PHYSICOCHEMICAL STUDIES ON SOL–GEL TRANSITION OF FOOD HYDROCOLLOIDS

KAORU KOHYAMA

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CONTENTS

INTRODUCTION

Lifelong dietary habits have been attributed as the leading factors to why life expectancy in Japan is the highest in the world. Japanese people enjoy many kinds of foods made from a variety of starting materials and ingredients, though usually in small quantities. One of the more peculiar characteristics of Japanese cuisine is the variety of textures. "Konnyaku" is a typical example; Japanese eat it merely to enjoy the texture, even though it is neither nutritious nor tasty from a biological or chemical point of view. Foreigners tend to avoid eating konnyaku because of its very curious texture and lack of taste.

Food hydrocolloids, the theme of this study, are utilized to modify food texture. Food research itself is an applied area of science, but basic research is necessary to elucidate the functional characteristics of food hydrocolloids to help facilitate their further development. Various components and agricultural products have been separately treated in food science, leaving researchers on different materials with relatively few opportunities to discuss recent findings. The annual meeting named "Symposium on Physical Properties of Foods", which celebrates its 20th anniversary in this year, has given researchers in different areas of food science just such opportunities. While not limited to hydrocolloids, the meeting is beneficial in that participants can discuss the problems of food hydrocolloids and exchange ideas about their properties in order to develop their utilization. This is the purpose of the international conference, "Gums and Stabilisers for the Food Industry" held every other year since 1981. Another international conference "Food Hydrocolloids '92 Tsukuba" was organized by Prof. E. Doi (Research Institute for Food Science, Kyoto University) as the chairman. It is certain that more people, both in academia and industry have taken an interest in food hydrocolloids, though only a few researchers are on this theme in Japan. The outlook on food hydrocolloids is described in the following chapter.

In this study, physicochemical properties of some food hydrocolloids are discussed. The physicochemical methods used in polymer science are adopted to elucidate sol and gel state of the hydrocolloids, with particular emphasis on the sol-to-gel and gel-to-sol transitions. Methodologies are discussed in Chapter II. The raw materials are chosen to develop the hydrocolloids in traditional Japanese foods for new areas; starch from sweet potato root (Chapter III), soybean proteins from domestic varieties (Chapter IV), and konjac glucomannan (Chapter V). The systems in this study could be considered as models of real foods. Since a practical food system includes water and a number of other components, interactions between two hydrocolloids or between the hydrocolloids and the other components such as sugars and water are investigated.

Chapter I
OUTLOOK ON FOOD HYDROCOLLOIDS

I-1. DEFINITION AND CLASSIFICATION

What are food hydrocolloids? Food hydrocolloids are literally hydrophilic colloids contained in food or colloidal particles dispersed in water. Since polysaccharides and proteins belong to colloidal materials, almost all foods contain hydrocolloids, except liquids such as oil and water, crystals such as sugar and salt, and dried foods not containing water.

Then, what are colloids? Two answers could be given (Nakagawa, 1990). The first is that colloids as a material, defined by Thomas Graham (1805–1869), who is considered a pioneer of colloid science, could be applied. Colloids are materials, like starch, gelatin and egg white, which show unusually slow diffusion (Graham, 1861), are retained by parchment membranes, and do not easily crystallize (Dickinson and Stainsby, 1982). The second answer is based on the view of colloids as a state according to Wolfgang Ostwald (1883–1943). A colloids is a system containing particles of size from about 1 nm to 1 μm (Dickinson, 1992), therefore this size is called the colloidal dimension (Nakagawa, 1990). Thus colloid chemistry treats the colloidal particles such as gold sol, micelles and polymers together with a similar approach (Nakagawa, 1990).

With reference to the first definition, food hydrocolloids would thus be viewed as macromolecules in foods. Since, chemically, they are either polysaccharides or proteins, the following methods in studying the food hydrocolloids could be useful; physicochemistry, biochemistry, nutritional science, and food engineering, etc. If we consider the second definition, food hydrocolloids may be colloidal dispersions such as gels, sols, foams, or emulsions. Both sol and gel are systems of solid particles of colloidal dimension dispersed in a liquid medium. A sol shows liquid-like behavior, while overall properties of a gel are solid-like (Dickinson and Stainsby, 1982). The methods of colloid and interface science, polymer science, and sensory evaluation may be the key.

Many food hydrocolloids are components from natural resources. The origin may be plants, animals or microorganisms. Many plant hydrocolloids are utilized for foods; for examples, starch from cereals or tubers, pectin from fruits, gum arabic and various seed polysaccharides, agar etc. from seaweeds. They produce not only saccharides but also proteins such as soybean globulins. Animal proteins, such as those found in milk, eggs, and muscle, represent a significant portion of proteins that humans consume. Recently, polysaccharides from sea animals including chitin and chitosan have drawn researchers' interest. Food researchers are also closely examining microbial polysaccharides, for instance, xanthan, curdlan and gellan.

Others are artificial colloids, made from natural colloids by modifying chemically or enzymatically, although to the author's knowledge, no food hydrocolloid has been totally synthesized for practical purposes. Cellulose itself does not dissolve in water, however the modified celluloses such as methylcellulose and sodium carboxymethylcellulose are water-soluble hydrocolloids and are allowed to be used for foods (Hidaka, 1990). Modified starch and alginate are another examples (Hidaka, 1990).

For practical purposes in the food industry, functions of food hydrocolloids are very important. Food hydrocolloids have been widely used for increasing viscosity, forming gels, binding, holding water, preventing syneresis and crystallization, foaming, emulsifying, stabilizing emulsions and dispersions, and making capsules, etc. Recently, more improved functions such as substituting fat and forming films are also applied for making low-calory foods and card-type foods.

I–2. ROLE OF FOOD HYDROCOLLOIDS

One may consider food hydrocolloids from two ways: first, that hydrocolloids may typify actual food systems, and secondary, that they function as food additives.

As mentioned above, almost all foods contain mostly water and numerous colloidal particles. Moreover, hydrocolloids are often the main components of foods. For example, cooked rice, the most important food in Japan, is considered a hydrogel of starch. Of course this may be a simplified view, however, hydrocolloid gels can be used as a model of real foods. Even though foods include many components which interact, the author believes that a simple system or model is perhaps the best approach to elucidate physicochemical properties of food.

On the other hand, hydrocolloids can be utilized as food additives which improve both taste and functional properties of foods. Fundamentally, foods should be hygienic and digestible. However, beyond these properties most consumers are conceived with organoleptic properties of foods, particularly texture. Since food texture is understood to be the sensations of touch or feel by the human hand and mouth (Kramer, 1973), the texture depends on thermal, structural and rheological parameters of food. To affect texture, hydrocolloids are usually added to food only in small amount, but can dramatically alter the texture. That is why food hydrocolloids that are added to foods are called texture modifiers.

I–3. FOOD HYDROCOLLOIDS IN JAPAN

Japanese consume a great deal of dietary fiber. The dietary fiber is found not only in vegetables and cereals, but also in seaweeds. In addition to cellulose materials from various of vegetables, other polysaccharides are part of the Japanese diet (Nishinari, 1988). Among them is agar, a polysaccharide extracted from red seaweeds, which has been utilized as a texture modifier since 17th century (Matsuhashi, 1990). Another polysaccharide, konjac glucomannan, has been used to make konnyaku gels for about 700 years (Kohyama, 1992).

There are also several gels made from proteins. Tofu (soybean curd) and other soy protein products, belonging to traditional Japanese foods, are common examples (Saio and Watanabe, 1978; Shurtleff and Aoyagi, 1979). Kamaboko, another example of protein gel which originated in Japan, is made from surimi or ground fish meat.

The gels mentioned above are habitually eaten because the texture is preferred. Especially, agar and konjac mentioned above have little or no nutritional value, but the texture is loved for many years. In the recent several decades, Japanese diet has been richer, we can afford enjoying various foods. Today's researchers are required to study the quality of food, including texture of agricultural products, as well as their quantity and the safety.

In those circumstances, the role of hydrocolloids in Japanese foods has become more and more important. Recent development in the food industry and knowledge about food hydrocolloids have produced numerous foodstuffs such as crab–kamaboko (kamaboko with crab taste and texture) and imitation ikura (salmon roe) (Nishinari, 1988).

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Capter II

METHODS IN PHYSICOCHEMICAL PROPERTIES OF FOOD SOLS AND GELS

Thermal and rheological properties of foods are very important in food manufacturing, processing and cooking. The two properties interact with each other; rheological properties much depend on the temperature. Transitions of gel–to–sol (melting) and sol–to–gel (gelation) are often induced by temperature change. Therefore, the mothods for determining the thermal and rheological properties of hydrocolloid sols and gels merit discussion.

In the first section, an automatic determination of the melting point for thermo–reversible gels and a theoretical approach to sol–to–gel and gel–to–sol transitions using a zipper model are described. Next, rheological observations of gelation phenomena including gelation time are presented. Some problems in rheological measurements of food gels commonly encountered are discussed in the third section.

II-1. THERMAL PROPERTIES

INTRODUCTION

Thermal properties of gels, particularly melting temperatures, are very important in food manufacturing, processing and cooking. Among the various which have been used to measure the melting points of gels include: the observation of flow when a gel in a glass tube is heated (Harrison et al., 1971; Wellinghoff et al., 1979), the measurement of displacement of a mercury drop or a small metal ball put on a gel (Paul, 1967; Takahashi et al., 1980; Okabe et al., 1985a) and the observation of the endothermic peak by differential scanning calorimetry (DSC) (Godard et al., 1978; Watase and Nishinari, 1985; Domsy et al., 1986). Djabourov et al. (1988a) studied the melting behavior of gelatin gels by optical rotation measurements. They found that the derivative of the optical rotation angle as a function of temperature had a peak which indicated a melting temperature of the helices. They correlated their data with those obtained from DSC endotherms.

The so-called falling-ball method is commonly used for determining the melting point. Here, the displacement of a small ball put on the surface of the gel is observed by a cathetometer, raising the temperature at a constant rate. Takahashi et al. (1980) studied melting point of gels using a ball of about 100 mg weight. Okabe et al. (1985a, b) used balls of 30 mg and 2 mg for low density polyethylene gel in organic solvents. This method was considered tedious in that it required an observer to carefully follow the displacement of a ball over a long period of time. The gel-to-sol transition temperature determined by the falling-ball method agrees with thermodynamic melting point method by DSC (Domszy et al., 1986). Tan et al. (1983) reported that the melting points of gels obtained by falling-ball method and by test tube tilting, were identical within ± 2 °C. We developed an automatic system for the determination of gel melting. This method is based on falling-ball method. A digital camera, in place of a human eye, observes the displacement of a small ball placed on the gel.

MATERIALS AND METHODS

Agarose samples

The agarose powders (Taiyo-Agarose AG-104903 (sample 1) and AGL-800 (sample 2), Taiyo-Agarose Co. Ltd., Shimizu, Japan) were used without further purification in this study. The characteristics of these powders are shown in Table II-1. The powders were allowed to swell overnight in distilled water in a 40°C water bath and a magnetic stirrer.

The sample was then preheated at 70 °C for 1h and dissolved completely at 100 °C for 1 h. The molds, glass tubes (7.7 mm dia. X 50 mm) with a pin hole bored at a 15 mm distance from the bottom, were set upside down in a 100 mL beaker. The hot agarose solution was poured into the beaker and cooled slowly to room temperature. The gel was annealed at 5 °C for 2 days and was left at room temperature for 1h before the measurement was taken. The concentration of gels was determined by dry weight method.

Measurement System

The entire system used in this study is schematically illustrated in Fig. II-1. A stainless steel ball was put on the gel. The surface of the gel was covered with silicone oil in order to prevent evaporation of solvent water. The heater sheet (Pattern B, Sakaguchi Dennetsu Co. Ltd., Tokyo) was wound around the glass tube. A thermocouple was inserted into the pin hole in order to monitor the temperature of the gel. The gel was heated from room temperature to the melting point at a constant rate, controlled by a micro-computer (REX-P100, Rika Kogyo Co. Ltd., Tokyo). The relationship between the output voltage of the thermocouple and the temperature was linear in the range used in this expetiment. The difference of temperatures between those of the controller and the thermocouple was not more than 0.1 °C.

The displacement measuring system consists of a digital camera system (SC700B, NEC Corporation, Tokyo) and a displacement data processor. The digital camera system is composed of a main body, containing a linear array of 256 photodiode cells, a lens system and a camera controller. The displacement data processor transforms signals from the camera controller into a signal proportional to the displacement of the ball.

Structure and an operation of the displacement data processor are illustrated in Fig. II-2. The data processor consists of four main blocks; a binary counter, a latch, a binary D/A converter and an amplifier. The constituent IC's are two SN74191's for the binary counter, two SN7475's for the latch, an MN333 for the binary D/A converter, and an LF356 for the amplifier. In addition to them, two auxiliary IC's, a NAND gate (SN7400) and an inverter (SN7404), were actually inserted into the circuit.

The operation of the displacement measuring system is explained in detail as follows. The gel and the ball are illuminated by a lamp and their optical image is formed in the plane of the photodiode array in the camera through the lens system. The camera is placed so that the photodiode array lies parallel to the direction of the displacement of the ball, and that the first cell corresponds to the lowest point in the field of view. Since the agarose gel is almost transparent and the stainless steel has a high reflectivity, the image splits into dark and bright areas, corresponding to the gel and the ball respectively, together with a sharp border between these two areas. The light intensities of the image at 256 cells in the photodiode array are scanned and read by the camera controller. By comparing with a certain preset threshold intensity level, the camera controller digitizes them and creates a sequence of one-bit pulses called light-levels (LL). The controller also provides some control signals; clock (CK), start (ST), enable (ENA), and end-of-scan (EOS).

In the beginning of one scanning the value of LL is "low", due to the low light intensity of the image of the gel. At the border between the dark and bright areas, however, the detected light intensity comes to exceed the threshold level and LL turns to "high". LL retains this value

until the end of the scanning, since the controller is designed to hold the LL signal once it turns to "high". Each step in the scanning is synchronized with CK. Then it follows that the position of the border can be detected by counting CK pulses with the binary counter during the period when LL is "low" and ENA is "high" in one scanning. ST and EOS indicate when the counting process should start and end, respectively. In this way the position of the ball is digitized into some integer between 0 to 255.

The scanning is repeated many times during the measurement. The latch retains a value of the output from the binary counter, until it is refreshed by EOS at the end of each scanning. The latched signal is subsequently fed into the D/A converter, being converted into an analog signal. Finally this signal is amplified by the operational amplifier so as to have a negative voltage (0 to −9.961 V) corresponding to the output (0 to 255) of the binary counter. This voltage signal, which is proportional to the vertical displacement of the border between the gel and the ball, is sent to a recorder for the simultaneous recording of displacement and temperature (Fig. II−3). The melting point (T_m) of the gel was determined as the temperature at which the displacement rapidly changed (Shown by an arrow in Fig. II−3).

Differential Scanning Calorimetry (DSC)

In order to test the correlation between the above method and DSC, measurements were carried out by use of a SSC580 apparatus with a DSC20 module (Seiko Instruments Inc., Tokyo). About 40 mg of the sample gels were hermetically sealed into a silver pan of 70 μL. Water was used as the reference. The temperature of the pan was raised from 20 to 100 °C using heating rates of 0.1, 0.5, 1.0, 1.5 and 2.0 °C/min. T_m of the gel was determined as the peak temperature of a DSC endotherm.

RESULTS

Dependence of Melting Point on Heating Rate

Melting points of gels shifted to higher temperatures using heating rates faster than 0.5 °C/min (Fig. II−4). Conversely, the melting point of the gel was not significantly effected at a heating rate less than 0.5 °C/min. Therefore, the heating rate was fixed at 0.5 °C/min hereafter.

