL-tofu, CMCa-tofu and CMMg-tofu) were done by using a Rheometer (Fudotech Co.Ltd, Japan) in China. Samples were cut into 30 mm-height and 36 mm-diameter ones. A 50-diameter plate plunger and 1mm/s crosshead speed were used. Stress-strain and stress relaxation curves of commercially produced tofu in China were analyzed by the same way as described in Chapter 2. For the limitation of equipment, creep tests were not done.

3.3 Results and Discussion

3.3.1 Stress-strain behavior of the commercial tofu in China

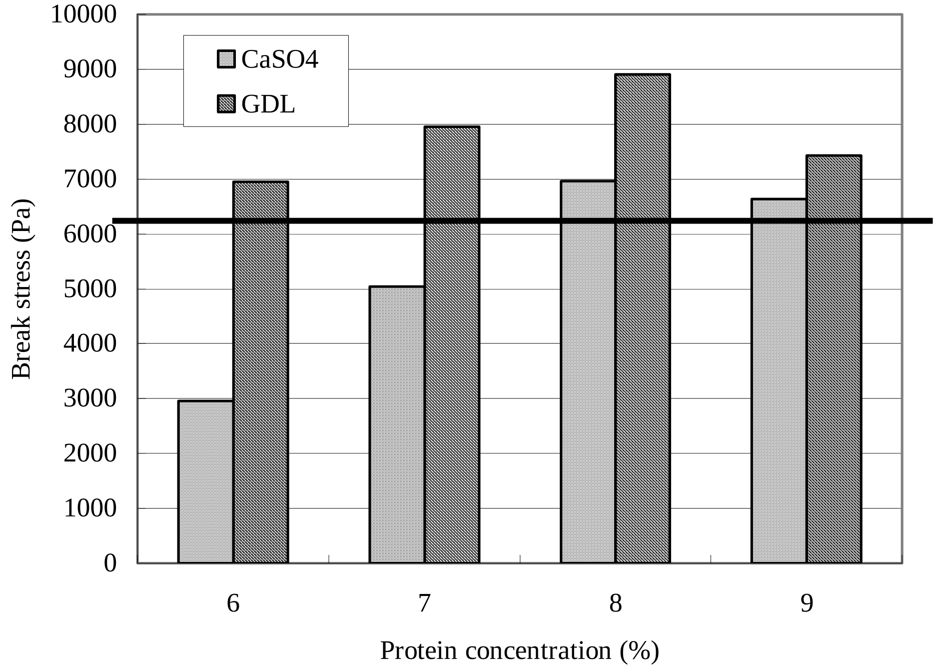
$CaSO_4$ (gypsum) and $MgCl_2$ (bittern) have been commonly used as coagulants for tofu production in China. Three kinds of commercial tofu were measured (CMGDL-tofu, CMCa-tofu and CMMg-tofu). Only CMGDL-tofu was an industrially produced tofu without a press. The other two were pressed and produced by family-style small factories. The stress-strain curves were recorded by Rheometer (Fudotech Co.Ltd, Japan). Results showed that CMGDL-tofu had the lowest hardness and CMMg-tofu was too hard to be measured for the stress-strain test (over the measurement range) (Table 3-1). Although GDL-tofu was harder than Ca-tofu with the same soymilk concentration when being produced in the laboratory (Chapter 2), for commercial tofu, CMCa-tofu was a little harder than CMGDL tofu. This is due to the fact that CMCa-tofu was a pressed type while CMGDL-tofu was produced without removing the whey, i.e., for a different processing method, they had different texture compared with tofu produced in the laboratory. CMMg-tofu was the hardest one. These data showed that some extremely hard tofu really exists in the local market in China. The processing method had a great influence on the texture of tofu. Even in the same place in China, the texture of different tofu changed greatly due to the processing method and usage of different coagulants.

Table 3-1 Break stresses of commercially produced tofu in China

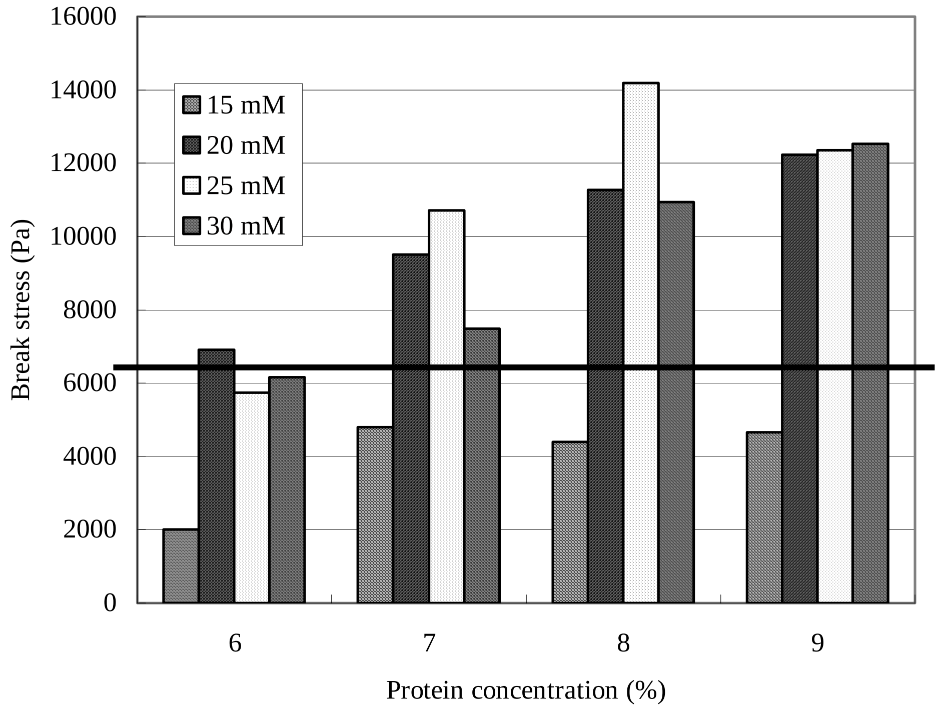
Coagulant	GDL	$CaSO_4$	$MgCl_2$
Break stress (Pa)	5660	6200	>20700

Taking the break stress of CMCa-tofu as an index, break stresses of Ca-tofu, GDL-tofu and Mg-tofu were compared with this index (Fig.3-1). For Ca-tofu, 8% and 9% protein tofu showed higher break stresses than CMCa-tofu. For GDL-tofu, 6%-9% protein tofu, for Mg-tofu, 6% (20 mM), 7% (20, 25 mM), 8% (20-30mM), 9% (20-30mM) tofu showed higher break stress values.

Further, the stress relaxation tests of the hard tofu were done.



(a) Ca-tofu and GDL-tofu



(b) Mg-tofu

Fig.3-1 Comparison between break stresses of commercial CMCa-tofu and Ca-tofu, GDL-tofu (a) and Mg-tofu (b) produced in laboratory. The line shows break stress value of CMCa-tofu

3.3.2 Stress relaxation behavior of commercial tofu in China

All three kinds of tofu were subjected to stress relaxation tests. Using the same methodology for tofu produced in the laboratory, it was found that these commercial tofu products could also be fit with the same 4-element Maxwell model (Fig. 3-2). Parameters were also obtained by a ‘least squares’ non-linear algorithm in Sigmaplot® as previously used. CMMg-tofu showed higher elastic parameters and viscous ones than other two (Table 3-2). The parameters of CMGDL-tofu and CMCa-tofu did not change much. Although Chinese prefer hard tofu, all the hard tofu do not have similar texture parameters. They are different due to having different processing conditions and coagulants. It has been reported that commercial momen tofu in Japan showed higher viscous and elastic parameters than kinugoshi tofu (Taneya et al., 1993). Because of the process differences, pressed tofu has a close network structure (Taneya et al., 1993) for having the press process, which may also cause the high values of elastic and viscous parameters of CMMg-tofu in this study.

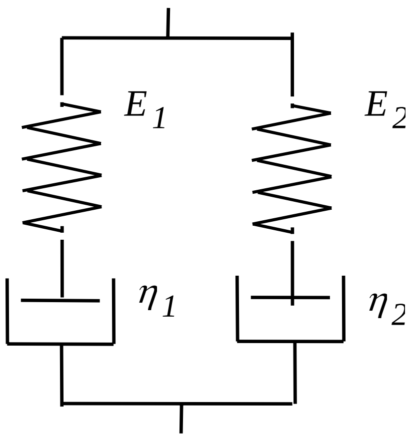


Fig. 3-2 4-element Maxwell model for stress relaxation behavior of tofu in local market in China

Comparison between stress relaxation parameters of commercial CM-Ca-tofu and Ca-tofu produced in laboratory was shown in Table 3-3. For 8%, 9% protein Ca-tofu, they showed similar or higher values of parameters comparing with CMCa-tofu. From both the break stress results and stress relaxation results, it was indicated that using kinugoshi production way, it is possible to produce tofu with similar texture as the commercial pressed $CaSO_4$-coagulated tofu.