Dependence of Melting Point on the Weight of Steel Ball

A heavy ball destroyed a gel mechanically and fell down before the temperature of the gel was raised. In the present study, a ball of 110 mg weight was too heavy for agarose gels. We used 32.4 mg ball bearings hereafter because lighter balls did not sink even after the gels (2 %) melted. Gels of low concentrations (*i.e.* 0.2 % and 0.4 %) were not examined using a ball of 32.4 mg.

Dependence of Melting Point on Concentration of Gels

The melting point of the gels raised with increasing the concentration. Using van't Hoff's law, Eldridge and Ferry (1954) proposed the following equation:

$$\log C = -\frac{\Delta H_m}{2.303RT_m} + \text{const.} \qquad \text{(II−1)}$$

where C is the concentration of gels, R is the gas constant, and T_m is the melting temperature in degrees Kelvin. $-\Delta H_m$ represents the heat absorbed on forming a mole of the junction zones that stabilize the network structure of the gel.

The plot of the logarithm of concentration C (wt.%) of agarose against the reciprocal of the melting point T_m is shown in Fig. II−5. Since the relationship was found to be linear, equation II−1 may be used to calculate the heat of reaction $-\Delta H_m$. $-\Delta H_m$ for the present samples were evaluated as 2.7×10^2 kJ/mol (sample 1) and 3.7×10^2 kJ/mol (sample 2) from the slopes of the best straight lines drawn through the observed points by the least square method.

DISCUSSION

Energy for Forming Junction Zones

It is not evident whether the underlying assumptions of the Eldridfe−Ferry analysis is applicable or not for all the thermoreversible gels, although the analysis has been widely used. The first application of the Eldridge−Ferry theory to determine the heat of reaction for agar−agar gels was performed by Tanii (1957). The $-\Delta H_m$ values for 7 kinds of commercial agars ranged from 2.2×10^2 to 1.1×10^3 kJ/mol were determined. Gels with higher melting points showed larger values of $-\Delta H_m$. According to Tagawa (1968), who obtained $-\Delta H_m = 8.9 \times 10^2$ kJ/mol for his agarose gel, the $-\Delta H_m$ value was not so different from that for agar−agar gels. This is consistent with established observations that agarose is a main component which governs the thermal and rheological properties of agar−agar gels (Tagawa, 1968). The observed values in the present work $-\Delta H_m = 2.7 \times 10$ and 3.7×10^2 kJ/mol reside at the lower value range of Tanii's data. This is reasonable since our observed melting points are low relative to Tanii's results (1957).

The enthalpy change $-\Delta H_m$ in Eldridgre−Ferry analysis represents the heat absorbed in forming a mole of junction points that stabilize the network structure of the gel. Since the applicability of the underlying assumptions is not well established in many cases, the observed value of $-\Delta H_m$ cannot have such a clear physical significance. However, there seems to be some relationship between $-\Delta H_m$ and the melting temperature T_m: the higher T_m of gels, the larger $-\Delta H_m$. Tan et al. (1983) examined the melting temperature of atactic polystyrene gels of various molecular weights, and found that gels of higher molecular weight showed a higher T_m. They also found that $-\Delta H_m$ increased with increasing molecular weight.

Comparison with DSC method

DSC measurement is widely used for determination of gel melting point because it can be automated and requires a small amount of sample (Watase and Nishinari, 1985, 1986; Domszy et al., 1986). The same agarose gels which were used for melting point measurements

by falling-ball method were used for DSC measurement in this work. But, because of the lower sensitivity at slow heating rates such as 0.5 °C/min, a clear endothermic peak which accompanies the melting or the transition from gel to sol was not observed. In gels of lower concentrations than 1.2 %, the endothermic peak could not be detected even at heating rate of 2.0 °C/min. When the heating rate was faster than 2.0 °C/min, the endothermic peak accompanying the transition from gel-to-sol was observed. However, the temperature of this endothermic peak shifted to higher temperatures with an increasing heating rate. Therefore, this peak temperature cannot be adopted as a melting temperature.

The DSC method is convenient for repeating measurements after a given time period or for observing both the melting and gelation behaviors of thermoreversible gels as also mentioned in Chapters III and V. Recently, a more sensitive DSC apparatus has become available. Relatively large amount of samples (~950 mg) can be used to detect the melting and gelation temperatures of dilute systems of gellan gum (Moritaka et al., 1992).

The method was effective automating the measurement of melting point of gels without impairing the accuracy.

Zipper Model

It has been widely accepted that most gels consist of somewhat crystalline regions, called junction zones, and somewhat amorphous regions. The structure of junction zones depends on the molecules which form the gels. Recently, the endothermic peaks in DSC curves for the gel-sol transition of thermoreversible gels were explained by using a zipper model (Nishinari et al., 1990).

As a model for structures of junction zones, the association of molecular zippers represents a rigid ordered molecular structure such as helices or extended molecules. The disappearance of crystalline region in gels is deemed as the opening process of molecular zippers. A junction zone consists of either an association of helices (single, double or triple) or that of rod-like molecules. The molecular forces which make these helices or rods aggregate are generally believed to be secondary forces such as hydrogen bonds rather than covalent bonds because the disruption of the covalent bond needs much higher energy than experimentally observed values. Each molecular zipper consists of N parallel links that can be opened from both ends (Fig. II-6). When the links 1, 2, ..., p are all open, the energy required to open the p+1 st link is assumed to be ε. Is is supposed that each open link can assume G orientations, *i.e.*, the open state of a link is G-fold degenerate, corresponding to the rotational freedom of a link. According to this treatment, the heat capacity C of such a system consisting of $\mathcal{N}$ zippers is written as follws:

$$\frac{C}{k} = \mathcal{N}(\log\frac{G}{x})^2 \left[\frac{2x}{(1-x)^2} + \frac{N(N+1)x^N\{-x^{N+1}+(N+1)x-N\}}{\{1-(N+1)x^N+Nx^{N+1}\}^2}\right] \qquad \text{(II-2)}$$

where k is the Boltzmann constant, G is the degree of rotational freedom of a link, $x = G\exp(-\varepsilon/\tau)$, where ε represents the energy required to open the link, and τ is the product of k and the absolute temperature. First application of this model to gelatinization of starch observed in heating DSC curve is described in Chapter III.

An endothermic peak accompanying a gel-to-sol transition in a heating DSC curve as well as an exothoemic peak accompanying sol-to-gel transition in a cooling DSC curve is equivalent to the maximum of the heat capacity. When the temperature is raised from a lower temperature than the gel-to-sol transition temperature (T_s), G should start from the lower value G_g corresponding to the gel state. The gel would expand, giving rise to an increase in the rotational freedom. However on the contrary, when the temperature is lowered from higher temperatures than sol-to-gel transition temperautre (T_g), G will start from the higher value G_s corresponding to the sol state. Therefore, the opening of molecular zippers begins to occur at small G values in the heating process, while gelation by cooling will take place with a decreasing G, starting from large G values at higher temperatures. The average effective value of G is small in heating process and is large in cooling. As a first approximation, therefore T_s is determined by a certain average $G_{av,g}$ of G for gel state and T_g is determined by an average $G_{av,s}$ of G for sol state. Apparently, $G_{av,g} < G_{av,s}$; hence, T_s is expected to be higher than T_g.

In the case of heating, the transition caused by the opening of the zippers will start as soon as the temperature arrives at (the tail of the C-T curve corresponding to) $G = G_g$. Upon cooling, on the contrary, the pair-wise coupling cannot start very easily because of the difficulty for a long molecule to find its partner in the appropriate positions for the zipper construction. Hence supercooling may take place during the course of cooling. It is, therefore, reasonable to assume that the transition is sharper in cooling than in heating.

Table II-1. Characteristics of agarose samples.

	sample 1	sample 2
moisture content (%)	10.5	10.8
ash (%)	0.2	0.2
calcium (%)	0.03	0.03
sulfate (%)	0.15	0.15
gel strength [a] (kgf/m^2)	$7.4X10^3$	$8.0X10^3$
viscosity [b] (Pas)	$5.4X10^{-1}$	$5.5X10^{-1}$

a) 1.5 % gel, at 20 °C by a Japanese Agar Gel Tester
b) 1.5 % sol, at 80 °C by a Brookfield viscometer

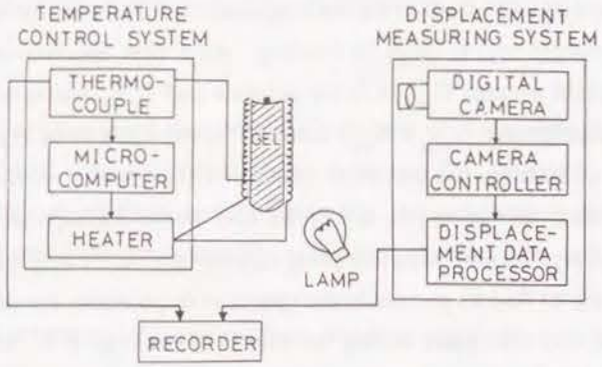

Fig. II-1. Schematic diagram of gel melting point measuring apparatus.

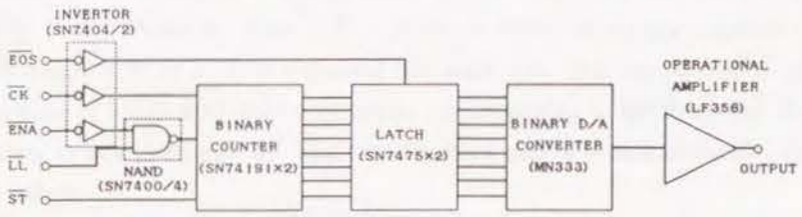

Fig. II-2. Block diagram of the displacement data processor.

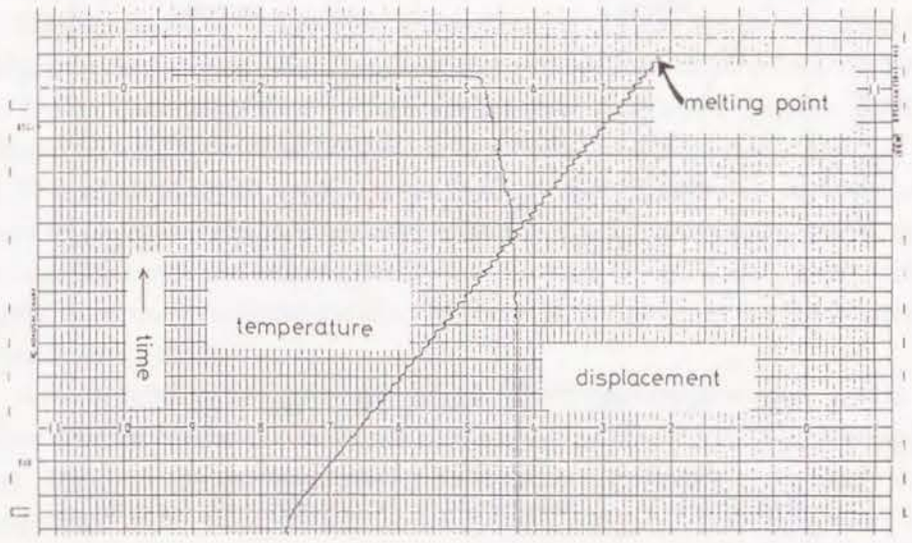

Fig. II-3. A typical output pattern of measuring system.

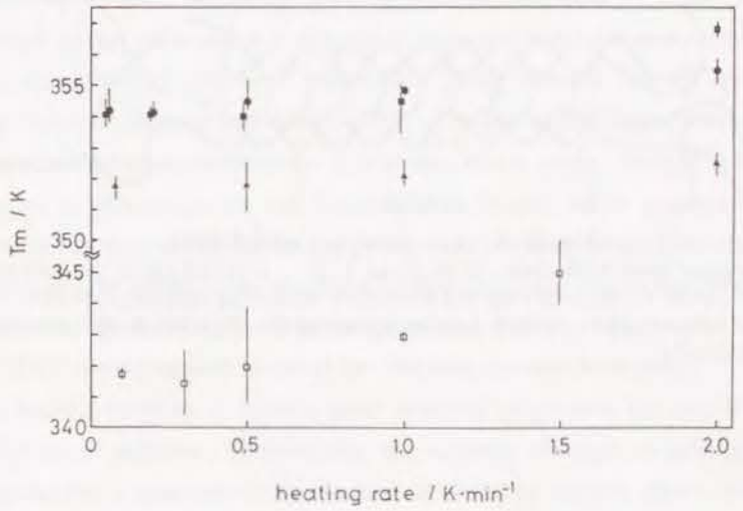

Fig. II-4. Dependence of gel melting points T_m on the heating rate.
Weight of stainless steel ball: 32.4 mg.
Concentration of agarose gels: sample 1□; 1.284 wt.%,
sample 2▲; 0.952 wt.%, ■; 1.534 wt.%, ●; 2.048 wt%.
Bars attached to symbols represent the experimental error.

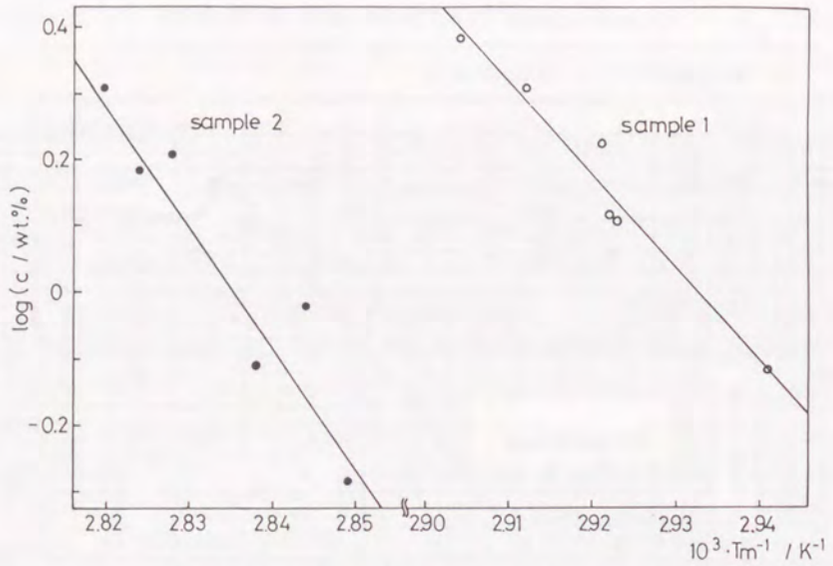

Fig. II–5. Eldridge–Ferry plot for agarose gels.
Heating rate: 0.5 °C/min, weight of stainless steel ball: 32.4 mg.

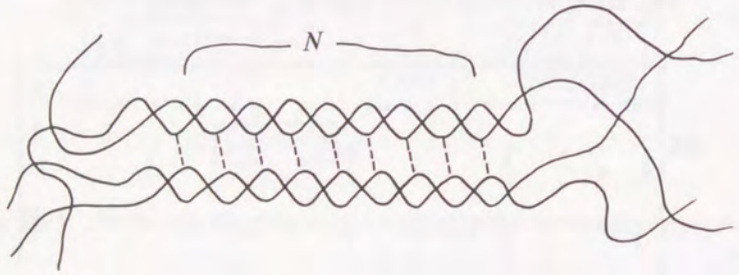

Fig. II–6. A single zipper with N links.
Links can be opened from both ends. If the links 1, 2, ..., p are all open, the energy required to open the p+1 st link is ε. Helices may not necessary be double helices; they may be single helices or triple helices. Here, double helices represent the association of somewhat ordered structure symbolically.

II–2. SOL–TO–GEL TRANSITION AND GELATION TIME

The definition of a 'gel' remains somewhat ambiguous. A gel, as it is said, is easier to recognize than to define (Dickinson and Stainsby, 1982; Almdal et al., 1993). Phenomenologically, a gel is like a jelly, a soft and solid–like material.

Classical theory for gels by Flory and Stockmayer explains that the sol–to–gel transition or gelation is due to the formation of networks of indefinite extent (Flory, 1942; Stockmayer, 1943). Their definition of polymer gels are three–dimensional polymers of infinitely large molecular weight, which are insoluble in all solvents that do not cause degradation (Flory, 1941). This polymer network structure is one typical form of food gels. However, the network is formed by physical aggregation of chains in most food gels, for examples, gelatin, agar and carrageenan (Dickinson and Stainsby, 1982). Another form of a gel is the network formed by aggregated dispersion of colloidal particles. Milk curd is an example of this type of gel (Dickinson and Stainsby, 1982).

A recent definition of a gel by Almdal et al. (1993) states: A gel is a soft, solid or solid–like material which consists of two or more components; one of which is a liquid, in substantial amount. The solid–like gels are characterized by a storage modulus which exhibits a pronounced plateau on the order of at least several seconds, and by a loss modulus which is considerably smaller than the storage modulus in the plateau region (later the details about storage and loss moduli are described). According to Almdal et al. (1993), a material which exhibits a storage modulus of 10^8 Pa is considered to be too rigid and does not fall under this definition.

Since sol–to–gel transition is analogous to a second–order thermodynamic phase transition and a typical critical phenomenon, percolation theory characterizes the critical point with the following variables (Martin and Adolf, 1991). The probability that a monomer belongs to the infinite network becomes nonzero for gels at the critical point. Weight average molecular weight or degree of polymerization and the correlation length, which measures the size of the largest cluster of the macrolinks in the gel phase, read infinite values (Stauffer et al., 1982). Therefore, time corelation function obtained by dynamic light scattering (Martin and Adolf, 1991; Lang and Burchard, 1991; Coviello and Burchard, 1992) or small angle X–ray scattering (Ando et al., 1993) measurements is useful for checking the transition point.

A mechanical property is anothor good indicator of gelation because it sensitively reflects the initiation of gelation. Theoretically, the viscosity diverges to infinity slightly below the gelation point and a nonzero elastic modulus is observed slightly above the gelation point (Stauffer et al., 1982). Adam et al. (1981) first applied the percolation theory for analizing viscosimetric data for two types of gelation phenomena (a radical copolymerization and a polycondensation), and obtained the critical exponent value for viscosity data which was in good agreement with three–dimensional percolation predictions. Besides numerous studies on synthetic polymers (Martin and Adolf, 1991), the gelation phenomena in biopolymers including

agarose (Tokita et al., 1987), pectin (Axelos and Kolb, 1990), gelatin (Djabourov et al., 1988a, b), and the casein micelle (Tokita, 1989) have been analyzed. Gautier-Manuel et al. (1987) reported that a sharp increase in the viscosity followed a continuous increase from zero in elastic modulus for silica, experimentally. More recently, concentration dependence of viscosity and dynamic viscoelasticity for gelatin was analyzed by an application of the percolation theory (Kumagai et al., 1993). Some rheological methods were applied for determining the gelation time in this work.

Low-shear Rate Viscosity

As mentioned above, viscosity sharply increases to infinity at the gelation point (Stauffer et al., 1982). However, experimental observations of this phenomenon were scant (Adam et al., 1981; Gauthier-Manuel et al., 1987; Axelos and Kolb, 1990; Kumagai et al., 1993). Adam et al. (1981) pointed out the difficulty in removing the effect of the hydrodynamic interactions to culculate the divergence of the viscosity. A very low shear rate is necessary for an accurate measurement of viscosity over a wide range. The gelation process of soybean 7S or 11S globulin in the presence of glucono-δ-lactone (GDL) (the behavior is described in deteil in Chapter IV) was traced with a rotational viscometer (Low-Shear 30, Contraves, Türich). A sample vessel of 12 mm dia. X 8 mm was held a constant temperature with a circulater (RMS6, Lauda, Pfarrstrasse, Germany). A sol sample (~0.8 ml) was put into the vessel. A coaxial measuring bob with 11 mm diameter inserted into the vessel. The vessel was then rotated with a low shear rate of 0.11 s^{-1}, which is the 7th dial among 30 shear rates supported by this apparatus ranging from 3.5 X 10^{-3} to 2.5 X 10^{2} s^{-1}. The viscosity was calculated from the indicated torque. The minimum detectable viscosity was 1.5 mPas. The sharp increase in viscosity began at a certain time after the addition of GDL but before the storage modulus (observed with a Rheometrics's RFS-II, see below section) began to rise both for the 7S and 11S sols (data not shown). However, nonzero storage modulus was observed before the detected viscosity became infinite, perhaps due to the difference in shear systems of the two apparatuses. Unfortunately, the viscometer did not have oscillatory facilities which is necessary to measure the viscoelasticity.

Damped Oscillation Rheometer

There are several rheological methods to observe gelation phenomena. However, these method, such as the latter method, often lack the sensitivity to a measure viscosity change at the initial stage of gelation. Few apparatuses can continuously measure a very low (from water level) viscosity change at a low cost.

Recently, Kaibara and Date (1985) developed a new type of rheometer consisting of a cylindrical tube suspended from a torsion wire and filled with the sample liquid. Fig. II-7 shows the schematic diagram of the damped oscillation rheometer (Reo-2, Shinku-Riko, Inc., Yokohama). The rheological system was the same as the original version, Reo-1 (Kaibara and

Date, 1985) designed for hemorheology, however we modified it to change the sample temperature appropriate for food science. A tube containing the sample was set on a holder with an 11.8 mm diameter in the upper chamber. The measuring system consisting of the coil and tube was suspended from a torsion wire passing through an aluminum tube. Fortunately, various kinds of vessels of various sizes were available for this apparatus. A polypropylene tube of 1.5 mL (No. 72692, Sarstedt, Nümberecht, Germany) was used as a sample container. A direct current was introduced to the coil to produce an initial rotational displacement (θ_0) to the measuring system and then the holder was locked at a certain fixed angle. The current was discontinued at the measuring time set by a micro-computer. Subsequently, the system started rotational oscillations in a magnetic field, thus producing an induced electromotive force. As a result, a damped oscillation curve was obtained. An example of the curve is shown in Fig. II-8. The output voltage was amplified and fed to a computer. The logarithmic damping factor (LDF), which is defined in equation II-3, is derived.

$$\ln(\theta_1/\theta_3) = \ln(\theta_3/\theta_5) = \text{---} = \ln(\theta_2/\theta_4) = \ln(\theta_4/\theta_6) = \text{---} = \text{LDF} \qquad \text{(II-3)}$$

where θ_i (i=1, 2, 3, ---) is the displacement angle of i-th oscillation (see Fig. II-8). The initial displacement was repeatedly given and the displacement angle was set at 10 degrees in these traials. The apparatus was totally controlled by a micro-computer. The temperature of the sample chamber was kept at 60 °C by a thermo-controller. The temperature scanning was also available by means of the controller.

Fig. II-9 shows changes in the LDF as well as the period of the damped oscillation curves plotted against the viscosity for various liquids (methanol, ethanol, and 8 standard oils of viscosity). The LDF value increased slightly to a maximum at approximately 3.7 mPas in viscosity, and then decreased sharply with increasing viscosity. The period increased and then levelled off with increasing the viscosity. These observations were similar to those reported by Murata et al. (1987).

Although the relationship between absolute viscosity and the LDF value for Newtonian liquids was analyzed (Murata et al., 1987), that for non-Newtonian liquids has not been derived. Since an increase in viscosity is observed in the gelation process, the LDF should increase at the first stage of a gelation and then decrease. In order to follow the gelation phenomenon, particularly gelation time, then change in LDF would be sufficient. Differences in the absolute LDF value caused by sample containers, therefore, were not important for the present experiments, thus disposable tubes with caps may be used to prevent the samples from drying out. Gelation of soybean proteins with GDL was tested. The increase in LDF was detected before the viscosity (detected by a Low-Shear 30 mentioned above) began to increase (data not shown). The mid point between the LDF maximum and its minimum was delayed according to the gelation time determined by the storage modulus measurement (see the next section). One of the reasons considered was that the low thermal conductivity of the atmos-

phere around the sample container. Even though the amount of the sample was small (1.0 mL), the thermo-controller indirectly heated the sample liquid. This apparatus could be improved more practical applications in food science.

Dynamic Viscoelasticity

Sol-to-gel and gel-to-sol transitions of a thermoreversible gel were observed by a dynamic viscoelasticity measurement with two Rheolograph Sol apparatuses (Toyoseiki Seisakusho, Tokyo) (Kaibara and Fukada, 1976, 1983). A block diagram for the instrument is shown in Fig. II-10. A hot sol (~1.6 mL) was put into the parallel plate-type cell with internal dimensions 2.6 X 15 X 45 mm. Then, the measuring blade (0.6 mm thickness X 10 X 25 mm) was inserted into the cell. The sample temperature is lowered from temperature higher than the sol-to-gel transition temperature at a constant cooling rate and then heated until the gel melted at the same rate with a thermo-module controlled by a micro-computer. The sample solution was then subjected to sinusoidal shear oscillations. One of the apparatuses applied an oscillation of 2 Hz and an amplitude of 125 μm, so that the shear strain was 0.125. Another allowed for the selection of amplitude (25, 50 and 100 μm) and frequency (0.1, 0.3, 1.0 and 3.0 Hz). The stress, as a response, was divided into a component in phase with the strain (real part, storage modulus) and a component $\pi/2$ out of phase with the strain (imaginary part, loss modulus) by a computing circuit. Values for the storage and loss moduli (G' and G'') were recorded.

A gelation process was also investigated by the same apparatus to observe G' and G'' at a constant temperature as a function of time. Both G' and G'' increased with time during the gelation process. A typical sol shows smaller G' values than G''. However, G' increases more rapidly than G'' and exceeds G''. The storage modulus keeps increasing with increasing cross-link density while the loss modulus goes through a slight maximum. Both moduli level off as the reaction comes to completion (Winter and Chambon, 1986). Therefore, the gelation time has been defined by the cross-over of the moduli (Djabourov et al., 1988b; Axelos and Kolb, 1990; Cuvelier et al., 1990; Lin et al., 1991; Fernandes et al., 1992). However, the cross-over point depended on the frequencies for gelatin in water; *i.e.*, a shorter gelation time was observed at lower frequencies (Djabourov et al., 1988b). A large shear rate by high frequencies may destroy the network structures formed at the initial stage of the gelation. On the contrary, a longer time required for one measurement at lower frequencies may result in failing to detect the very moment of the gelation. Since the above mentioned apparatus did not support lower frequencies than 0.1 Hz unfortunately, a more precise rheometer, RFS-II (Rheometrics, Piscataway, NJ, USA) was used to measure the same system used in Chapter IV. A sample solution (~10 mL) was poured into a double Couette-type of cell at 60 °C. Then rotational oscillations (10^{-2}–10^{2} rad/s) with a strain of 0.025 were applied. This apparatus can detect a modulus of $>10^{-2}$ Pa. Both G' and G'' increased more than 10^{3} times in several seconds near the gelation point (data not shown). Therefore the gelation time was defined as the time at which G' steeply

increased in this study, the error caused by relatively high frequency and strain of a Rheolograph Sol apparatus seemed to be negligible.

Winter and Chambon (1986) proposed a new method for detecting the transition point in a crosslinking reaction of polydimethylsiloxane. Detected dynamic moduli at the transition points have the following relationship (Winter, 1987):

$$G' = G''/\tan(n\pi/2) \qquad \text{(II-4)}$$

where n is the network specific relaxation exponent. When the end-linking networks are stoichiometrically balanced, n becomes 0.5, therefore the storage modulus has the same value as the loss modulus at the transition point for any frequency. While some stoichiometrically imbalanced systems have a different relaxation exponent, where $n>0.5$, the crossover of G' and G'' occurs bofore the critical point. Then, the critical point must be defined as the point at which $\tan\delta=G''/G'$ is constant for all frequencies below a limit frequency (Martin and Adolf, 1991).

Most food gels involve both physical and chemical networks (Dickinson and Stainsby, 1982). Generally, food systems are heterogeneous and metastable, *i.e.*, thermodynamically unstable (Dickinson and Stainsby, 1982). Their properties may not be constant during a measurement for a long time or at a low frequency, since the systems are not in equilibrium. Therefore, the method of Winter and Chambon (1986) for determining the critical gel point of an equilibrium system does not always appropriate for food systems. The author is not aware of the best method for defining the sol-to-gel transition point for foods, however, an increase in G' as mentioned above can closely show the gelation point.

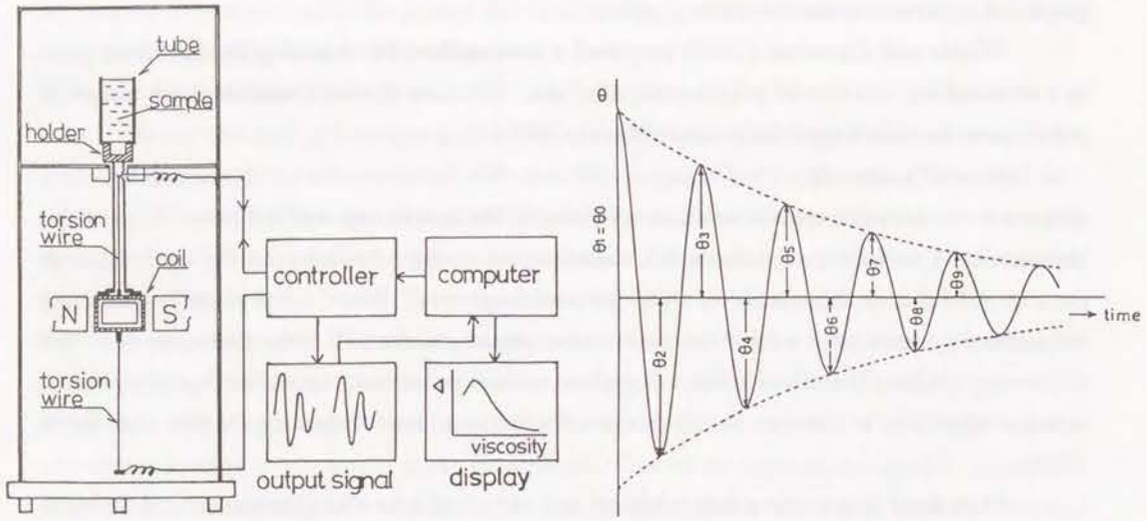

Fig. II–7.(left) A schematic diagram of the damped oscillation rheometer.
Fig. II–8.(right) The damped oscillation curve.

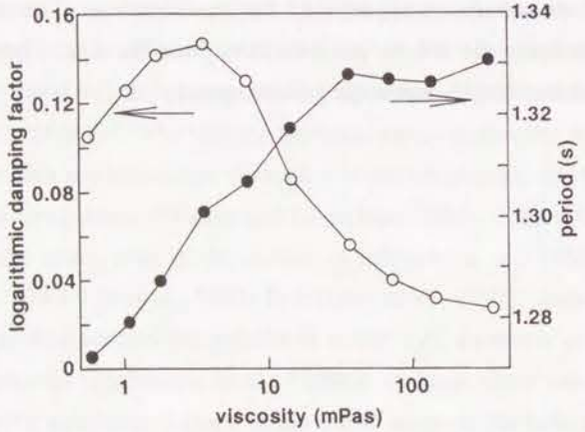

Fig. II–9. Logarithmic damping factor (○) and the period (●)
for liquid samples with various viscosities.
Sample liquids are methanol, ethanol and 6 kinds of standard oils.
1 mL liquid in a glass tube was released at torsion angle of 10 degrees at 25 °C.
The LDF and period were mean value from first 10 times of oscilation curves (θ_1–θ_{10}).