Table 3-2 Parameters of four-element Maxwell model for stress relaxation of commercially produced tofu in China

Coagulants	GDL	$CaSO_4$	$MgCl_2$
E_1 (10^3 Pa)	3.7	4.9	10.5
E_2 (10^3 Pa)	2.2	3.1	8.4
η_1 (10^6 Pa•s)	2.8	2.0	16.7
η_2 (10^4 Pa•s)	2.2	2.3	9.0
τ_1 (s)	747	406	1595
τ_2 (s)	10.0	7.4	10.6

Table 3-3 Comparison between stress relaxation parameters of Ca-tofu produced in lab and commercial one

Protein concentration	6%	7%	8%	9%	Commercial
E_1 (10^3 Pa)	3.2	4.2	4.5	4.0	4.9
E_2 (10^3 Pa)	2.0	3.3	3.1	2.2	3.1
η_1 (10^6 Pa•s)	1.1	1.8	3.5	4.1	2.0
η_2 (10^4 Pa•s)	3.4	5.3	5.7	7.0	2.3
τ_1 (s)	341	502	810	1021	406
τ_2 (s)	17	19	30	32	7.4

3.4 Conclusions

Based on above results, following results could be drawn:

(1) There are different types of tofu being distributed in local market in China. With different coagulants and processing methods, texture of tofu is also different.

(2) Fairly hard tofu really exists in the local market in China. Tofu in China is much harder than that in Japan. It reflected the fact that Chinese prefer hard tofu.

(3) It is possible to produce tofu using kinugoshi production way, which has similar texture as the commercial pressed Ca-tofu.

Chapter 4 Effect of okara-addtion on the texture of tofu

4.1 Introduction

During tofu production, okara (soybean residue) is generated. Okara is a residue left from ground soy beans after the extracted water fraction is used to produce soy milk and tofu, which is also called "tofukasu" (Matsumoto and Take, 1980), or soy pulp ("douza" (Chinese), "bejee" (Korean), and "tempe gembus" (Indonesian)) (Liu, 1997). In the tofu production industry, it is known that 1 kg of soybeans will generate 1.3 kg okara. In Japan, 1.3 million tons of okara was generated in 1994 (Yoshii et al., 1995). Previously, 80% of okara was eaten by human beings or used as animal feed, but this trend has changed and the disposal of okara has become more and more difficult. Most of the tofu factories in Japan even pay for the disposal of okara.

Many studies have therefore been conducted on how to utilize it more effectively (reviewed by O'Toole (1999)). Okara was fermented by different microorganisms to extract useful products and has been used as silkworm food and in the production of ceramics in some cases. However, there are few reports about the addition of okara to soymilk for tofu production. Numata et al. (1997) used ultrafine pulverized soybean powder and found that the smaller soybean powder had less influence on the texture of tofu and good quality tofu could be produced by small soybean powder. Generally speaking, the addition of okara influences the production of tofu and affects its texture. Furthermore, okara with smaller size resulting from ultrafine pulverization or enzymatic treatment is expected. So far, the effects of adding okara on the texture of tofu with different coagulants have not been reported. To evaluate the texture of tofu, viscoelstic tests (stress-strain, stress relaxation tests) were conducted on tofu made from different soymilk concentrations with various types of coagulants, such as 30 mM GDL and 30 mM $CaSO_4$ and on some commercial tofu in local Chinese markets in previous chapters and described in Chapter 2 in detail. It was found that this viscoelastic test was effective for evaluating the texture of tofu. Thus, the same texture evaluation method was also employed in this study. It was reported that okara from different resources had different components (O'Toole, 1999).

To insure that the same okara would be used in this study, a kind of paste-like commercial okara product (Proplus-SY^{TM}, Fuji Oil Co., Ltd) processed by ultrafine pulverization was used. Thus, the effects of okara addition on the texture of tofu coagulated with different coagulants were investigated in this chapter.

4.2 Materials and Methods

4.2.1 Materials

Tofu made in the laboratory was used in this study. The soy protein isolate (SPI, 90% protein content, FujiproE^{TM}) (Fuji Oil Co., Ltd., Osaka, Japan) was used to prepare soymilk. Okara was a paste-like commercial product (Proplus-SY^{TM}, Fuji Oil Co., Ltd., Osaka, Japan) processed by ultrafine pulverization, with compositions shown in Table 1 (Fuji Oil Co., Ltd., 2001). It is rich in dietary fiber, as high as 5.7%. Analysis-grade Glucono-delta-lactone (GDL) and food-grade $CaSO_4 \cdot 2H_2O$ were purchased from Wako Pure Chemicals Industries Co., Ltd., (Osaka, Japan) and used without further purification.

Table 4-1 Compositions of okara product Proplus-SY (g/100g)

	moisture	protein	lipid	sugar	ash	dietary fiber	isoflavone
wet base	86.7	4.3	2.8	0	0.5	5.7	0.014
dry base	------	32.1	21.0	0	3.8	42.8	0.1

4.2.2 Tofu Production in the laboratory

Tofu production in the laboratory was based on the Japanese industrial kinugoshi (silken) tofu production method (Watanabe, 1997) as reported previously (Table 2-1, Cheng et al., 2002). Tofu produced from 7% protein soymilk (solid content is about 8%) had a high hardness of about 8,000 Pa when using 30 mM GDL as a coagulant (Cheng et al., 2002), so soymilk concentration was set as 7% protein (including the protein from okara when adding okara) in this study. Figure 4-1 showed the procedures. SPI was dissolved in distilled water to prepare soymilk and 0, 5, 10, 15, 20, 25% (w/v) okara was added. Soymilk was heated to 100℃ and held at that temperature for 3 minutes. It was then cooled to below 10℃ and a coagulant was added. The soymilk and okara mixture were poured into a steel tank where glass molds (20 mm in height, 50 mm in diameter) had been placed in advance to shape the columnar tofu samples. The soymilk was then heated to 75℃ in a water bath and kept for more than 40 minutes to let the soymilk coagulate. The samples were stored at room temperature for 60 min before the viscoelastic tests. The concentrations and types of coagulants used in this study were: 30 mM GDL, 15 mM $CaSO_4$ + 15 mM GDL, 30 mM $CaSO_4$ + 30 mM GDL, 60 mM $CaSO_4$ and 60 mM GDL. Tofu made from these coagulants was abbreviated as 30GDL, 30CG, 60CG, 60Ca and 60GDL tofu, respectively.

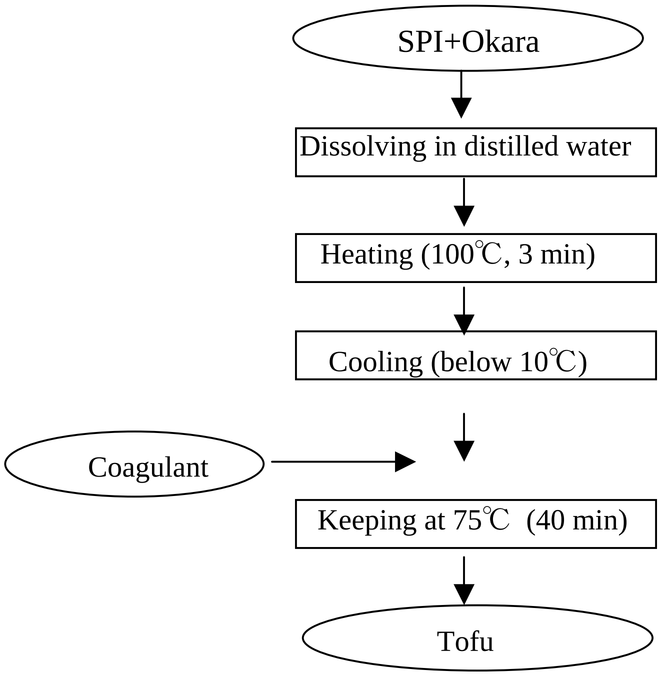


Fig. 4-1 Process procedures of okara-added tofu (silken tofu production method) in laboratory

4.2.3 Measurement of viscosity of okara-added soymilk

Viscosity of soymilks with different okara addition ratio was measured to understand the influence of addition on viscosity by Viscometer LV2000 (Cannon Instrument Company, PA, USA). The rotation speed was 30 rpm for all the cases. The experiments were repeated for more than 3 times.

4.2.4 Viscoelastic tests for okara-added tofu

Stress-strain and stress relaxation tests were performed on the tofu with a Texture Analyzer (TA-XT2I, Stable Micro Systems, UK) with a 5 kg load cell. The samples were 20 mm in height and 50 mm in diameter. A 70 mm in diameter flat plunger was used to compress the samples in the stress-strain test at 1 mm/s. After the linear range was determined by stress-strain profiles, a relaxation test was conducted. The stress relaxation period was 600s for all samples. All experiments were repeated at least three times.

4.2.5 Dynamic viscoelastic tests of okara-added tofu

Dynamic viscoelastic tests were also conducted to okara-added tofu. Dynamic viscoelastic tests will help to get insight of the effect of okara addition on the texture of tofu. ARES (Advanced Rheometrics Expansion System, Rheometric Scientific™, UK) (Fig.4-2) was used for dynamic viscoelastic tests. Elastic moduli at a certain frequency and effects of frequency on the elastic moduli were investigated. Time-course of elastic index was recorded. Test conditions were: test time, 1500s; strain, 0.5%. A 25-mm diameter parallel plate was used. Tofu coagulated by 60 mM $CaSO_4$ or 60mM GDL with different okara addition ratios was tested.

4.2.6 Analysis of viscoelastic test

4.2.6.1 Stress-Strain

Stress-strain test was analyzed by the same way described in *2.2.5.1*.

4.2.6.2 Stress Relaxation

Stress relaxation curves were fitted with viscoelastic models as described in *2.2.5.3*.

4.2.6.3 Dynamic viscoelastic test

In dynamic viscoelastic test, storage modulus and loss modulus can be directly obtained from the curve and loss tangency can be obtained from following equation:

Loss tangency = loss modulus / storage modulus   (1)

(a) Appearance of ARES

(b) Appearance of experiment

Fig.4-2 Appearances of ARES (a) and experiment (b)

4.3 Results and Discussion

4.3.1 Stress-strain behavior of okara-added tofu

Stress- strain behavior is usually investigated for the textural evaluation of many food materials. Break stress, break strain and some other indices could be obtained from the stress-strain behavior. Break stresses of 60Ca-tofu with different amounts of okara added are shown in Fig. 4-3.

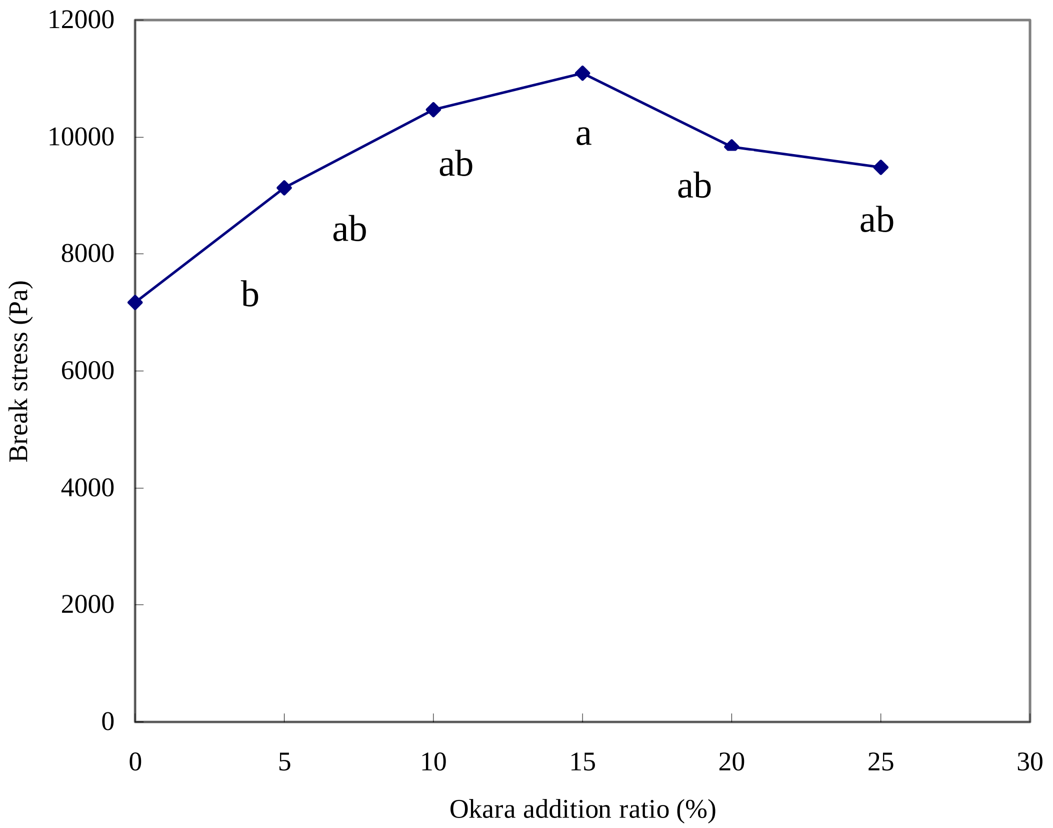


Fig.4-3 Effect of okara-addition on the break stress of 60mM $CaSO_4$-coagulated tofu. a-b, Different letter means significantly different at level $p<0.05$

Initially, break stress increased significantly with an increasing okara ratio and then it decreased. Okara is rich in dietary fiber (Table 4-1), which will negatively influence tofu texture when using common okara made from the soymilk or tofu production factories. Common okara has a large particle size and these particles inhibit the formation of a good protein network. In this study, however, a commercial ultrafine pulverized okara product was used, with a particle size of only 30 to 40 μm, although still rich in dietary fiber (Table 4-1). The okara used in this study also has a high viscosity (195.0 Pa･s at 120℃) (Fuji Oil Co., Ltd., 2001). Figure 4-3 showed that the addition of okara improved the break stress of 60Ca-tofu. However, it was not always the same when other types of coagulants were used (Fig.4-4). GDL-coagulated tofu exhibited a significant decease in break stress with an increasing ratio of okara. Compared with GDL samples, the mixture of GDL and $CaSO_4$ reduced the decrease of break stress. In other words, adding okara had less influence on the tofu coagulated by a mixture of GDL and $CaSO_4$ than on GDL-coagulated tofu. When using the same coagulant, a higher concentration of coagulant resulted in a higher break stress (comparisons between 30GDL-tofu and 60GDL-tofu, and between 30CG-tofu and 60CG-tofu). GDL presented a different response to okara addition than $CaSO_4$ did. Addition of high-viscosity okara strengthened the structure of tofu with $CaSO_4$ as the coagulant, but weakened it when GDL was the coagulant.

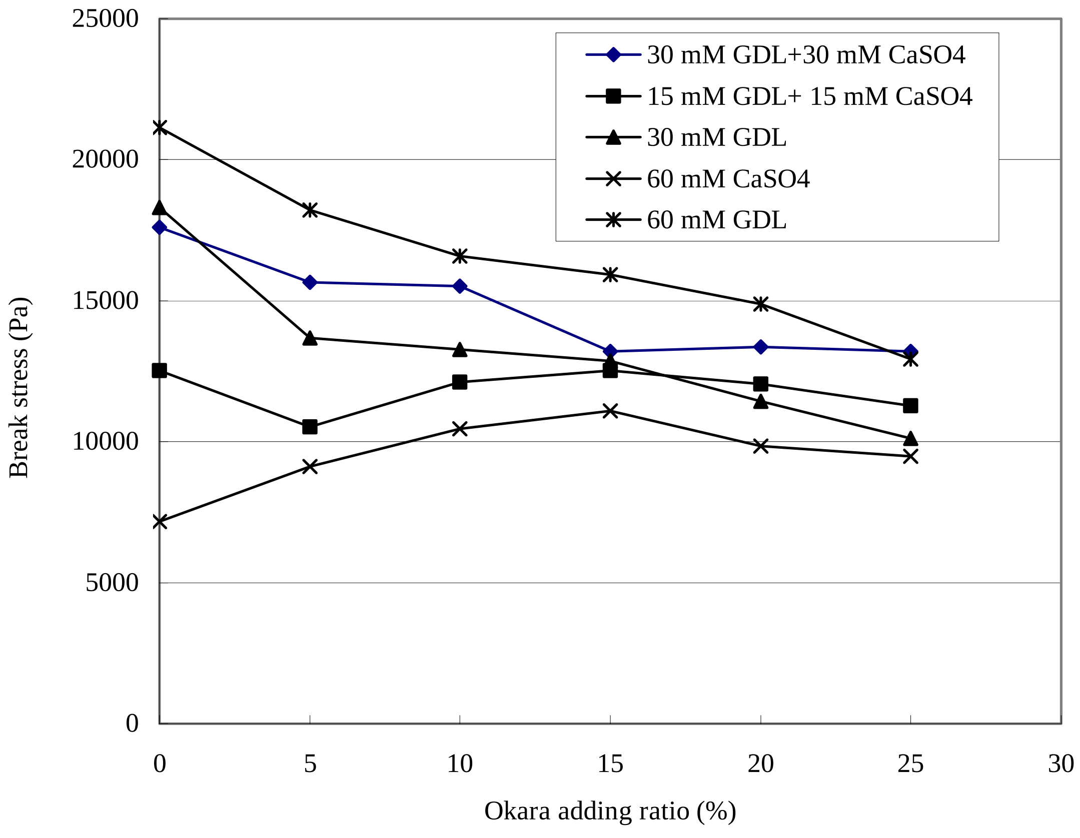


Fig.4-4 Effects of okara-adding and types of coagulants on the break stress of tofu