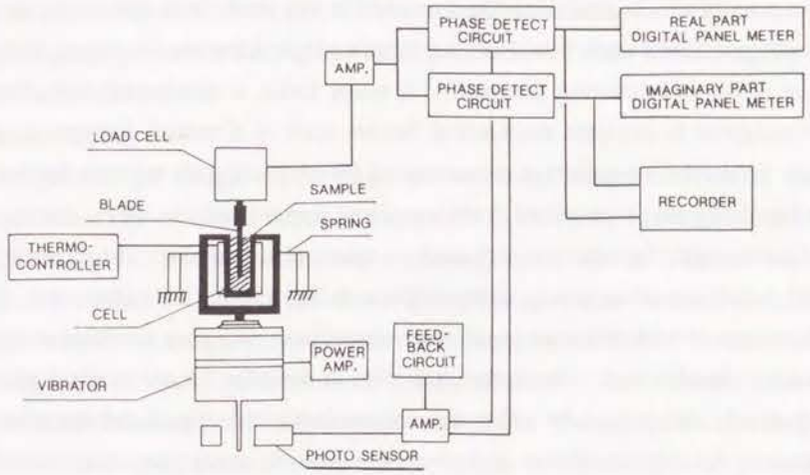

Fig. II–10. A block diagram of a Rheolograph Sol.

II-3. RHEOLOGICAL METHODS FOR FOOD GELS

The purpose of rheological methods discussed in this study is to effectively describe the fundamental gel properties such as elastic modulus and breaking stress. Since texture is the most important factor in evaluating the quality of many foods, a number of objective methods have been investigated to evaluate mechanical factors such as firmness, toughness, shortness, chewyness etc. However, no objective measurement for evaluating all textures has been established. The breaking force obtained from emprical apparatuses is often shown as "jelly strength" or "gel strength" in food industry such as agar and kamaboko. Those methods which are empirically based are often poorly defined (Szczesniak, 1963). Therefore, it is difficult to compare data measured with different apparatuses since these data give insufficient information and are not easily corroborated. The author has tried to describe the rheological properties of gels most objectively and physically rather than organoleptically. Small deformation rheology including dynamic Young's modulus and creep compliance measurements was taken even though it may not be closely related to any organoleptic properties. Food processing and its allied industries (agriculture, transportation, storage, and commerce and catering) require a physicochemically accurate description and a common recognition, because today's food industries often deal with another materials. The author feels that the growth of the food industry depends on such consistency.

LARGE DEFORMATION RHEOLOGY

Tensile, puncture and compression testings of gels were carried out using a Rheoner RE-33005 (Yamaden Co. Ltd., Tokyo). This apparatus is designed for uniaxial compression and tensile tests of food such as those performed with the Instron type universal testing machine (Bourne et al., 1966; Ross-Murphy, 1984). The maximum value of the load is 20 kgf.

Puncture Testing

The center of the commercially available tofu gel was vertically compressed with a cylindrical plunger at a compression rate of 1.0 mm/sec at 20 °C. The apparent stress τ was evaluated from the load value at any given point divided by the cross-sectional area of the plunger. The strain was the ratio of the depressed distance of the plunger to the initial thickness of the sample. Fig. II-11 shows that the apparent breaking stress increased with decreasing the plunger diameter. The detected force becomes the sum of the force vertically reacted by a sample to the base of the plunger and the friction given to the side of the plunger. It is clear that the friction effected the result. However, it is not easy to estimate the two forces separately and calculate the true stress and strain. Therefore, the consideration of plunger size is necessary.

Tensile Testing

To avoid slip of a sample chuck, a ring-shaped gel sample was hung on two polyacetal

bars arranged vertically. The gel ring was initially deformed under its own weight and then vertically elongated at a constant rate. This method was more useful than compression in evaluating breaking properties for highly elastic gels such as konjac and kamaboko. An example is described in Chapter V.

Compression Testing

A cylindrical gel sample on the stage was vertically compressed with a flat plunger of 40 mm diameter. The breaking stress of the gels was calculated from the load value at the breaking point divided by the initial cross-sectional area of the gel. The stress calculated in this way is called as the nominal stress. Nominal stress is not a true stress derived by a detected force by the area at the moment of compression. However, nominal stress has been widely adopted because it is easily calculated. Breaking strain was determined as the ratio of the deformation at the breaking point to the initial height. The Young's modulus was defined as the slope of each stress-strain curve at small strain range in which the stress is proportional to the strain. The breaking energy was calculated from the area under the stress-strain curve, and normalized per unit volume (m^3).

Fig. II-12 shows the nominal stress *versus* strain curve for kinugoshi tofu with different sizes. Though the ratio of the sample diameter to the height ranged from 0.625 to 1.875, the curves almost superimposed each other. Of course, if the ratio becomes smaller or larger, the curve may vary due to effect of the surface etc. Apparent breaking stress in the puncture test depended on plunger diameter (Fig. II-11), but in the compression test it was independent of sample diameter (Fig. II-12) within our experimental conditions. Therefore, tensile or compression testing was applied mainly in this study.

It has been established, both theoretically and experimentally, that rheological properties are also influenced by test temperature and time. Generally, materials exhibit solid-like behavior when examined at lower temperature and shorter time intervals (Dickinson, 1992). The author examined the effects of compression speed and test temperature using kinugoshi tofu as a sample. Detailed data are not shown here, however the results depended on those conditions. The temperature and time-scale must be described as experimental conditions.

SMALL DEFORMATION RHEOLOGY

Dynamic Viscoelasticity

Cylindrical gels with a 20 mm inner diameter and a 30 mm height were prepared. The sinusoidal vibrations of a small amplitude of 100 μm and a frequency of 3 Hz were given to the lower end of the cylindrical gel using a Rheolograph Gel (Toyoseiki Seisakusho, Tokyo) (Nishinari et al., 1980). The block diagram is shown in Fig. II-13. In order to prevent evaporation of water and to hold the sample at a constant temperature the sample was placed between the upper and lower plates immersed in silicone oil. The temperature of the oil was controlled

by means of a circulator. The stress produced in the sample was detected by a strain gauge, then amplified and introduced to the computing circuit. By this device, the stress was divided into the component in phase with the applied strain (real part, E') and the component with leading phase $\pi/2$ to the applied strain (imaginary part, E''). These values were indicated in meters directly.

Static Viscoelasticity

A creep measurement was taken also using a Rheoner RE-33005 (Yamaden Co. Ltd., Tokyo) with a 200 gf load cell. A cylindrical gel was put on the stage, and it was uniaxialy applied by a constant load with a flat plunger. From preliminary tests, the load values were chosen to give a strain of several percent which was in the linear region between stress and strain. The vertical displacement of the stage was recorded until 1 μm order every 0.1 s. Creep curves (compliance vs. time) were analyzed using a mechanical model which consisted of Maxwell bodies and Voigt bodies. In most cases, a simple four-element model consisting of a Maxwell body connected in series with a Voigt body was used. After a set time period, the sample gel was unloaded to observe a creep recovery behavior.

Stress relaxation, another basic method for stadying static viscoelasticity, was not carried out in this study because it is too time intensive. Since some sample gels such as tofu and carrageenan showed syneresis, gels would change physically during the measurement.

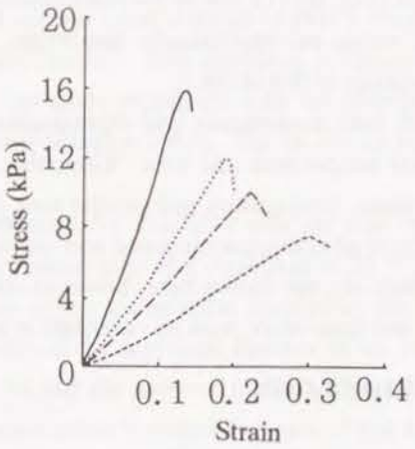

Fig. II-11. Stress-strain curves observed in a puncture testing of kinugoshi tofu at 20 °C. Sample thickness: 25 mm. Puncture speed: 1.0 mm/s. Cylindrical plunger diameter: ———, 3 mm; ·····, 5 mm; ·—·——·, 8 mm; --------, 16 mm.

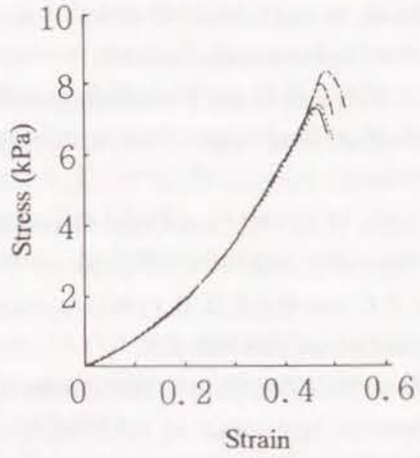

Fig. II-12. Stress-strain curves observed in a compression testing of kinugoshi tofu at 20 °C. Compression speed: 1.0 mm/s. Sample height: 16 mm. Sample diameter: ———, 10 mm; ·····, 12 mm; --------, 20 mm; ·—·——, 30 mm.

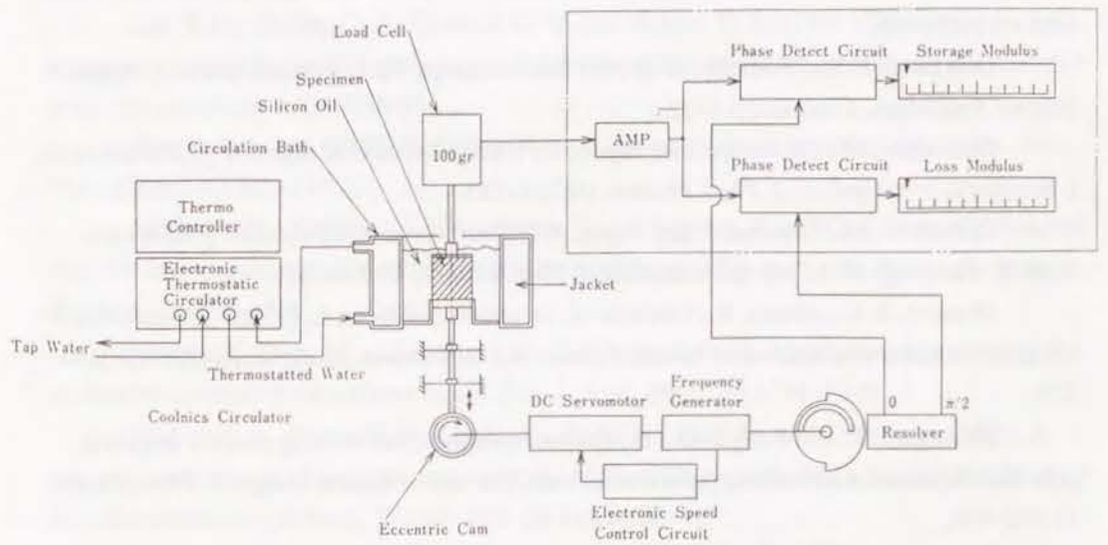

Fig. II-13. Block diagram of a Rheolograph Gel.

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Chapter III
GELATINIZATION AND RETROGRADATION OF SWEET POTATO STARCH

The sweet potato (*Ipomoea batatas*) has been regarded as one of the most important biomass crops because both cultivation and harvest are relatively easy. This crop is promoted not only for food or feed but also for alcohol production. Its cultivation for provisions in the case of famine has been encouraged for several centuries by the Japanese government.

The main components of sweet potato are carbohydrates. Starch makes up about 70 % of its dry matter. The gelatinization properties of starch are important in food processing and cooking (Honbo, 1977; Creamer et al., 1983; Takahashi et al., 1985). Sweet potato starch is consumed in a gelatinized state. Few investigations have been reported on the basic physicochemical properties of sweet potato starch (SPS) (Fukui et al., 1964; Madamba et al., 1973; Wada et al., 1979; Kitada et al., 1988) in comparison with starch extracted from wheat (Wada et al., 1979; Longton and LeGrys, 1981; Ghiasi et al., 1982; Roulet et al., 1988; Wootton and Ho, 1989), rice (Wada et al., 1979; Nakazawa et al., 1984; Biliaderis et al., 1986; Chungcharoen and Lund, 1987) or corn (Kalb and Sterling, 1961; Evans and Haisman, 1979; Wada et al., 1979; Owusu–Ansah et al., 1982).

The carbohydrate contents for nine varieties of Japanese sweet potato roots were studied by Taira and Yasui (1987). Dry matter of the roots mainly consists of carbohydrate (89.7 – 94.0 %). Starch is the main component (66.8 – 78.5 %). The remaining dry matter are water–soluble sugars (8.2 – 15.3 % of total dry matter). The other carbohydrates are water–insoluble dietary fibers (6.17 –7.69 % of total dry matter).

The insoluble carbohydrate content is about 10 % starch, and is higher than in case of potato or taro. Palmer (1982) reported the presence of cellulose, pectic substances and hemicelluloses in cooked sweet potatoes. Since fiber consists of several polysaccharides, it may greatly effect the physical properties of the main component of sweet potato starch. Physicochemical studies of model systems which contain starch and various fibers at the same ratio may therefore be useful. The model systems we used consisted of sweet potato starch and several types of modified celluloses for food use at a ratio of 9:1. Gelatinization and retrogradation properties of the model systems were examined in the first part of this chapter. The rheological properties of blends of wheat starch and xanthan gum (Christianson et al., 1981; Sajjan and Rao, 1987; Alloncle et al., 1989), guar gum (Christianson et al., 1981; Sajjan and Rao, 1987; Alloncle et al., 1989), locust bean gum (Sajjan and Rao, 1987; Alloncle et al., 1989), carrageenan (Tye, 1988) and sodium carboxymethylcellulose (Christianson et al., 1981) have been studied. These hydrocolloids significantly increased viscosity and decreased the retrogradation

rate. Since fiber does not generally dissolve even in hot water, the texture of starch paste would change and become less smooth when fiber is added to starch. The effects of methylcellulose which is known to be water-soluble are also investigated.

The content of soluble sugars in sweet potatoes were found to be about 20 % of starch. Sucrose is the main sugar component, while glucose and fructose are minor components (Taira and Yasui, 1987; Van Den et al., 1986). When sweet potatoes are processed or cooked, these sugars may have influence on the gelatinization or retrogradation properties of starch. The second part of this chapter deals with the gelatinization and retrogradation properties of the starch from the viewpoint of the effect of water-soluble sugars.

Since sugars influence the gelatinization and retrogradation behaviors of wheat starch, they influence strongly the quality of baked products such as breads and cakes. An investigation of the pertinent literature reveals that these effects have been the subjects of many studies using several analytical techniques; differential scanning calorimetry (DSC) (Wootton and Bamunuarachchi, 1980; Spies and Hoseney, 1982; Slade and Levine, 1987), loss of birefringence (Bean and Yamazaki, 1978; Bean et al., 1978; Spies and Hoseney, 1982), rheological studies including amylography (D'Appolonia, 1972; Bean and Yamazaki, 1978; Bean et al., 1978; Cheer and Lelievre, 1983) and nuclear magnetic resonance (NMR) (Hansen et al., 1989). Sugars are known to function as anti-staling ingredients (Slade and Levine, 1987). It is well known that sugar prevents staling of rice cake, and shifts the gelatinization temperature higher. Some researchers have reported that the enthalpy of gelatinization of starch decreased by the addition of sucrose (Wootton and Bamunuarachchi, 1979; Chungcharoen and Lund, 1987). These bread and confectionery systems contain high concentrations of sugar and may not contain sufficient water to necessary for starch gelatinization.

Although the content of water soluble sugars in sweet potato is about 10 % in dry matter, it may change the properties of the starch, and so it is also important to study the effect of sugars of low concentration on gelatinization and retrogradation. Cheer and Lelievre (1983) reported that the viscosity, yield stress and rigidity of several percent wheat starch paste showed maxima at the sucrose concentration of about 20 %. The viscosity maxima at 20 % concentration of various sugars were also observed by Bean and Osman (1959). DSC measurements were carried out to clarify the effect of sugars on the gelatinization and retrogradation of sweet potato starch over a wide concentration range of sugars. DSC study makes it possible to determine the gelatinization temperature and heat of gelatinization (Shiotsubo and Takahashi, 1984) and also to analyze the retrogradation process at a molecular level (Roulet et al., 1988). Though there have been a few DSC studies on sweet potato starch (Wada et al., 1979; Kitada et al., 1988), the interaction between starch and other components of sweet potato has not yet been examined.