GDL-tofu has a slightly different gel formation mechanism than Ca-tofu. It was reported that the network of GDL-tofu consisted of flocculent aggregates, and that that of Ca-coagulated tofu had a spongy structure with tight frames (Saio, 1979). In tofu gel formation, at first, the hydrophobic regions of the protein molecules in the native state are located inside and are exposed to the outside by heat denaturation; and then in the case of $CaSO_4$, after addition of Ca^{2+}, proteins chelate with Ca^{2+} and aggregate, then forms the gel. While for GDL, GDL was converted into gluconic acid with heating, and the released H^+ neutralizes the net charges of the heat denaturing proteins, and causes the gel formation of GDL-coagulated tofu (Kohyama et al., 1995). The Ca-coagulated tofu has a Ca bridge that may differentiate the structure of Ca-coagulated tofu from that of GDL-coagulated tofu. The different responses of GDL and $CaSO_4$ to the okara addition may partly result from the differences in their gel formation mechanisms. The dissolubility of $CaSO_4$ in water is very low (0.2g/100g at 20℃) and that of GDL is high (59g/100g at 20℃). The addition of okara increased the solid content of soymilk. Although part of the dietary fiber is not insoluble in the solution, the fiber can form a uniform suspension due to the small size of the particles, which could increase the viscosity of soymilk (Fig.4-5).

The increase of viscosity could have prevented the precipitation of $CaSO_4$ and resulted in the uniform distribution of $CaSO_4$ in the soymilk. It may also facilitate the gelation and formation of tofu. Increasing the ratio of okara also increased the concentration of polysaccharides in soymilk. Karim et al. (1999) reported the effect of carrageenan, a kind of polysaccharide, on yield and properties of tofu, and found that Ca-tofu and GDL-coagulated tofu responded differently to the addition of carrageenan. The changes of polysaccharides in soymilk resulting from the addition of okara in our study may also have had an important role in the different responses of Ca-tofu and GDL-coagulated tofu to the addition of okara.

For all the cases, hardness of almost all the tofu exceeded 10,000 Pa, even after adding 25% okara. It was reported in chapter 3 that the hardness of some of the tofu distributed in the local markets in China as 6200 Pa. Although the measurement methods are a little different, it was suggested that even adding 25% okara product could still provide tofu with enough hardness.

The break strain of tofu with okara –added with different coagulants was also recorded in this study (Fig.4-6). In all the cases, the break strain decreased when the okara ratio increased. The decrease in break strain reflected the fact that the tofu became more fragile. Break stress and break strain reflect the different properties of viscoelastic materials. For 60Ca-tofu, the break stress increased with the increasing okara ratio, while break strain decreased. These two were not consistent in this study. Kohyama et al. (1992) studied the break stress and break strain of tofu at different temperatures and found that at higher temperatures, the break stress of tofu became lower but the break strain increased and they were not always consistent, which is in agreement with our results. It was also suggested that break strain was an important index, which reflects whether the foodstuff is fragile or not. Thus, to comprehensively evaluate the texture profile of tofu, it is also necessary to consider the break strain index if possible, especially when the changes of breaks strain are not consistent with those of break stress.

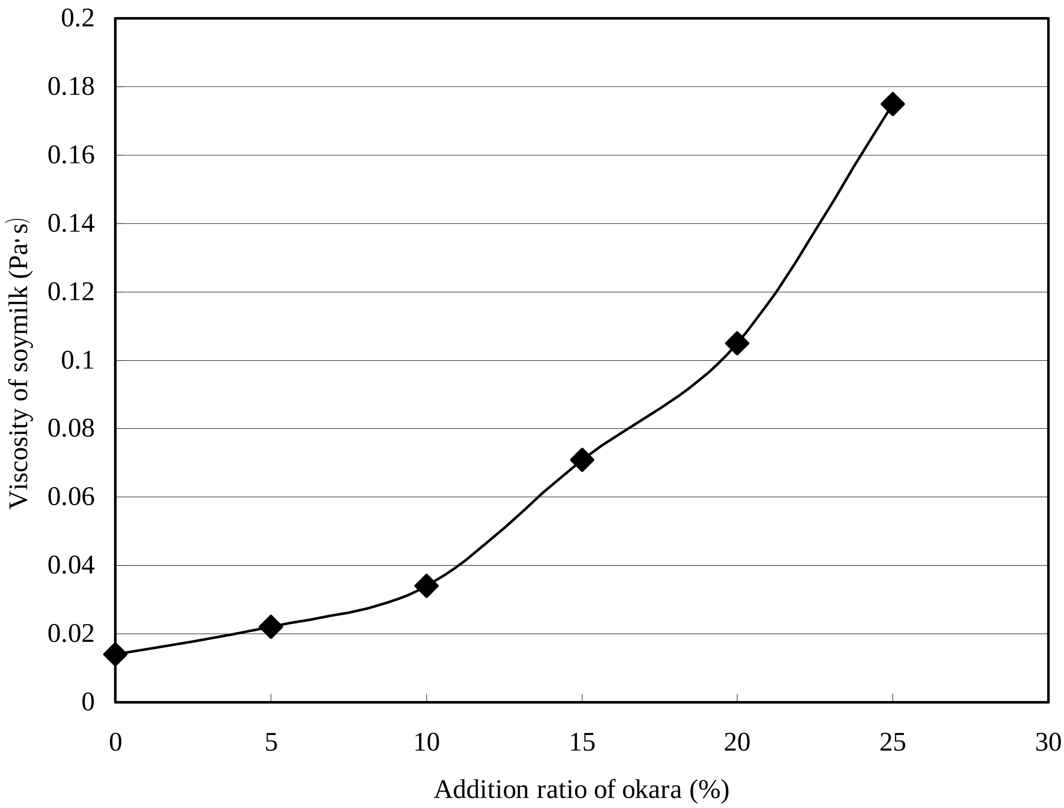


Fig.4-5 Effect of okara addition on the viscosity of soymilk

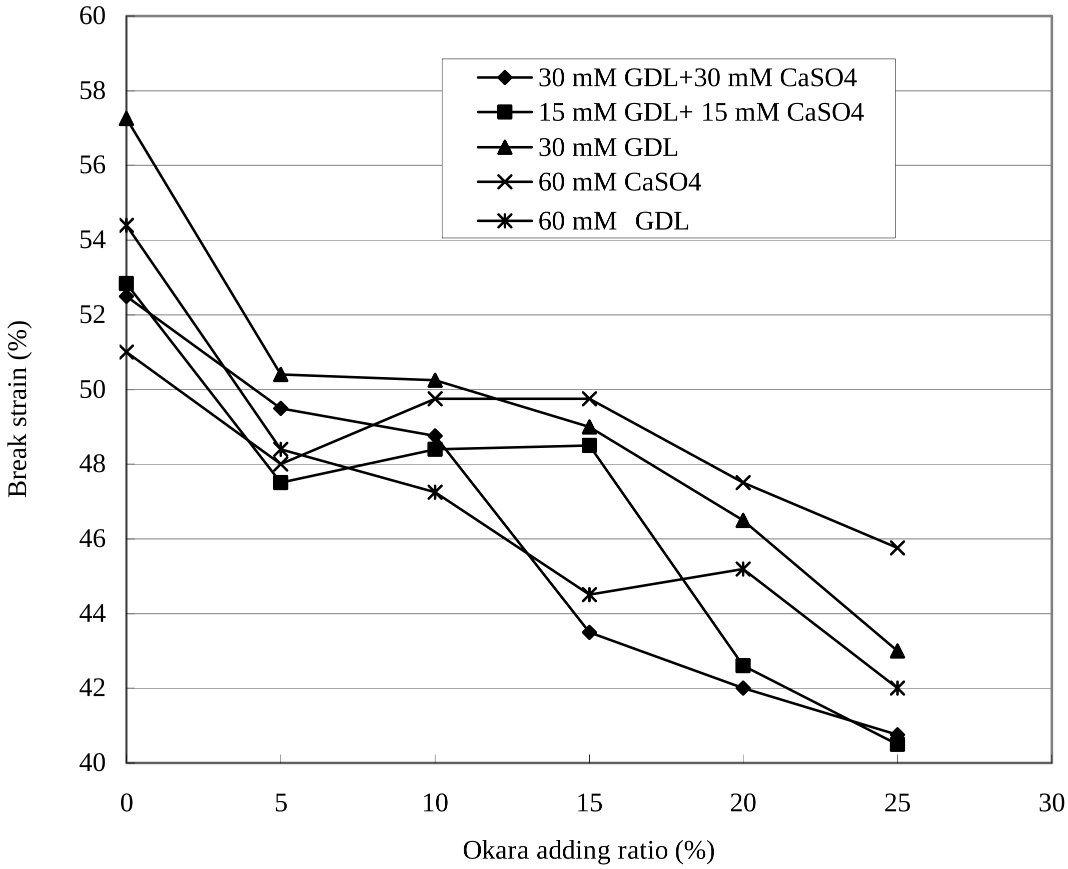


Fig.4-6 Effects of okara-adding and the types of coagulants on the break strain of tofu

4.3.2 Stress relaxation behavior of okara-added tofu

In a typical stress relaxation curve of okara-added tofu, apparent elasticity decreased with time (Fig.4-7). It did not reach an equivalent stress even after 600 seconds. After calculation, it was found that the four-element Maxwell model (Fig. 4-8) could fit the stress relaxation behavior of tofu with okara added. The relationship between apparent elasticity and viscous and elastic parameters can be expressed in Equation 2 (Mohsenin, 1986; Kawabata, 1989):

$$E(t) = E_1 e^{-\frac{t}{\tau_1}} + E_2 e^{-\frac{t}{\tau_2}} \qquad (2)$$

Where $E(t)$ is apparent elasticity; t is time (s); E_1, E_2 are the elasticity of two Maxwell models (Pa); and $\tau_i = \eta_i / E_i$ (i = 1,2) is the stress relaxation time (s).

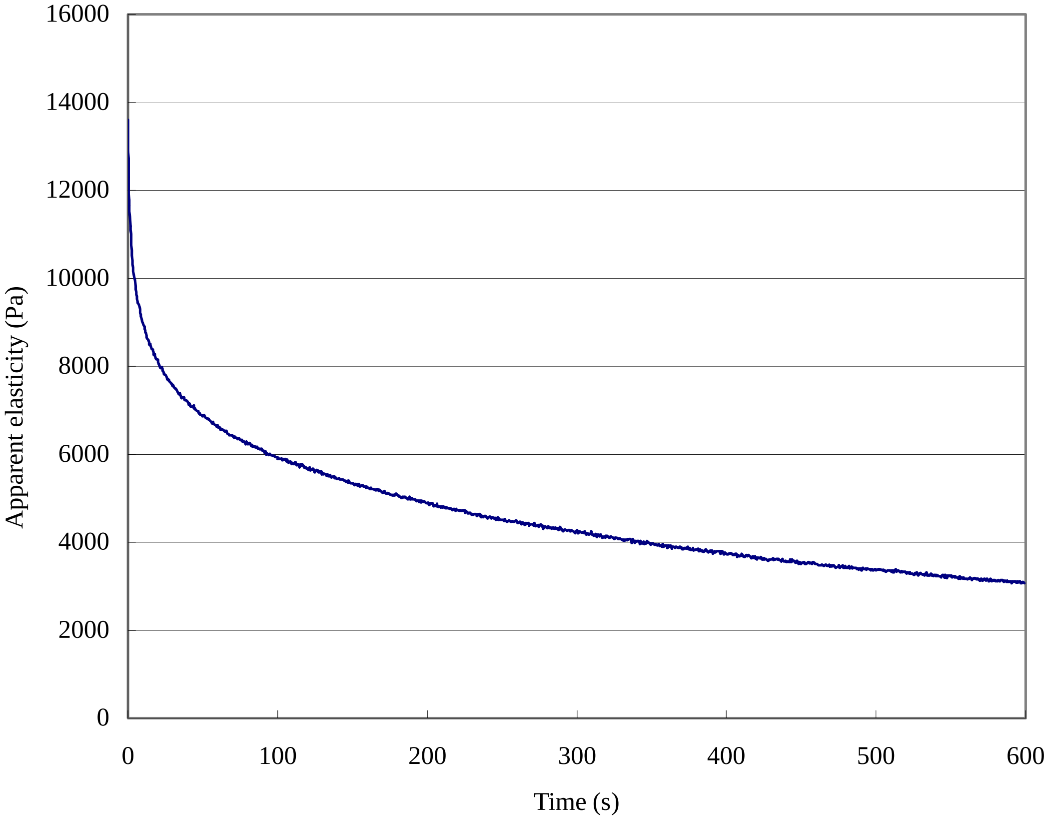


Fig.4-7 A typical stress relaxation curve of okara-added tofu

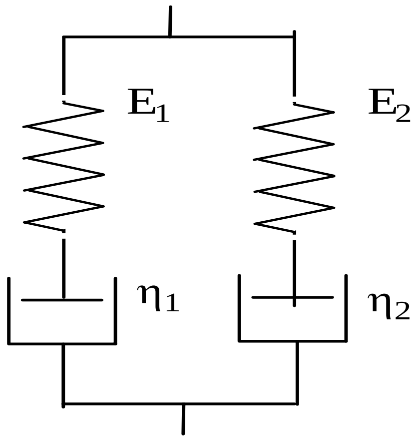


Fig.4-8 Characteristics of 4-element Maxwell model for okara-added tofu. *E*, elastic modulus of spring model, *η*, viscous modulus of dashpot model

After determining the model, parameters for the models were calculated. The parameters of 60Ca-tofu, 60CG-tofu, and 60GDL-tofu are shown in Tables 4-2, 4-3, and 4-4. The 60Ca-tofu with okara added (Table 4-2) had higher *E*, *η* and relaxation time than tofu without okara. This suggested a firmer tofu structure after okara addition. For 60CG-tofu (Table 4-3), a 15% okara ratio produced the highest *E* and *η* values. While for 60GDL-tofu (Table 4-4), increasing the okara ratio deceased the parameters, which correlated with the result of break stress (Fig.4-4). Both 60CG-tofu and 60GDL-tofu (Tables 4-3 and 4-4) exhibited higher values of *E* and *η* than 60Ca-tofu, but not a higher relaxation time. GDL was not desired as a coagulant by many consumers, but it could provide a harder tofu than $CaSO_4$ (Chapter 2). The parameters obtained from the stress relaxation were highly correlated with the strength and the degree of the cross-linking of the network (Tang et al., 1998). The increase of the relaxation time of 60Ca-tofu might reflect that the structure of the tofu gel is becoming firmer with increased okara ratios in this study. The high viscosity of the okara product used in this study may also play an important role in the texture changes of okara-added tofu.

The formation of tofu gel is influenced by many factors, such as the component of soy protein, concentration of soymilk, phytic acid, heating time and temperature, and type and concentration of coagulants (Watanabe, 1997). This study also indicated that both large (stress-strain) and small deformation (stress relaxation) behaviors depended greatly on the soymilk properties and the type and concentration of the coagulant, suggesting that okara could be used to produce hard tofu. If this could be implemented, it would be beneficial in hard tofu production and in reducing environmental loads. To obtain more information about the effect of okara addition on the texture of tofu, dynamic viscoelastic tests were also conducted.

Table 4-2 Parameters of 4-element Maxwell model for stress relaxation property of okara-added tofu（coagulant: 60 mM $CaSO_4$）

Okara	0%	5%	10%	15%	20%	25%
E_1 (10^3 Pa)	2.5	2.4	3.6	3.9	3.0	3.9
E_2 (10^3 Pa)	1.8	1.8	2.2	2.1	1.5	2.0
η_1 (10^6 Pa·s)	1.4	1.6	3.1	4.2	3.1	4.4
η_2 (10^4 Pa·s)	3.8	4.2	6.1	6.1	4.4	5.2
τ_{rel1} (s)	571	680	852	1077	1032	1146
τ_{rel2} (s)	20	24	28	29	29	26

.