III-1. EFFECT OF CELLULOSE DERIVATIVES

MATERIALS AND METHODS

Materials

Sweet potato starch (SPS), chemically modified edible celluloses, standard type carboxymethylcellulose (CMC) and methylcellulose CP4000 (MC) were purchased from Wako Pure Chemicals Industries Ltd. (Osaka). Physically modified celluloses, Avicel SF [powdered microcrystalline cellulose, MCC (Thomas, 1986)] and alkaline soluble fibrous cellulose, ASC (Hisano et al., 1989) were obtained from Asahi Chemical Industries Co. Ltd. (Kawasaki). Both of MCC and ASC are chemically pure celluloses. The particle size of MCC ranges from 6 to 10 μm while ASC is fibrous because it is made by spinning an alkaline solution of cellulose. Only MC is water-soluble, but the other 3 cellulose derivatives are insoluble even in hot water. The moisture content of SPS was 16 % and those of cellulose derivatives were less than 10 % by heating at 105 °C for 5 h at 10^{-2} mmHg. These carbohydrate samples were used without further purification. Water was distilled using a glass apparatus.

Rheological measurement

Sample paste (10–20 g) was prepared just before each rheological measurement. SPS or the 9:1 mixture of SPS and various cellulose derivatives was dispersed in water by a motor stirrer at 200 rpm (LR41-B, Yamato Science Ltd., Tokyo) in a round bottom flask with removable top and a teflon stopper for 30 min at room temperature (~25 °C). The mixing blade of the stirrer was designed to fit the curvature of the flask bottom to prevent precipitation of starch. The suspension was then heated for 10 min in a water or an oil bath and cooled to room temperature for 30 min. The mixture was stirred continuously at 200 rpm.

1.55 mL of the sweet potato paste was filled in the cell of a Rheolograph Sol (Toyoseiki Seisakusho, Tokyo). The surface of the sample paste was covered with silicone oil in order to prevent the evaporation of water and the sample was cooled from room temperature to 5 °C by a programmable thermomodule for 19 min. Then, the paste was subjected to the 2 Hz sinusoidal shear oscillation with amplitude of 125 μm or 50 μm. The shear strain was 0.125 or 0.050 respectively. This is sufficiently low so that the rheological measurements might be conducted within the range of linear viscoelasticity. The storage and loss moduli G' and G'' at 5 °C were recorded as a function of time to examine the retrogradation process.

G' and G'' were also monitored as a function of temperature to observe the changes in dynamic moduli with temperature. SPS and MC were mixed at various ratios and heated with water in the flask with removable top as described above. The samples were filled in the cell and heated from room temperature to 85 °C at the heating rate of 1 °C/min. Those measurements were repeated several times and the average values determined.

Differential Scanning Calorimetry

Differential scanning calorimetry (DSC) measurement was carried out by use of a DSC

apparatus (SSC580 with DSC10 module, Seiko Electronics Co. Ltd., Tokyo). Sweet potato starch and a modified cellulose were mixed in the ratio of 9 to 1. Then, 20 mg of powdered carbohydrate mixture and 40 mg of water were directly weighed into a silver pan of 70 μL and the pan was sealed hermetically. A pan containing the equal amount of water was used as a reference. The temperature was raised from room temperature to 120 °C at the heating rate of 1.0 °C/min. The temperature and enthalpy (ΔH) of gelatinization were determined from peak temperature, T_p, and the endothermic peak area respectively. After the first run heating, the pan was cooled immediately from 120 to 5 °C and then stored at 5 °C for various periods. The temperature was raised again from 5 to 120 °C at the same heating rate in the second run. The reproducibility of DSC was good enough such that the experimental errors were smaller than the differences among various samples.

RESULTS AND DISCUSSION

Fig. III–1 shows the storage modulus (G') and the loss modulus (G'') of 8 w/w% sweet potato starch as a function of time after heating at various temperatures for 10 min. The time t = 0 was taken as the time when the measurement began just after the 8 w/w% SPS samples were cooled to 5 °C from each heating temperature by a micro-computer for 19 min after leaving the samples stirred continuously at room temperature for 30 min. The samples heated at a temperature lower than 72 °C did not gelatinize and precipitated within 2 h after they were cooled to 5 °C. The precipitation was not observed when the sample was heated at temperatures higher than 74 °C. However, the elastic moduli of samples which were heated at 74 and 76 °C were small. Therefore, SPS is not gelatinized completely at temperatures lower than 76 °C. The loss modulus of the sample heated at 78 °C was especially large and both the storage and loss moduli decreased with the lapse of time. The remarkable decrease in storage and loss moduli suggests that this sample is not fully gelatinized either. The completely gelatinized SPS, whose storage modulus was increasing and loss modulus was decreasing gradually with time, was obtained when 8 % SPS was heated at temperatures higher than 80 °C. Fig. III–2 shows the heating temperature dependence of the initial storage modulus (G') and the initial loss tangent (tanδ). Measurements were taken just after the time when the samples were cooled to 5 °C from 120 °C. Values of tanδ for samples heated at lower temperatures were very large as in the case of liquid-like materials. Both the storage modulus was maximum and the tanδ was minimum at 80 °C, the lowest temperature for complete gelatinization to occur. In the completely gelatinized starch samples, the storage modulus decreased and the tanδ increased with increasing heating temperature.

Gelatinization and retrogradation properties of the model systems studied by DSC are shown in Table III–1. The gelatinization temperature T_p and enthalpy of gelatinization ΔH_1 for starch alone were independent of starch concentration when the concentration was less than 40

w/w%, although the data are not shown here. T_p was almost the same in all the systems examined. Kitada et al. (1988) reported that T_p determined by DSC for 25 w/w% sweet potato starches ranged from 72.5 to 74.3 °C and was independent of the place of production. Their T_p was higher than ours, 72.4 °C, because their DSC heating rate, 5 °C/min, was faster than ours (1 °C/min). The effect of heating rate on gelatinization temperature was discussed by Shiotsubo and Takahashi (1984).

The apparent endothermic enthalpy, $\Delta H_{1,app}$, estimated from the area enclosed by the DSC endothermic peak and the base line for four mixed systems, was smaller than that for SPS alone (4th column, Table III–1). However, gelatinization enthalpies should be estimated as the enthalpies/mg of starch since modified celluloses including MC did not show any exo- and endotherm at the temperature range studied. Gelatinization enthalpies $\Delta H_{1,s}$ of the system which contained MCC, ASC and CMC showed no large differences in comparison with the $\Delta H_{1,s}$ (5th column, Table III–1) of SPS alone, while the SPS–MC mixture seems to require more energy to gelatinize starch. Our $\Delta H_{1,s}$ values were smaller than those obtained by Kitada et al. (1988) (15.0–16.1 mJ/mg), presumably because of their faster heating rate (5 °C/min). Biliaderis et al. (1986) observed that the enthalpy of gelatinization for 50 % starch-water mixture increased with increasing heating rate. After the first run DSC heating, retrogradation of starch proceeded during storage at 5 °C. The heat required for melting the retrograded starch (33 w/w%) ΔH_2 was estimated from the area of the endothermic peak at the second run DSC heating. The heat ΔH_2 increased with storage time and saturated for 14 days storage at 5 °C. Therefore, the retrogradation of starch-cellulose mixture were evaluated from the peak area/mg starch, $\Delta H_{2,s}$, after storing at 5 °C for 14 days. Values of $\Delta H_{2,s}$ were larger for SPS mixture with MCC, ASC, CMC than for SPS alone (7th column, Table III–1). The retrogradation ratio, defined as $\Delta H_{2,s}/\Delta H_{1,s}$ X 100 (8th column, Table III–1), showed this tendency more clearly.

Fig. III–3 shows the time dependence of storage and loss moduli after 8 w/w% dry matter sample was heated at 83–84 °C. The heating temperature was chosen because it is the lowest temperature at which the mixture showed maximum storage modulus after the starch gelatinized, and the retrogradation would proceed most rapidly at that temperature. G' and G'' of 5, 7, 8 and 10 w/w% starch paste did not reach at the equilibrium value and were increasing gradually upon storage.

According to Miles et al. (1985), the retrogradation of starch proceeds in two crystallization stages. The first stage, in which the rigidity and crystallinity of starch gels develop rapidly, was dominated by amylose gelation. The second stage, which is shown by a long-term increase in the elastic modulus of starch gels, was attributed to the thermo-reversible crystallization of amylopectin. The slower stage continued 1 wk at 26 °C for 20 % starch gels. If the starch concentration was much higher and temperature was lower, the retrogradation would proceed faster. The dynamic storage modulus even for 50 % starch gel, which does not contain

enough amount of water for gelatinization, was still increasing after 6 days storage at 4 °C (Roulet et al., 1988). The limiting plateau values for the dynamic moduli of starch gels have not been obtained since it is difficult to measure rheological properties without bacterial degradation of starch over long measuring time. This was possible for DSC measurement since the samples were hermetically sealed into sample pans.

The addition of modified celluloses except MC increased the storage and loss moduli. Retrogradation was accelerated by MCC, ASC and CMC. These molecules act as hydrated filler particles and therefore exist as discrete entities in the starch gel network. They might accelerate the retrogradation ratio by absorbing water from the gel matrix (Zeleznak and Hoseney, 1986). However, the amount of water absorbed by the cellulose derivatives was low since the increase of gelatinization temperature, T_p, was not observed in any systems. Biliaderis et al. (1986) observed an increase in T_p with increasing starch concentration was induced by the lack of water. Altough those celluloses bound the water in the gel matrix, the effect should not be significant. Water-soluble MC is regarded as more hydrophilic than the water-insoluble polysaccharides, but it greatly decreased retrogradation kinetics. Therefore, the insoluble cellulose derivatives should accelerate retrogradation by the other mechanism. These molecules may form nuclei for crystallization in starch paste. Since retrogradation of starch paste is a crystallization process, it would be accelerated by the presence of nuclei. Our CMC sample was not soluble in water since it was the free acid form. Although CMC has hydrophilic hydroxyl groups, the solubility depends on the degree of substitution (DS). Most commercially available samples of sodium CMC have a DS range from 0.4 to 1.4 (Keller, 1986). Such a water-soluble CMC added in starch paste makes the retrogradation slower (Christianson et al., 1981). Tomioka and Matsumura (1987) suggested from viscometry of dilute solutions of MC and sodium CMC that sodium CMC molecules may form extended coils while MC may form compact coils. The effect on retrogradation of these celluloses may be different because of different molecular forms in water. It has been reported that added hydrocolloids increased viscosity of starch paste (Christianson et al., 1981; Sajjan and Rao, 1987; Tye, 1988; Alloncle et al., 1989). Both water-soluble and water-insoluble polysaccharides such as MCC, ASC, and CMC increased the dynamic viscosity of gelatinized starch, even when the latter showed smaller effect than the water-soluble hydrocolloids. The accuracy of the retrogradation ratio estimated from dynamic viscoelasticity measurement is about 10 % and that from DSC is 15 % (Roulet et al., 1988). Therefore, the magnitude of the promotion for retrogradation could not be estimated quantitatively for MCC, ASC and CMC. In contrast to these three celluloses, MC dramatically decreased the storage modulus and increased the loss modulus of starch paste. MC was considered to prevent retrogradation of starch.

The results mentioned above indicate that MC showed peculiar effects on the gelatinization and retrogradation of starch. Temperature dependence of elastic moduli of SPS-MC

system mixed at various ratios is shown in Fig. III-4. The concentration 3 % was chosen so that the modulus remained within range for the measuring equipment. The storage and loss moduli G' and G'' of the paste made from starch alone decreased with increasing temperature. G' and G'' of the systems containing MC began to increase about 55 °C, it was responsible for the commencement of gelation of MC. The temperature at which G' and G'' began to increase shifted lower with decreasing MC concentration. This tendency was similar to the experimental observation that the gelling temperature of MC shifted lower with increasing MC concentration (Nagura et al., 1981). The increase of storage and loss moduli at higher temperature became smaller with decreasing MC ratio in the specimens. It seems that there was no special ratio of starch to MC at which a significant synergistic effect was found. The unexpected effect of MC on starch retrogradation was caused by the characteristics of MC; it is a sol at low temperatures and a gel at high temperatures. At low temperatures at which retrogradation of starch occurs, MC would have dispersed well as a sol, while other celluloses including CMC precipitated. The sol state of MC prevented retrogradation by the same manner as with other water soluble polysaccharides.

Table III-1. Gelatinization temperature and enthalpy of gelatinization for sweet potato starch (SPS)-cellulose-water mixture. (total polymer concentration: 33 w/w%)

Sample	Starch Content	T_p (°C)	First run		Stored for 14 days at 5°C		Retrogradation ratio[b]
			$\Delta H_{1,app}$ (mJ/mg sample)	$\Delta H_{1,s}$[a] (mJ/mg starch)	$\Delta H_{2,app}$ (mJ/mg sample)	$\Delta H_{2,s}$ (mJ/mg starch)	
SPS	1.0	72.4	11.0	11.0	8.0	8.0	72.7
SPS-MCC	0.9	72.5	9.7	10.7	7.5	8.3	77.6
SPS-ASC	0.9	72.7	10.0	11.1	8.0	8.9	80.2
SPS-CMC	0.9	72.4	10.2	11.3	8.1	9.0	79.6
SPS-MC	0.9	72.6	10.8	12.0	5.7	6.3	52.5

[a] $\Delta H_{i,s} = \frac{\Delta H_{i,app}}{\text{starch content}}$, (i = 1, 2).

[b] Retrogradation Ratio = $(\Delta H_{2,s}/\Delta H_{1,s}) \times 100$.

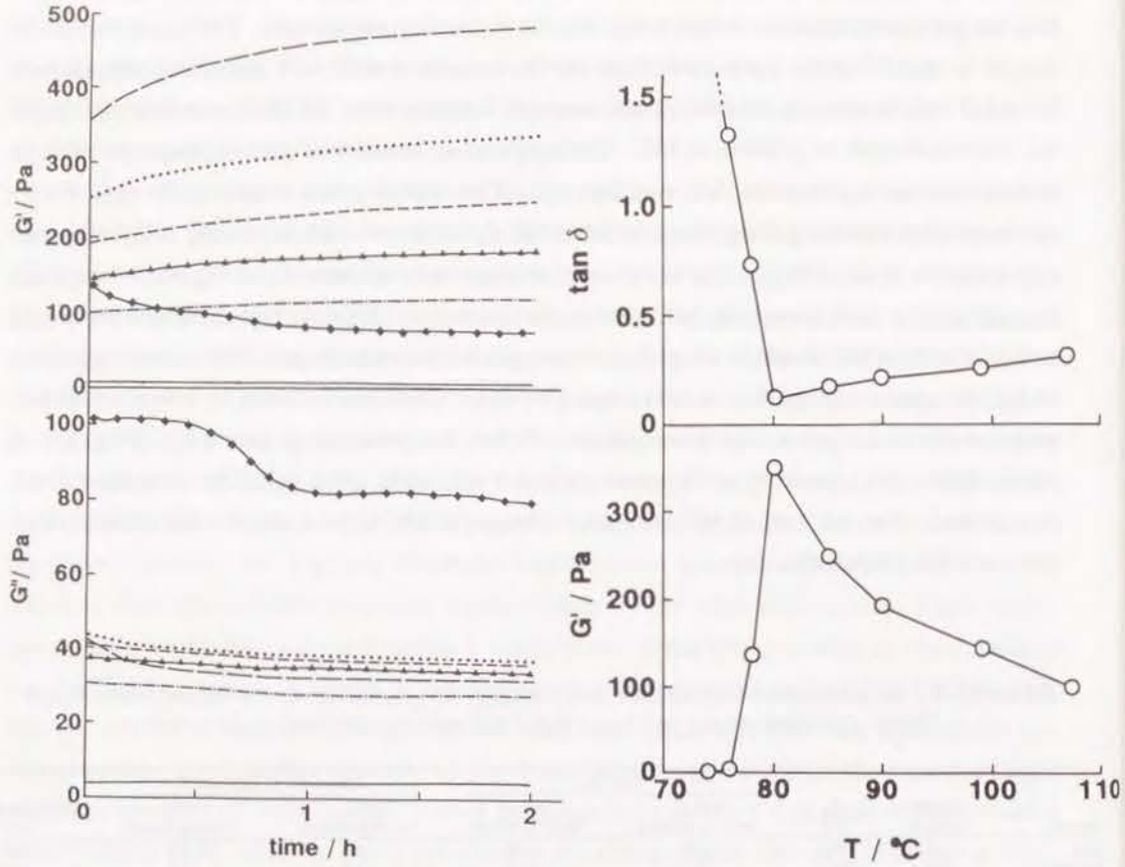

Fig. III–1.(left) Storage modulus G' and loss modulus G'' for 8 w/w% sweet potato starch pastes as a function of time at 5 °C.
Shear strain: 0.125. Heating time: 10 min. Heating temperature: ————, 76 °C; -•-•-•-, 78 °C;- – – – –, 80 °C; ·····, 85 °C;--------, 90 °C; -•-•-•-, 99 °C; —·—·—, 107 °C. Samples are cooled to 5 °C from each heating temperature by a micro-computer for 19 min after leaving the samples at room temperature for 30 min.

Fig. III–2.(right) Heating temperature dependence of storage modulus G' and tanδ (G''/G') for 8 w/w% sweet potato starch paste at 5 °C.
Shear strain: 0.125. Heating time: 10 min. Samples are cooled to 5 °C from each heating temperature by a micro-computer for 19 min after leaving the samples at room temperature for 30 min.

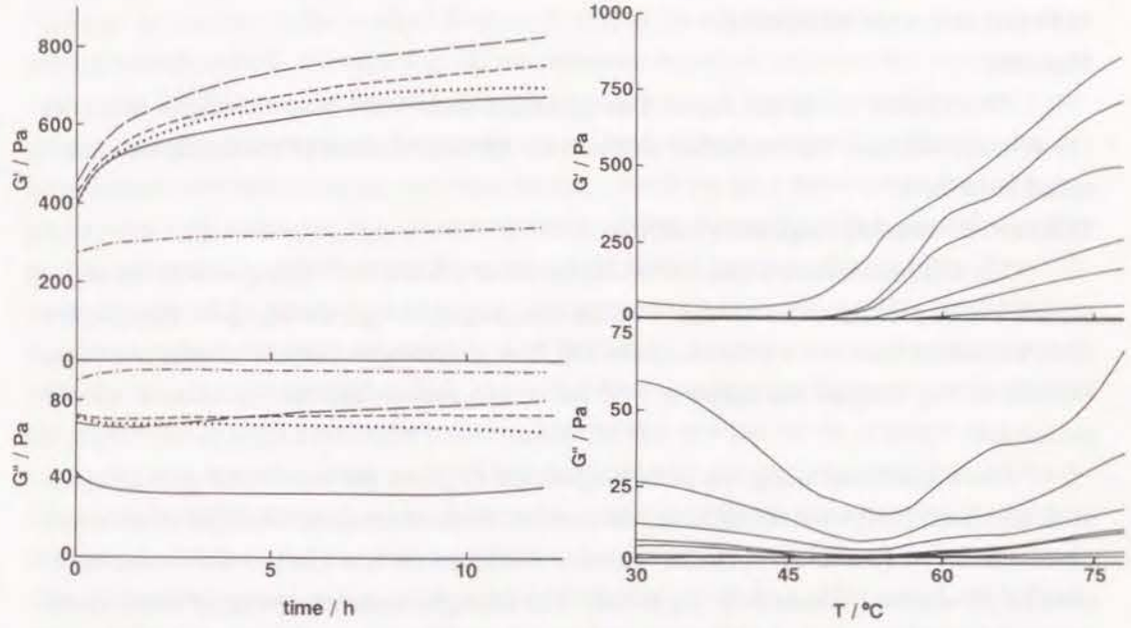

Fig. III–3.(left) Time dependence of storage modulus G' and loss modulus G'' for sweet potato starch (SPS) – cellulose mixture at 5 °C.
Total polysaccharide concentration: 8 w/w%. Shear strain: 0.050. Samples are heated at 83 °C for 10 min and cooled to 5 °C for 19 min after left at room temperature for 30 min. ————, SPS alone; --------, SPS–microcrystalline cellulose (9:1); – – – – –, SPS–alkaline soluble cellulose (9:1), ·····, SPS–carboxymethylcellulose (9:1); —·—·—·, SPS–methylcellulose (9:1).

Fig. III–4.(right) Temperature dependence of storage modulus G' and loss modulus G'' for sweet potato starch (SPS) and methyl cellulose (MC) mixture.
Total polysaccharide concentration: 3 w/w%. Shear strain: 0.050. Heating rate: 1 °C/min. Samples are cooled to room temperature for 30 min after being heated in boiling water for 10 min. Ratio of SPS to MC are 10:0, 9:1, 8:2, 7:3, 6:4, 5:5 and 0:10 from lower to upper curve for both of G' and G'', respectively.

III–2. EFFECT OF SOLUBLE SUGARS

MATERIALS AND METHODS

Materials

Sweet potato starch and sugars were purchased from Wako Pure Chemicals Industries Ltd. (Osaka) and used without further purification. Moisture content of the starch was determined as 16 %.

Differential Scanning Calorimetry (DSC)

The DSC measurement was carried out by use of a Seiko DSC as described in the above section. Sweet potato starch and sugar solution were directly weighed onto a pan. The temperature was raised from room temperature to 130 °C at a heating rate of 1.0 °C/min. After the first run heating, the pan was stored at 5 °C for various periods, and then the second run was carried out.

In a preliminary study, the peak temperature (T_p) and the area of the gelatinization peak/mg starch (ΔH) were found to be independent of starch concentration for lower starch concentrations (< 40 w/w%). A similar tendency was found for rice starch at lower concentrations (< 50 w/w%) (Biliaderis et al., 1986). The retrogradation may proceed faster in the system with a high starch concentration. 20 mg of sweet potato starch and about 40 mg of sugar solution were put in a DSC pan, so that starch concentration might become 33 w/w% in the present work.

RESULTS AND DISCUSSION

Typical heating DSC curves of 33 w/w% sweet potato starch in sucrose solutions are shown in Fig. III–5 for various sucrose concentrations. The heating DSC curves were more symmetrical for lower sucrose concentrations (< 20 w/w%) as observed in a starch–water system whose starch concentration was lower than 40 % (Shiotsubo and Takahashi, 1984; Biliaderis et al., 1986). Shiotsubo and Takahashi (1984) also reported from differential thermal analysis of 17 w/w% potato starch that T_p shifted to higher temperatures with increasing heating rate but it was independent of heating rate below 1 °C/min. According to them, if the endothermic curve was symmetrical and the heating rate was slow enough to provide information about the equilibrium state. Therefore, half the starch specimen gelatinized at the temperature T_p. Determination of the gelatinization temperature from DSC heating curves is often based on the onset temperature at which the heating DSC curve begins to deviate from the baseline (Wada et al., 1979; Nakazawa et al., 1984). We use mainly the peak temperature T_p in the present work because it can be determined much more precisely than the onset temperature, and T_p does not depend on the concentration of starch in the concentration range lower than 40 w/w% (Shiotsubo and Takahashi, 1984). The T_p of sweet potato starch was reported as 76.9 °C by Wada et al. (1979) and 72.5–74.3 °C by Kitada et al. (1988). These were higher than ours

(72.4 °C), presumably since both of them used a faster heating rate (5 °C/min) than ours (1 °C/min). In addition, in the work of Wada et al. (1979) the starch concentration was probably much higher than 40 %, although they did not determine the starch concentration acculately.

Fig. III–5 shows that the peak temperature together with the onset temperature (T_o) and the final temperature at which the endotherm returned to the baseline (T_f) shifted to higher temperatures with increasing sucrose concentration. Since the peak became broader and more asymmetric with increasing sucrose concentration, neither T_o nor T_f shifted parallel with the peak temperature T_p. Wootton and Bamunuarachchi (1980), however, reported that T_o and T_f were not affected by the increase of sucrose concentration up to 45 %. Another small peak or shoulder appeared at higher temperatures in a DSC curve for a starch–sucrose–water system with sucrose concentration higher than 20 w/w%. Peak broadening at higher temperatures and the appearance of small peak might be induced by the lack of water for the gelatinization.

As in a fringed–micelle model which has been used for partially crystalline polymers (Wunderlich, 1981), starch consists of somewhat crystalline regions and somewhat amorphous regions. Gelatinization of starch is considered to be a process in which the crystalline region is changed into amorphous region, as observed by birefringence (Bean and Yamazaki, 1978; Bean et al., 1978; Spies and Hoseney, 1982) or X–ray diffraction (Miles et al., 1985; Roulet et al., 1988) measurements.

Recently, the endothermic peaks in DSC curves for the gel–sol transition of thermoreversible gels were explained by using a zipper model (Nishinari et al., 1990). The disappearance of crystalline region in starch–water systems which consist of N single zippers can be assumed as the opening process of molecular zippers with N parallel links. According to this treatment, the heat capacity C of such a system is given by equation II–2 (see Section II–1).

Assuming the order of magnitude for the energy ε as $2300k$, which is the approximate value for hydrogen–bonding energy, and the number of links N as 100, we could fit the calculated curve using equation III–1 to the experimental DSC heating curve (Fig. III–6). From this curve fitting, we obtained the rotational freedom of a link G for gelatinization curves for starch–water systems with various concentrations of sucrose. Results are shown in Table III–2. As is seen clearly from Table III–2, G decreased with increasing concentration of sucrose. Since hydrogen bonds may be created by the addition of sucrose, and the mobility of links will decrease, this is reasonable. Of course, the fitting of an experimental curve can not be carried out perfectly because starch is polydisperse and there must be the distribution of the number of links N and of the energy ε. The calculated curve obtained by using only one set of N, N, ε and G is too sharp for an experimental curve. However, as a first approximation, we could find values of G assuming a reasonable N, ε and N, and as a result G was found to be a decreasing function of sugar concentration. Experimental DSC curves for gelatinization became broader with an increasing sucrose concentration. This may be interpreted as follows: the distribution

of the energy required to open the link ε and the rotational freedom of a link *G* will become broader as a result of newly created hydrogen bonds by the addition of sucrose.

Fig. III-5 shows that the gelatinization temperature shifted to higher temperatures with increasing sucrose concentration. A similar tendency was observed in the presence of glucose or fructose as shown in Fig. III-7. T_p shifted to higher temperatures in the following order: sucrose > glucose > fructose. The order is the same as reported by Slade and Levine (1987) for the system which contains equal amount of starch, sugar and water. The amount of water immobilized by various sugars was ranked in the following order: sucrose > glucose > fructose. Sugar concentrations in the edible part of sweet potato are nearly 10 to 20 % of starch (Taira and Yasui, 1987), that is several percent of sugars to 33 % starch. Even such a small amount of sugar can increase the gelatinization temperature. There are two possible reasons for the increase in T_p by the addition of sugar: (i) interaction of sugar molecules with molecular chains in starch to stabilize the crystalline region of starch; (ii) hydration of sugar molecules decreases the effective water, which is equivalent to the effect that starch concentration becomes higher. The experimental findings, that T_p and the gelatinization enthalpy (ΔH_1) of the first run DSC heating do not depend on starch concentration in water-rich condition, suggest that the second factor is less important at least when sugar concentration is low. Dynamic hydration numbers, which is the number of water molecules in hydration cosphere, of sucrose, glucose and fructose are 25.2, 18.6 and 16.5, respectively (Uedaira et al., 1989). The water which forms the co-sphere of sugar becomes 1.65, 1.86 and 1.33 g/g of sugar. As was mentioned above, T_p was found to be almost independent on starch concentration below 40 w/w%. The quantity of water involved in the cosphere of sugars cannot increase the effective concentration of starch higher than 40 w/w%. Therefore, hydrated water by the sugar of low concentration is not enough to increase gelatinization temperature by the immobilization of water molecules.

The gelatinization enthalpy (ΔH_1) increased with increasing sugar concentration up to 10 % as illustrated with closed circles in Fig. III-8. Our findings are different from those of Wootton and Bamunuarachchi (1980), and Chungcharoen and Lund (1987) who found that sucrose decreased gelatinization energy. When sugar concentration is low, the system contains sufficient free water for gelatinization. The increase of T_p and ΔH_1 suggests that the crystalline region of starch is stabilized by sugar at low sugar concentrations. When the sugar concentration is higher than a level which available water in the system begins to be lacking, *i.e.*, the effective starch concentration exceeds 40 w/w%, the gelatinization energy ΔH_1 ceased to increase. The endothermic peak temperature of the system with insufficient water shifted to higher temperatures, and the peak became broader with decreasing water content. At a certain water level, the second small peak or a shoulder appeared at a higher temperature than the main endothermic peak temperature. These phenomena were observed when sucrose concentrations exceeded 20 w/w% (Fig. III-5). Since the hydration number of sucrose is reported to be about

6.5 (mole of water/mole of sugar) (Bociek and Franks, 1979), the hydrated water corresponds to about 7 w/w% in 20 w/w% sucrose solution. Accordingly, non-hydrated water is nearly 73 (= 80-7) w/w% in 20 w/w% sucrose solution and is about 29 mg (= 40 mg X 0.73) in each DSC pan used in our experiment. The ratio of starch to non-hydrated water is 20:29. This is nearly the same starch concentration (40 w/w%) below which T_p and ΔH_1 are independent of starch concentration in a starch-water system. Therefore, the shift of the peak temperature to higher temperatures at lower sucrose concentration than about 20 w/w% is mainly attributed to stabilization of the starch crystalline region by sugar. The increase of viscosity, yield stress and rigidity observed in starch-sugar systems (Bean and Osman, 1959; Cheer and Lelievre, 1983) might be caused by the same mechanism of stabilization. In the system which contains a higher sugar concentration, sugar does not seem to effectively stabilize the crystalline region of starch because the ΔH_1 did not increase with increasing sucrose concentration beyond 20 w/w% as shown in Fig. III-8 (solid circle). Beyond a sucrose concentration of greater than 20 %, the lack of effective water due to the hydration of sugar molecules becomes the dominant factor in the increase of gelatinization temperature.

The heat required for re-gelatinization (ΔH_2) of 33 w/w% sweet potato starch as a function of storage time is shown in Fig. III-9. The degree of retrogradation which was estimated by the area of the endothermic peak at the second run tended towards the saturated value after 14 days storage at 5 °C for 33 w/w% starch. Saturated re-gelatinization energy of the starch was about 73 % of gelatinization energy (ΔH_1) which was required for the first run DSC heating. Since amylose did not show any heat flow in heating DSC measurement, the endothermic peak was attributed to gelatinization of amylopectin. According to Miles et al. (1985), the retrogradation process of starch consists of two crystallization stages. In the first stage, the fast development of gel structure and crystallinity in starch gels was found to be dominated by amylose gelation. Additionally at this stage, the shear modulus and the X-ray peak intensity for amylose and starch gels did not decrease by heating. Therefore, this stage can not be detected by DSC measurement. In the second stage, the crystallinity in starch gels continues to develop slowly mainly in amylopectin. This was related to the endothermic peak in the second run DSC heating and to the slow development of the shear modulus. This change was thermally reversible: the X-ray peak intensity and the shear modulus fell, by heating, to the value just after the first stage. Since the DSC pan was sealed, amylopectin could not be decomposed. A smaller value of the saturated re-gelatinization energy than that of the gelatinization energy suggest that some amylopectin granules crystallize thermo-irreversibly.