Table 4-3 Parameters of 4-element Maxwell model for stress relaxation property of okara-added tofu (coagulant: 30mM $CaSO_4$ + 30 mM GDL)

Okara	0%	5%	10%	15%	20%	25%
E_1 (10^3 Pa)	4.8	6.5	7.1	10.8	8.9	7.8
E_2 (10^3 Pa)	3.5	4.6	4.3	5.9	4.5	4.0
η_1 (10^6 Pa·s)	2.8	5.6	5.4	11.0	9.6	8.3
η_2 (10^4 Pa·s)	9.6	13.2	13.6	17.1	13.6	12.6
τ_{rel1} (s)	570	866	763	1022	1072	1070
τ_{rel2} (s)	27	29	32	29	31	31

Table 4-4 Parameters of 4-element Maxwell model for stress relaxation property of okara-added tofu (coagulant: 60 mM GDL)

Okara	0%	5%	10%	15%	20%	25%
E_1 (10^3 Pa)	6.8	5.4	5.8	5.9	5.8	5.3
E_2 (10^3 Pa)	5.1	3.8	4.5	4.9	4.6	5.3
η_1 (10^6 Pa·s)	4.4	3.1	2.8	2.8	3.1	1.9
η_2 (10^4 Pa·s)	14.9	10.2	10.6	12.9	11.9	12.7
τ_{rel1} (s)	637	574	487	471	534	363
τ_{rel2} (s)	29	27	24	26	26	24

4.3.3 Dynamic viscoelastic behavior of okara-added tofu

After okara-added tofu (0-25%) were produced, the storage and loss moduli were observed at 1 Hz and 0.5% strain as functions of time. A typical time course of these two paramters was shown in Fig.4-9. The storage and loss moduli seemed to reach equilibrium and did not change during 1500s measurement period. Nagano et al. (1994a) studied the dynamic viscoelastic properties of glycinin and β-conglycinin gels from soybeans. They measured the storage and loss moduli of glycinin and β-conglycinin at 1 Hz and 5% strain as functions of time. The storage and loss moduli of these two proteins reached equilibrium after 2 hrs. The equilibrium means the good formation of gel network (Nagano et al., 1994a, b, c).

Figs.4-10, 4-11 showed double-logarithmic plots of the storage and loss moduli *vs* frequency of tofu with different okara addition ratios. In these figures, for both Ca-tofu and GDL-tofu, with the increase of frequency, both storage modulus and loss modulus showed a plateau and then increased. For all the cases, storage modulus showed a higher value than loss modulus. As is often observed, G' as a function of frequency shows a plateau when the system behaves like a gel while G' decreases with decreasing frequency when the system behaves like a sol (Nagano et al., 1994a,b,c). The plateau indicates the presence of a network structure. On the other hand, G'' for a gel often shows a shallow dip, as observed in β-conglycinin gels coagulated in the presence of glucono-delata-lactone (Kohyama and Nishinari, 1993). When storage modulus is larger than loss modulus, the behavior is typical of a solid. In this study, for all the samples, storage modulus showed a higher value than loss modulus, which means that a good network exists in the gel for all the cases. These results suggested that the addition of okara did not destroy the formation of a good gel. In Fig.4-10, it was also shown that moduli of 20% and 25% okara-added Ca-tofu had a sudden increase comparing with 15% one. It seemed that addition of okara above 15% for Ca-tofu caused some structure changes of tofu and induced this sudden increase. In previous part, it was indicated that addition of okara above 15% decreased the break stress of Ca-tofu comparing with 15% one (Fig.4-5). Both of these two results might result from the structure change of tofu for a higher addition of okara. GDL-tofu showed different results with Ca-tofu, with the increase of okara addition ratio, moduli did not change much (Fig.4-11). Comparing data between Ca-tofu and GDL-tofu, GDL-tofu showed higher values of moduli, which is highly correlated to the results of break stresses (Fig.4-4).

As industrial waste, the large quantities of okara produced annually pose a significant disposal problem. It is still rich in nutrients and can be fermented by different methods to provide for human consumption (Matsumoto and Take, 1980; Kronenberg and Hang, 1996) or for extracting antioxidants or biosurfactants (Ohno et al., 1995; Yokota et al., 1996) or some other useful products. So far, however, there are no practical cases for these studies, and okara is not utilized on a large scale. If okara could be successfully added to soymilk to produce tofu, large quantities of okara could be used because tofu is one of the daily foods in some Asian countries.

4.4 Conclusions

In this chapter, following conclusions could be drawn:

(1) Higher concentration of coagulants ($CaSO_4$ and GDL) resulted in a higher break stress. Partial replacement of $CaSO_4$ by GDL could also help to produce hard tofu.

(2) It is possible to add okara to soymilk for producing a fiber-rich tofu. Furthermore, adding paste-like okara could help to produce a harder $CaSO_4$-coagulated tofu. Even adding 25% okara did not affect the texture of Ca-coagulated tofu and tofu coagulated by mixed GDL and $CaSO_4$. Results of dynamic viscoelastic tests revealed that okara addition did not destroy the formation of a good gel for tofu.

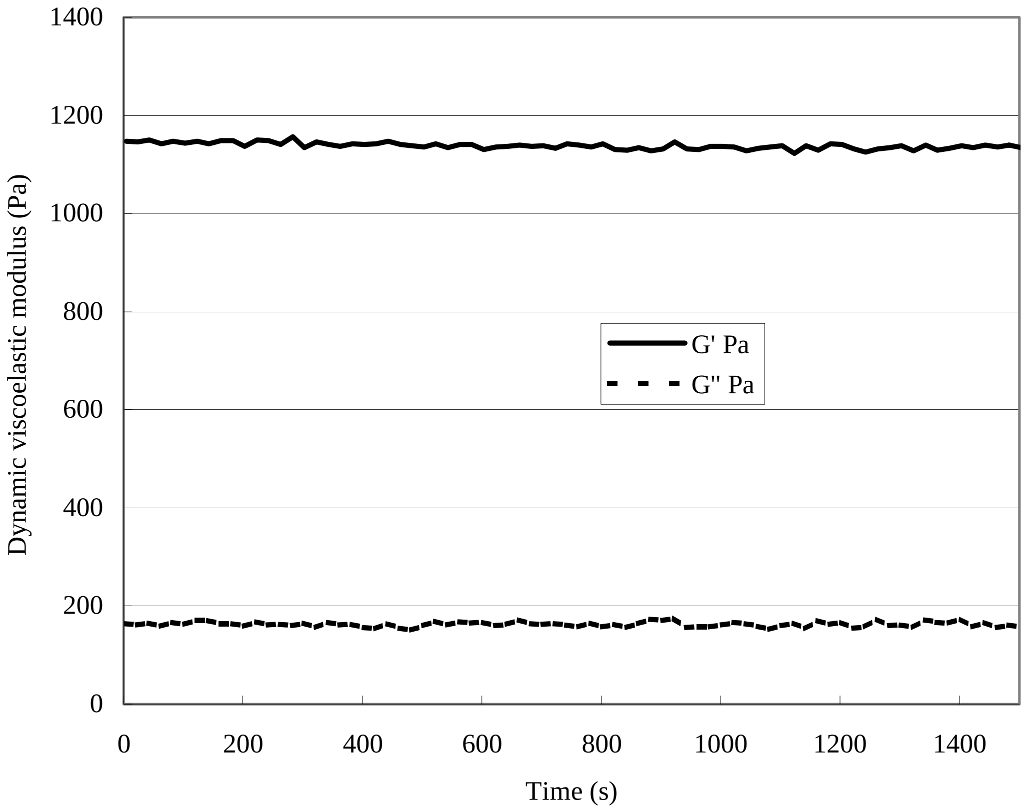
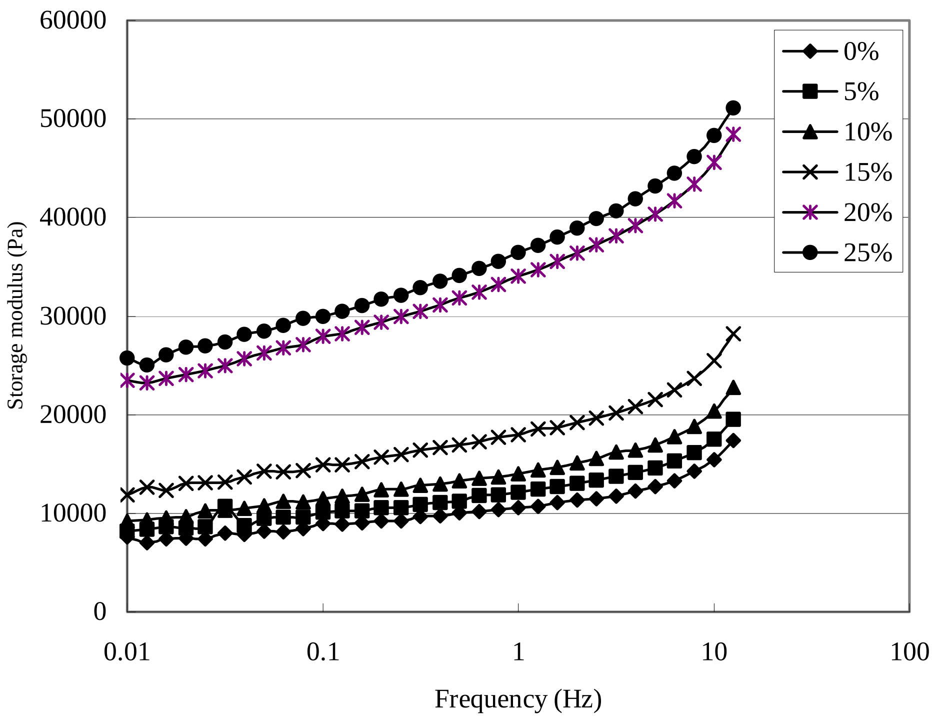
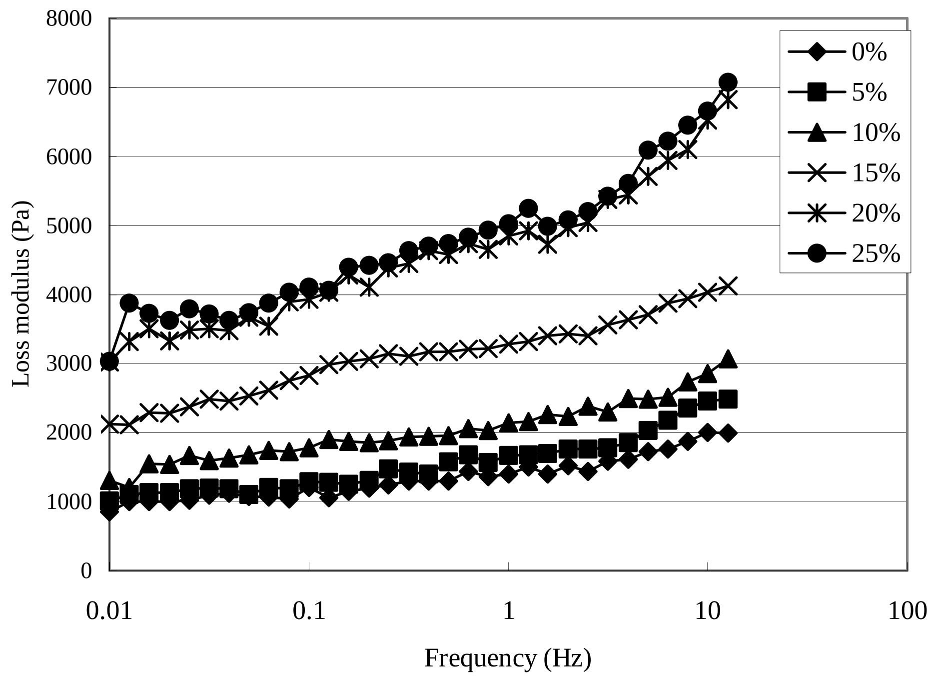