In contrast with ΔH_1, the heat required for re-gelatinization (ΔH_2) after storage for 14 days at 5 °C decreased with increasing sugar concentration (Fig. III-8). The retrogradation ratio was estimated by $\Delta H_2/\Delta H_1$, and plotted against sugar concentration in Fig. III-10. The retrogradation ratio decreased with increasing concentration of sugars. At low concentrations

of sugars, the retrogradation ratio did not vary for three sugars. Therefore, retrogradation ratio decreased in the order sucrose > glucose > fructose at higher sugar concentrations. Slade and Levine (1987) reported that the extent of recrystallization of 1:1:1 starch:sugar:water mixture after 8 days storage at 25 °C increased in the order fructose > water alone > glucose > sucrose. Our results showed that fructose also prevents retrogradation, although it was not as effective as glucose and sucrose. Recently, Katsuta et al. (1992a, b) also reported that: (i) soluble sugars retarded the retrogradation of rice starch gels, and (ii) sucrose effectively retarded retrogradation better than glucose and fructose. They added that sugars containing larger number of equatorial OH groups stabilized the tridymite structure of water by hydrogen bondings. Thus, motions of gelatinized starch chains were inhibited to make crystalline region. However, their system contained 50 % starch so that the water should be insufficient for gelatinization. In our case, the water content is high enough such that water freely moved in the system. The mechanism of retrogradation inhibition is considered to be as follows: (i) sugar molecules interact with starch molecular chains to stabilize the amorphous and entangled matrix of gelatinized starch (Spies and Hoseney, 1982; Slade and Levine, 1987); (ii) sugar molecules reduces water structure by hydration to decrease the amount of free water. As stated above, only the first factor would exist at low sugar concentrations. The effect of sucrose is more effective than glucose and fructose as in the T_p increase. Low concentrations of water soluble sugars made the retrogradation of starch slower, so that the softness of cooked sweet potato may last.

Table III–2. Relationships between the degree of rotational freedom G and sucrose concentration calculated by equation III–1 with $\varepsilon=2300\,k$ and $N=100$. $\mathbb{N}$ was chosen so that the calculated curves fit best with observed heating DSC curves.

Sucrose concentration / wt.%	G
0	760
1	744
2	740
5	735
10	725
20	685
30	620
50	490

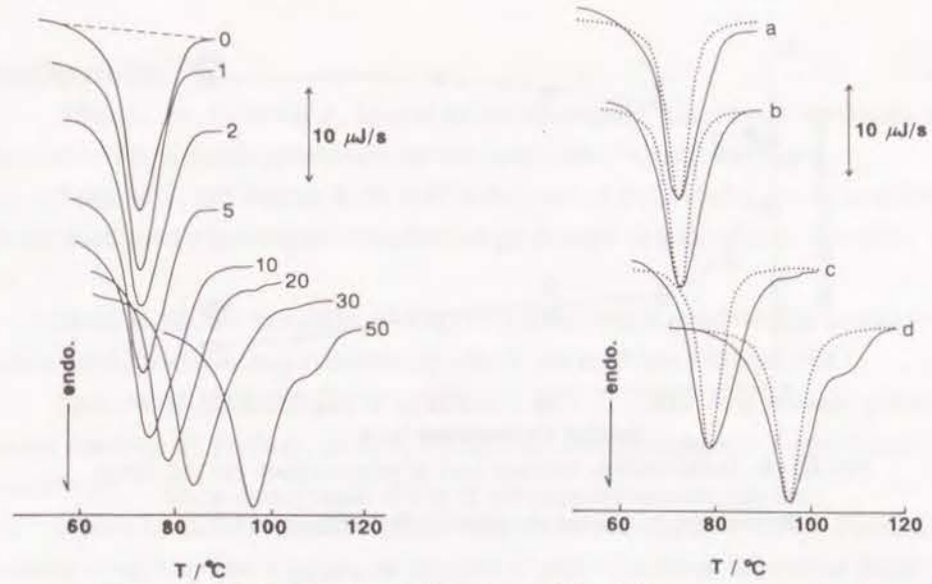

Fig. III–5.(left) Heating DSC curves of 33 w/w% sweet potato starch in sucrose solution.
Each curves were standardized to 1 mg starch. Heating rate: 1 °C/min. Figures at the right of each curve represent sucrose concentration in w/w%.

Fig. III–6.(right) Examples for observed heating DSC curves (solid lines) and the best fitted calculated curves (dotted lines) by using equation II–2.
Each curves were standardized to 1 mg starch. Starch content: 33 w/w%. Heating rate: 1 °C/min. (a) sucrose 0 w/w%, G=760, $\mathbb{N}$=3.0 X 10^{15} (in 1 mg starch); (b) sucrose 2 w/w%, G=740, $\mathbb{N}$=3.0 X 10^{15}; (c) sucrose 20 w/w%, G=685, $\mathbb{N}$=3.1 X 10^{15}; (d) sucrose 50 w/w%, G=490, $\mathbb{N}$=3.3 X 10^{15}.

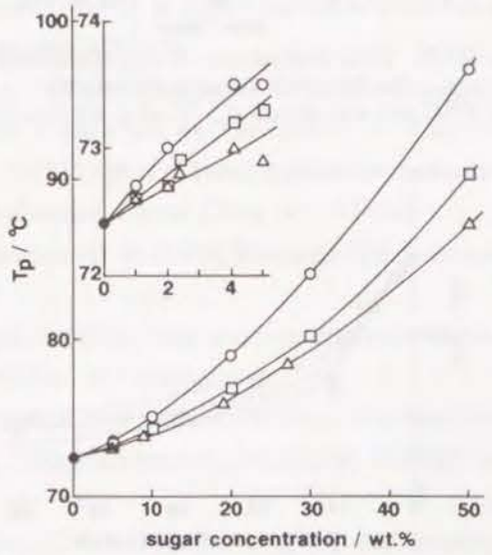

Fig. III–7. Peak temperature T_p as a function of sugar concentration.
Starch content: 33 w/w%. Solvent:○, sucrose; □,glucose; △, fructose.
(Inset) Magnification at low sugar concentration range.

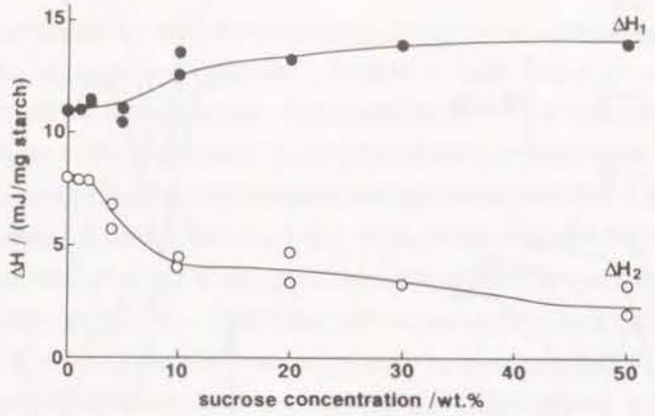

Fig. III–8. Relationships between heat of gelatinization per mg starch and sucrose concentration for 33 w/w% sweet potato starch.
●, first run; ○, second run after 14 days storage at 5 °C.

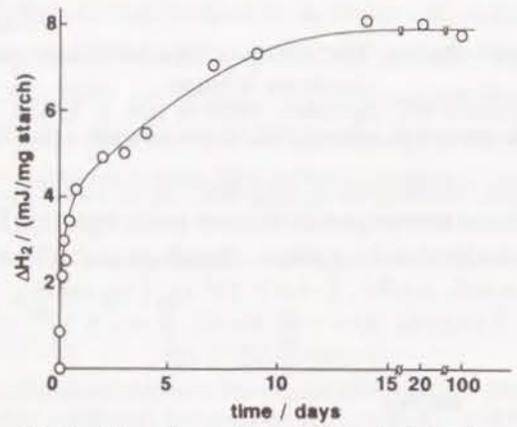

Fig. III–9. Time dependence of regelatinization energy for 33 w/w% sweet potato starch.
DSC pan was stored at 5 °C after the first run.

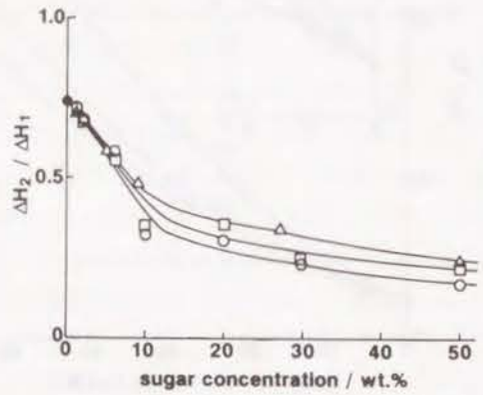

Fig. III–10. Retrogradation ratio ($\Delta H_2/\Delta H_1$) for 33 w/w% sweet potato starch as a function of sugar concentration.
○, sucrose; □, glucose; △, fructose.

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Chapter IV
GELATION PROCESS OF TOFU

From ancient times, soybeans (*Glycine max*) have been utilized for food in many Asian countries. They have served as one of the most important protein sources in Japan and China (Shurtleff and Aoyagi, 1979). More than 50 % of the soybean yield in Japan has been used for tofu manufacture. Tofu is a gel–like food made from soybean milk and a coagulant. Soymilk has traditionally been coagulated with Nigari or Sumashiko. Nigari, also known in the West as bittern, is the residue after extraction of sodium chloride from sea water and consists of magnesium chloride with the trace minerals in sea water. Sumashiko is crude calcium sulfate prepared from gypsum. Recently, glucono–δ–lactone (GDL) became a more popular coagulant than refined calcium sulfate or natural Nigari, especially in tofu–processing factories. There are two types of tofu in Japan. One is momen tofu, which refers to tofu that is made by using a cotton–cloth ("momen" in Japanese) filter, and the other is softer kinugoshi tofu or silken tofu. In both soymilk is boiled and mixed with coagulant. Kinugoshi tofu is allowed to stand until it becomes firm without removal of the whey. However, momen tofu is made from soymilk curds. After the bulk of the whey is removed, the curds are scooped into a cloth–lined molding box with draining holes and pressed for about 30 min to remove the residual whey. Usually, the protein content of Japanese tofu is 5.0 % for kinugoshi and 6.8 % for momen (Resources Council, Science and Technology Agency, 1982). Water content of tofu varies from 84 to 90 %.

A novel method for evaluating the properties of soybean for making tofu is investigated. There have been many investigations on the processing of tofu, based on the measurements of the gel strength (Saio et al., 1969; Hashizume et al., 1975; Saio and Watanabe, 1978; Hara and Negishi, 1987). Gel strength is the breaking force for fully gelled samples. This method is widely used by tofu makers using a curdmeter or a texturometer. It is demonstrated that the gelation of soymilk with GDL could be monitored in real time by dynamic viscoelasticity measurements. The dynamic shear modulus is compared with the breaking stress in the first part of this chapter. In order to clarify the effect of acidic subunit A_4 of 11S globulin, the gelation process of soymilk prepared from twelve varieties of soybean was compared.

It has been reported that 11S globulin principally determines the breaking force of tofu (Saio et al., 1969; Saio and Watanabe, 1978) by virtue of sulfhydryl bonds. Rheological studies by Mori and his co–workers (Utsumi et al., 1982; Nakamura et al., 1984, 1985a; Mori et al., 1989) on heat–induced gels of soybean 11S globulin are also based on texturometer measurements. The gelation properties of 11S globulin observed in the dynamic viscoelasticity measurement are described in the second part. Several factors influencing the gel properties are also

discussed.

Soybean proteins contain two major globulins, 7S and 11S (Wolf et al., 1961; Saio and Watanabe, 1978; Brooks and Morr, 1985), which show different thermal transition temperatures (German et al., 1982; Varfolomeyeva et al., 1986; Damodaran, 1988) and gel forming properties (Hermansson, 1986; Morr, 1990). In the last part of this chapter, the gelation of 7S globulin in the presence of GDL is investigated by a dynamic viscoelastic measurement and a compression testing and discussed in comparison to the 11S–GDL systems.

IV–1. EVALUATION OF TOFU–QUALITY

MATERIALS AND METHODS

Soybeans

Five varieties of soybeans, Enrei, Okushirome, Tamahomare, Akiyoshi and Tsukuizairai, produced in Japan in 1988 were used as samples. Soybeans having the A_4 acidic subunit of the 11S globulin (6 varieties) and lacking the A_4 subunit (6 varieties) were also used. They were stored in sealed bags at 0 °C until tested.

Preparation of Soymilk

For the hardness measurement soymilk was prepared as described by Hara and Negishi (1987). Six times the weight of water was added to soybeans to simulate the processing of kinugoshi tofu, the protein content of the soymilk being about 5 %.

Soymilk for the dynamic viscoelasticity measurement was prepared in a small scale. Soybeans (5 g) were immersed in 50 mL of deionized water for 18 hours at 20 °C. The mixture was homogenized in a Waring Blendor (Nihonseiki Seisakusho, maximum speed for 60 s) and then in a Polytron–type homogenizer (MPC–NS, Tokyo Rika, dial setting 5 for 60 s). The slurry was poured over a two–fold gauze, whereby the resulting filtrate was used as soymilk, the protein content being around 3 %.

Preparation of 11S Globulin

11S globulin was isolated by the method of Thanh et al. (1975) as follows. Soybeans were ground and defatted with *n*–hexane at 20 °C. The resulting powder was immersed in 63 mM tris(hydroxymethyl)aminomethane–hydrochloric acid (Tris–HCl) at pH 7.8 for 1 h, and then centrifuged to remove the insoluble fraction. 2 N hydrochloric acid was then added to lower the pH to 6.6. The extract was dialyzed against Tris–HCl buffer at pH 6.6 for 3 h at 2–3 °C, and finally centrifuged, before the precipitate was directly freeze–dried.

Analysis of Subunit Composition

The protein content of the soybeans was determined by the micro–Kjeldahl method. Soybean powder and 11S globulin samples obtained were examined by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS–PAGE). The concentration of polyacrylamide was 13 %, and Laemmli's buffer system in the presence of 5 M urea was used (Laemmli, 1970). The relative quantity of the protein subunits was determined by densitometry.

Measurement of Gel Hardness

The gel hardness was evaluated by the method of Hara and Negishi (1987) as follows. Soymilk was boiled for 4 min and then cooled. The solution (25 mL) was poured into a 30 mm–diameter glass tube and mixed with 1 mL of a glucono–δ–lactone (GDL) solution which had been freshly prepared with ice–cold water. GDL was adjusted to 0.4 % in the mixture. The tube was allowed to stand for 60 min at 70 °C, and cooled in an ice bath and tap water for 60 min each. The gel was then aged at the test temperature for 60 min before measurement.

The hardness of the gels was determined with a Neo-Curdmeter (I-io Denki Co. Ltd., Tokyo), the center of the gel in the tube being vertically compressed with a cylindrical plunger (8 mm dia.) at a compression rate of 3.6 mm/s. Gel hardness at 20 °C was evaluated from the load value at the breaking point. The hardness was calculated by equation IV-1 to compare to the dynamic shear modulus.

$$H=F\cdot g/A \qquad \text{(IV-1)}$$

where H is the hardness of the gel (Pa), F is the breaking force in kgf, A is the cross-sectional area of the plunger (5.02×10^{-5} m^2), and g is the acceleration of gravity (9.80 m·s^{-2}).

Dynamic Viscoelasticity

Soymilk or an 11S globulin aqueous solution (1-5 %) was heated in boiling water for 5 min. The dynamic viscoelasticity during the gelation process was measured with a Rheolograph Sol apparatus (Toyoseiki Seisakusho, Tokyo). The cell of the instrument was heated to 80 °C beforehand, and 1.5 mL of the sample solution was put into the cell. Then, 0.05 mL of a GDL solution, which had been freshly prepared with ice-cold water, was added to the sample. After stirring the mixture quickly, the measuring blade was inserted into the cell, and the surface of the sample was covered with silicone oil to prevent the evaporation of water. The sample solution was then subjected to 2 Hz sinusoidal shear oscillations with an amplitude of 125 μm to apply a shear strain of 0.125. Values for the storage and loss moduli were recorded as a function of time.