Fig.4-9 Typical time course of storage modulus and loss modulus of tofu at a frequency of 1 Hz and a strain of 0.5%

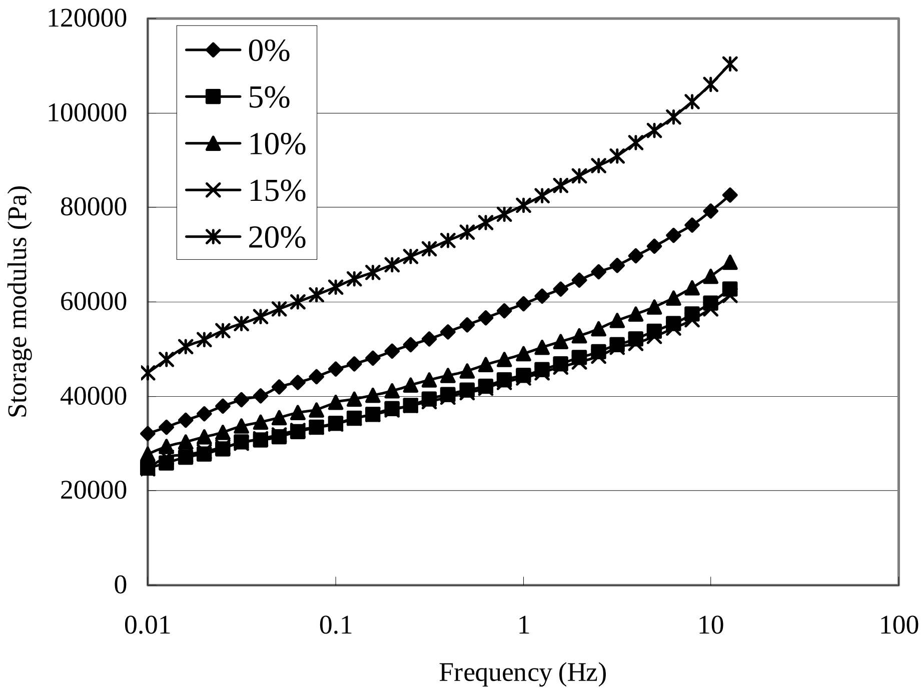


(a) Changes of storage modulus

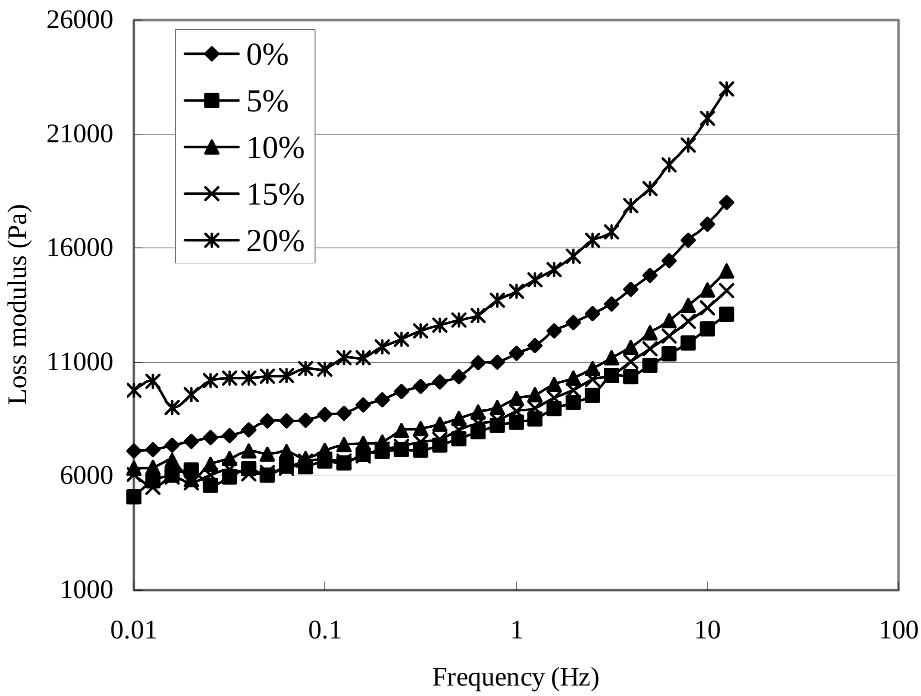


(b) Changes of loss modulus

Fig.4-10 Effect of frequency on storage and loss moduli of 60 mM okara added Ca-tofu



(a) Changes of storage modulus



(b) Changes of loss modulus

Fig.4-11 Effect of frequency on storage and loss moduli of 60 mM okara-added GDL-tofu

Chapter 5 General discussion and conclusions

In previous chapters, for improvement of tofu production in China, texture evaluation method of tofu, investigation of texture properties of Ca-tofu, GDL-tofu, Mg-tofu, and hard tofu in local Chinese market, investigation of effects of okara addition on texture of tofu and creep properties of tofu were done. Based on these results, in this chapter, general conclusions were drawn and discussed.

Stress-strain and stress relaxation tests were found to be effective for evaluation of tofu in this study (Chapter 2). Using these tests, texture properties of Ca-tofu, GDL-tofu and Mg-tofu with different protein concentrations were investigated (Chapter 2). It was suggested that the break stresses and stress relaxation times increased with increasing the protein concentrations. The increase of these parameters implied a firmer structure of tofu accompanying with the increase of protein concentration. Although other components in soymilks, such as lipid (Ono et al., 1996; Guo et al., 1999), phytic acid (Watanabe, 1997) can take influence on the gel formation of tofu, protein is the most important component. From this point, increasing the protein concentration can result in a harder tofu.

Although it was shown that increasing protein content could result in a hard tofu, in industrial practice, it is difficult to obtain too high a protein content from soybeans. And a higher concentration of protein results in a higher viscosity of soymilk, which will cause the difficult transportation of soymilk in industrial practice. Thus, the type and concentrations of coagulants are more important for hard tofu production. For coagulant, industrially used 3 types of coagulants ($CaSO_4$, GDL, $MgCl_2$) were used in this study. Tofu with different coagulant showed different characteristics. The break stresses of tofu coagulated by different coagulants and protein concentrations were shown in Table 5-1.

Table 5-1 Break stresses of different tofu

Coagulant concentration (mM)	30mM GDL*	30mM $CaSO_4$*	20mM $MgCl_2$**
Protein concentration	6-9%	6-9%	6-9%
Break stress (Pa)	6960-8900	2960-6960	6910-12200

* Data from Chapter 2, Table 2-1; **Data from Chapter 2, Table 2-2.

The break stress of commercial hard Ca-tofu in local markets in China is 6200 Pa (Chapter 3). From the point of stress-strain and stress relaxation behaviors, 8% Ca-tofu produced in the laboratory had similar parameters with the CMCa-tofu (Tables 2-1, 2-4, 3-1, 3-2). That is to say, it is possible to produce tofu without removing the whey, which has a similar texture to commercial pressed tofu when $CaSO_4$ is used as the coagulant. Using GDL and $MgCl_2$ can produce hard tofu (break stress 7000 to 12000 Pa) than $CaSO_4$ at the same protein concentration. Mg-tofu and GDL-tofu produced under proper conditions using the no-press production method can be sufficiently hard to meet the palatability standards of Chinese consumers. However, GDL tofu tastes somewhat acidic and is not preferred by consumers (Cheng et al., 1999). At the same time, the quick reaction of $MgCl_2$ with protein (Chapter 2) can cause difficulties in handling for the tofu production industry. Because $CaSO_4$ and GDL were reported to have a slower reaction with proteins (Watanabe, 1997), decreasing the content of $MgCl_2$ and then mixing $MgCl_2$ with them are thought to able to slow down the rapid reaction of $MgCl_2$. In China, Mg-tofu is preferred by many consumers, and this study demonstrated that $MgCl_2$ could provide hard tofu. Thus, for hard tofu production in China, when using $MgCl_2$ as a coagulant, mixture with GDL or $CaSO_4$ should be considered. The concentration of coagulants also took great influence on the texture of tofu. For $CaSO_4$ and GDL, generally speaking, the increase of coagulant concentration resulted in the increase of break stress. However, for $MgCl_2$, the range of optimal concentration was narrow. Too high concentration of $MgCl_2$ resulted in a quick reaction with soymilk and could not induce a good gel formation. Properties of different coagulants were summarized in Table 5-2. In industrial practice, the mixture of different coagulants can be considered and will be effective for hard tofu production.

Table 5-2 Properties of different coagulants for tofu production

coagulant	hardness of tofu	Optimal concentration range	Reaction with protein	Taste
$CaSO_4$	soft	wide	slow	good
GDL	hard	wide	slow	acidic
$MgCl_2$	hard	narrow	quick	bitter

The effects of okara addition on the texture of tofu were investigated. It was shown that different coagulants had different responses to the addition of okara. For Ca-tofu, a certain addition of the paste-like okara product increased the break stresses and relaxation times of 4-element models, which reflected a stronger structure of tofu with the addition of okara. But for GDL-tofu, at all addition ratios, the addition decreased the break stresses of tofu. These results showed the possibility of okara addition for hard tofu production. If this could be applied in industrial practices, it would help the hard tofu production, increase the nutrition value of tofu and reduce the waste output in tofu industry. Although the possibility of okara addition to hard tofu production was shown here, there were still some problems, such as cost of the process, how to control the high viscosity of soymilk for the addition of okara and how to get a uniform distribution of coagulants in high viscous soymilk. Before the real application, there are still many studies should be done.

For the improvement of tofu texture in China, from the view of soymilk concentration, types and concentration of coagulants, the addition of okara, following conclusions were drawn:

(1) A higher soymilk concentration (protein concentration) can lead to a harder tofu. The viscosity of soymilk also increased with increasing the protein concentration. A too high viscosity might cause the difficulties of pipe transportation. In industrial practice, the balance between protein concentration and a proper viscosity should be considered.

(2) Different coagulants showed different properties to the tofu production. For, $CaSO_4$ and GDL, increasing the amount of coagulant resulted in a harder tofu. $MgCl_2$ showed a narrow range of optimal concentrations and too high concentration of $MgCl_2$ caused a ductile break of tofu gel. When at the same concentration of protein, GDL and $MgCl_2$ can induce harder tofu than $CaSO_4$. The reaction of $MgCl_2$ with soy protein is rapider than GDL and $CaSO_4$.

(3) It is possible to add paste-like okara to soymilk for the production of tofu. At a certain range, the texture of tofu was not significantly affected by the addition of okara. $CaSO_4$ showed different response to the addition of okara comparing with GDL. 15% addition of okara increased the break stress of $CaSO_4$-coagulated tofu while the addition of okara decreased the break stress of tofu.

Based on the results in this study, it can be suggested that for industrial hard tofu production in China, using properly high concentration of soymilk, mixture of coagulants, such as $CaSO_4$ with $MgCl_2$, GDL with $MgCl_2$, increasing the concentration of coagulants like $CaSO_4$ and GDL and adding okara can be reasonable alternatives.

Abstract

Nowadays, in China, the problem of protein inadequacy still exists. To solve this problem, low cost, good quality vegetable proteins are considered to be one of the best choices. Among them, as a good vegetable protein resource, soybean has aroused the attention of many researchers. Tofu, a kind of soybean product, was invented in ancient time in China and nowadays, it is still a popular daily dish in some Asian countries like China, Japan, Korea, etc and is considered as a good vegetable protein resource. For the traditional food culture and the style of Chinese dishes, Chinese prefer hard tofu. At present, the production of tofu is mainly done by small-scale factories with handmade way. Such kind of factories can produce hard tofu meeting the palatability of Chinese consumers. But, the shelf life of tofu is very short, in summer, only half a day, which limits the distribution of tofu. At the same time, the labor density is higher, the quality control of tofu is mainly by experiences and a uniform quality is difficult to be realized. In 1980s', some industrial lines were imported. But, such kind of industrial production line uses glucono-delta-lactone (GDL) as coagulant. Tofu produced by this way is somewhat acidic, which is not preferred by consumers, and the texture of tofu is very soft, which is not suitable for most of the Chinese dishes. Lack of information on producing hard tofu by industrial line limit the industrial production of hard tofu in China. Based on the present situation of hard tofu production in China, this study takes the improvement of tofu texture in China as the objective.

To improve the texture of tofu, firstly, a proper evaluation method for the texture of tofu is necessary. Stress-strain, stress relaxation and creep tests were conducted. Results showed that parameters from stress relaxation and creep behaviors analyzed by using viscoelastic models (taking Maxwell model, serialized by a Spring model and Dashpot model, and Vigot model, paralleled by a Spring model and a Dashpot model as primary models) can reflect both the viscous and elastic changes of tofu with different coagulants and protein concentrations. It was suggested that this viscoelastic evaluation method was effective for the texture evaluation of tofu.

After determining the proper texture evaluation method for tofu, in this study, the effects of soymilk concentration (protein) concentration, types and concentration of different coagulants ($CaSO_4$, GDL, $MgCl_2$) on the texture of tofu and the effects of okara addition on the texture of tofu were investigated. For the improvement of tofu texture in China, from the view of protein concentration, types and concentration of coagulants, the addition of okara, following

conclusions were drawn:

(1) A higher soymilk concentration (protein concentration) can lead to a harder tofu. The viscosity of soymilk also increased with increasing the protein concentration. A too high viscosity might cause the difficulties of pipe transportation. In industrial practice, the balance between protein concentration and a proper viscosity should be considered.

(2) Different coagulants showed different properties to the tofu production. For $CaSO_4$ and GDL, increasing the amount of coagulant resulted in a harder tofu. $MgCl_2$ showed a narrow range of optimal concentrations and too high concentration of $MgCl_2$ caused a ductile break of tofu. When at the same concentration of protein, GDL and $MgCl_2$ can induce harder tofu than $CaSO_4$. The reaction of $MgCl_2$ with soy protein is rapider than that of GDL and $CaSO_4$.

(3) It is possible to add paste-like okara to soymilk for the production of tofu. At a certain range, the texture of tofu was not significantly affected by the addition of okara. $CaSO_4$ showed different response to the addition of okara comparing with GDL. 15% addition of okara increased the break stress of $CaSO_4$-coagulated tofu while the addition of okara decreased the break stress of GDL-tofu.

Based on the results in this study, it can be suggested that for industrial hard tofu production in China, using properly high concentration of soymilk, mixture of coagulants, such as $CaSO_4$ with $MgCl_2$, GDL with $MgCl_2$, increasing the concentration of coagulants like $CaSO_4$ and GDL and adding okara can be reasonable alternatives.

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