Analysis of the Gelation Process

The observed data were fitted to the empirical formula:

$$G'(t)=G'_{sat}[1-\exp\{-k(t-t_0)\}] \qquad \text{(IV-2)}$$

where G'_{sat} is the saturated value of the storage modulus, k is the rate constant of gelation, t_0 is the gelation time, and t is time. The origin of the time, $t = 0$, was taken as the time when GDL was added to the soybean solution. The gelation time was defined as the point where G' began to deviate from the base line. Rate constant k was estimated from curve fitting by the least squares method. Calculations were done with a SALS program (ver. 2.5) (Nakagawa and Oyanagi, 1980) on a time-sharing system at the Computer Center of the Agriculture Forestry and Fishieries Research Secretariat (Tsukuba).

RESULTS AND DISSUCUSION

Subunit Composition

The protein content and subunit composition of the five varieties of soybeans used in this experiment are shown in Table IV-1. α', α and β are subunits of 7S globulin, and A_3 and A_4 are the acidic subunits of 11S globulin according to the naming method of Nielsen (Kitamura et al., 1984). A designates the other acidic (A_{1a}, A_{1b} and A_2) and B basic subunits of 11S globulin. The A_5 subunit was not observed, probably because little or none was present, and it

therefore had little effect on the subunit composition. The Enrei and Okushirome varieties lacked an A_4 subunit, while Akiyoshi and Tsukuizairai contained an A_4 subunit. As shown in Table IV-1, Tamahomare contained a small amount of A_4, which seems to have come either from the background as an experimental error or from the other protein bands, since Tamahomare is known as an A_4-lacking variety.

Table IV-2 shows the protein content and subunit composition of six soybeans with A_4 subunit and six soybeans without A_4. Since the protein content affects the gelation characteristics of soymilk, varieties whose protein content was almost the same were chosen for the present study. However, the protein content of Hakubidaize was a little higher than that of the other eleven varieties. Six varieties with sample numbers from 1 to 6 lacked the A_4 subunit and the other six varieties with sample numbers from 7 to 12 contained the A_4 subunit. Since A_4 subunit together with the lower molecular weight acidic A_5 subunit forms intermediate subunit A_5-A_4-B_3 (Momma et al., 1985), samples 1 to 6 don't contain the A_5 subunit, while samples 7 to 12 show the A_5 band in SDS-PAGE pattern.

Gelation Curve

Both the storage and loss moduli began to rise after a certain time (t_0), and the observed curves seem to fit first-order reaction kinetics. Fig. IV-1 shows a typical gelation curve for soymilk, concentration C_g of GDL being fixed at 0.4 %. All the observed curves appear to have reached equilibrium values after one hour. The loss modulus showed no significant difference among the varieties as shown in Table IV-3. However, the saturated storage modulus depended on varietal difference, so that the gelation curves were different among the soybean varieties. Storage and loss moduli $G'(3600)$ and $G''(3600)$, and mechanical loss tangent $\tan\delta = G''/G'$ at time $t-t_0 = 3600$ s are shown together with rate constant k in Table IV-3. Samples 1 to 6 without the A_4 subunit show larger values of $G'(3600)$ and G'_{sat} than samples 7 to 12 with the A_4 subunit. Some exceptions are noticeable: however, as is shown in Table IV-3, values of $G'(3600)$ for sample 5 (Tamahomare) and for sample 6 (Tamatsukuri 11 go) are slightly smaller than those for samples 1-4. This may be attributed to the fact that the A_3 content of samples 5 and 6 was lower than that of samples 1-4, as first suggested by Nakamura et al. (1985b). Values of $G'(3600)$ for samples 7, 9 and 12 was higher than that of samples 8, 10 and 11. These trends, however, were not clearly defined, and may also have been affected by the composition of the 11S globulin. Loss modulus G'' was no different among the twelve varieties. Therefore, values of $\tan\delta$ are smaller for the tofu gels prepared from varieties without the A_4 subunit. In other words, the former gels were more brittle and more solid-like than the latter gels. Samples 7 to 12 with the A_5 subunit showed slightly higher values for the rate constant than the varieties concentration of GDL. The rate constant could be determined mainly by the concentration of GDL coagulant, as in the case of the gelation of casein by rennet at constant temperature (Tokita et al., 1982a).

It is apparent that we could have obtained better results if we increased the number of parameters; *e.g.*, if we replaced empirical equation IV–2 by the sum of two exponentially increasing functions. However, the physical meaning of the parameters obtained from such a procedure is not simple due to the complicated nature of soymilk. In the case of equation IV–2, rate constant k indicates the speed of gelation, and G'_{sat} is the final pseudo–equilibrium elastic modulus of the tofu gel.

Comparison between Saturated Storage Modulus and Hardness

The calculated values for saturated storage modulus G'_{sat} and gel hardness by a curdmeter are shown in Table IV–4, G'_{sat} correlating well with the hardness (r=0.998). Soybeans which form tofu gels with 0.4 % GDL and have a breaking strength higher than 100 gf/cm^2 or 9.8 X 10^3 Pa have been practically evaluated as sufficient for making tofu (Hara and Negishi, 1987). The linear relationship between G'_{sat} and the hardness implies that those soybean varieties with an over 170 Pa storage modulus at 80 °C during dynamic viscoelastic measurements were suitable for tofu processing. The viscoelastic method is convenient for measuring the gelation process in real time, and will shorten the time needed for evaluating the products and will improve the efficiency during processing. The direct monitoring of gelation for practical use in tofu–making is also possible if an appropriate instrument can be developed at an attractive price.

Gelation of 11S Globulin

11S globulin was separated from the five varieties of soybeans but, unfortunately, these fractions contained about 10 % of an impure component originating from the β–subunit of 7S globulin according to SDS–PAGE patterns. The gelation curves at 80 °C for 11S globulin solutions are shown in Fig. IV–2, protein concentration C_p being 5.0 % and C_g being 0.4 %. The curves were different according to variety, although rate constant k was almost the same with the same concentration of GDL, except for Tsukuizairai which gelled at a slower rate. The dependence of saturated storage modulus G'_{sat} on the 11S concentration in the presence of 0.4 % GDL for Enrei and Tsukuizairai is shown in Fig. IV–3. G'_{sat} increased with increasing C_p, the values for G'_{sat} varying according to variety, although the amount of 11S globulin was the same.

The gelation kinetics of the 5 % 11S globulin solution were investigated in the presence of various concentrations of GDL for Enrei and Tsukuizairai (Fig. IV–4). Rate constant k increased with GDL concentration. It has been reported that, for the clotting of casein in the presence of rennet, the amount of coagulant (rennet) governed the rate of gelation (Tokita et al., 1982a). In this case as well, the amount of coagulant (GDL) governed the rate of gelation of 11S globulin.

The saturated storage modulus was not same for all varieties, even when the 11S globulin concentration, coagulant and other experimental conditions were identical. Nakamura et al. (1984) have reported that the hardness of heat–induced 11S globulin gels differed between different varieties at the same concentration of 11S globulin. They also pointed out that the gel hardness increased with the content of an acidic subunit of high molecular weight in the total 11S globulin. It is well established that a higher molecular weight fraction in a polymer forms a gel with higher strength than a lower molecular weight fraction (Mitchell, 1980; Watase and Nishinari, 1983). The proposal by Nakamura et al. (1984) is therefore plausible. Since the A_3 subunit has the highest molecular weight of the acidic subunits, the variety with the highest content of A_3 should form the gel with the highest gel strength. However, our results showed otherwise. For example, the 11S globulin gel prepared from Tsukuizairai, which has a higher A_3 subunit content than Enrei, was not stronger than that of Enrei. The content of the other acidic subunits in 11S globulin was not determined, and their effect could possibly have been greater even if they had a lower molecular weight. The composition of the acidic subunits of 11S globulin may affect gel properties by interaction between subunits. Our findings lead to the conclusion that the quality of not only heat–induced gels but also of coagulated gels in the presence of GDL is affected by the subunit composition of the 11S globulin.

As shown in Fig. IV–4, differences in subunit composition caused different gelation rates, the gelation rate not being simply governed by either the A_3 or A_4 subunit content or by the total 11S globulin concentration. It also seems to have been affected by the combination of subunits contained in 11S globulin. Since the gelation rate is important in processing tofu, studies on the effect of subunits on the gelation rate would be valuable from both academic and practical view points. Further investigation is necessary to clarify the detailed relationship between viscoelasticity during the gelation of 11S globulin and its subunit composition.

Table IV–1. Protein content and subunit composition of the soybean varieties.

Variety	Protein content (%)	α' (%)	α (%)	β (%)	A_3 (%)	A_4 (%)	A (%)	B (%)	11S subunit content (%)
Enrei	33.7	8.8	13.2	15.4	3.3	0	26.4	22.5	17.6
Okushirome	32.8	10.5	16.3	13.7	5.3	0	21.1	17.9	14.5
Tamahomare	31.9	8.2	10.3	12.9	3.4	0.9	24.1	22.4	16.2
Akiyoshi	33.8	7.3	9.9	12.8	4.7	4.4	20.4	19.0	16.4
Tsukuizairai	31.4	7.9	12.4	15.5	5.0	6.7	20.9	16.3	15.4

The content of each α', α, β, A_3, A_4, A, and B subunit in a protein was determined by densitometry.

Table IV–2. Protein content and difference in the subunit composition of soybean varieties used in this experiment.

No.	Variety	Protein content (%)	$\alpha'+\alpha$ (%)	β (%)	A_3 (%)	A (%)	A_4 (%)	B (%)	A_5 (%)
1	E-4-2	39.48	7.8	7.2	5.3	19.6	0	17.9	0
2	Suzuyutaka	38.96	16.4	9.6	5.4	18.8	0	16.7	0
3	Tachinagaha	38.80	15.7	8.7	5.4	17.0	0	17.5	0
4	Enrei ET	39.79	15.6	11.1	5.1	17.6	0	13.6	0
5	Tamahomare	37.75	15.6	10.1	3.8	16.0	0	12.1	0
6	Tamatsukuri 11 go	38.13	14.9	9.1	3.7	15.9	0	12.1	0
7	Miyagishirome	39.05	11.5	8.4	3.2	14.4	4.6	13.9	1.7
8	Williams	36.88	11.9	7.5	3.9	14.5	4.6	14.7	2.4
9	Hakubi	36.84	14.0	7.1	4.8	16.8	6.1	14.8	1.7
10	Sekaiichi	39.62	11.4	9.9	3.1	16.7	6.4	16.4	2.4
11	Hakubidaizu	43.10	11.2	5.2	5.0	16.4	7.6	18.2	2.5
12	Picket	39.23	13.6	6.9	5.2	14.6	5.3	16.9	2.5

Table IV–3. Storage and loss moduli at t = 3600 sec, saturated storage modulus G'_{sat} and rate constant k estimated from curve fitting by equation IV–2.

No.	Variety	G' (3600) (Pa)	G'' (3600) (Pa)	tan δ	G_{sat} (Pa)	k (sec^{-1})
1	E-4-2	154	24	0.160	137.5±4.0	0.00321
2	Suzuyutaka	173	29	0.171	153.7±3.5	0.00283
3	Tachinagaha	171	24	0.141	151.3±3.6	0.00301
4	Enrei ET	136	22	0.157	119.8±3.7	0.00316
5	Tamahomare	123	21	0.175	110.4±2.6	0.00254
6	Tamatsukuri 11 go	120	20	0.169	103.4±3.2	0.00323
7	Miyagishirome	135	24	0.171	119.2±3.6	0.00347
8	Williams	120	22	0.183	106.6±3.5	0.00347
9	Hakubi	137	22	0.157	121.2±3.2	0.00302
10	Sekaiichi	132	26	0.200	122.6±3.1	0.00349
11	Hakubidaizu	119	25	0.208	106.7±3.2	0.00336
12	Picket	134	20	0.154	118.0±3.9	0.00321
	Mean of nos. 1–6	146.2	23.2	0.162	129.4	0.00300
	Standard deviation of nos. 1–6	21.3	2.9	0.011	19.4	0.00025
	Mean of nos. 7–12	129.5	23.2	0.179	115.7	0.00334
	Standard deviation of nos. 7–12	7.2	2.0	0.020	6.6	0.00017

Table IV–4. Saturated storage modulus (G'_{sat}) and hardness of soymilk–GDL gels.

Variety	G'_{sat} (10^{-2}Pa)	Hardness (10^{-4}Pa)
Enrei	2.6	1.2
Okushirome	1.8	1.0
Tamahomare	1.9	1.0
Akiyoshi	2.1	1.1
Tsukuizairai	1.6	0.98

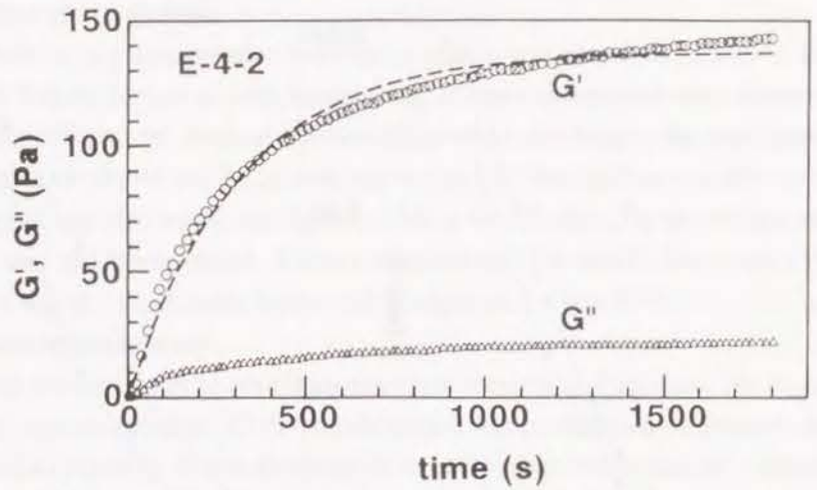


Fig. IV–1. Typical gelation curve after the gelation time for soymilk prepared from E–4–2. Measuring temperature was 80 °C. ○, experimental storage modulus G';△, experimental loss modulus G''; – – – –, calculated storage modulus by curve fitting (kinetic equation IV–2).

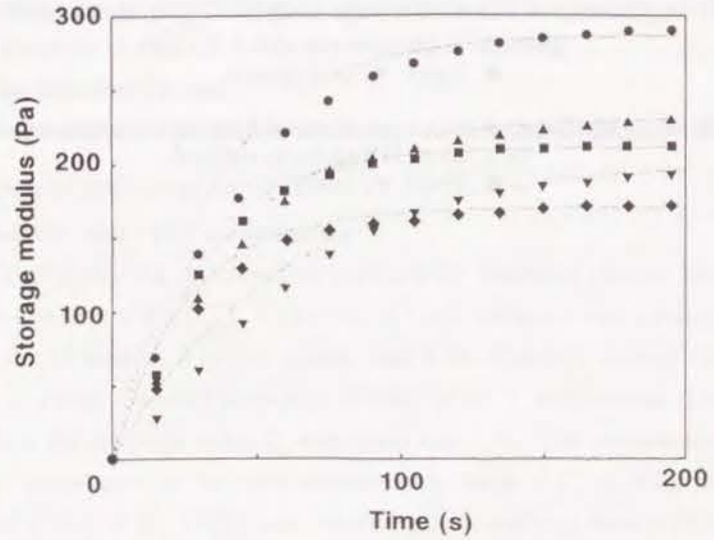


Fig. IV–2. Gelation curves for 11S protein prepared from different varieties of soybean.
Measuring temperature was 80 °C, and concentration of 11S globulin was 5 % and that of glucono–δ–lactone was 0.4 %. ●, Enrei; ▲, Akiyoshi; ■, Tamahomare; ▼, Tsukuizairai; ◆, Okushirome; ·····, calculated storage modulus by curve fitting (see the experimental section for kinetic equation IV–2).

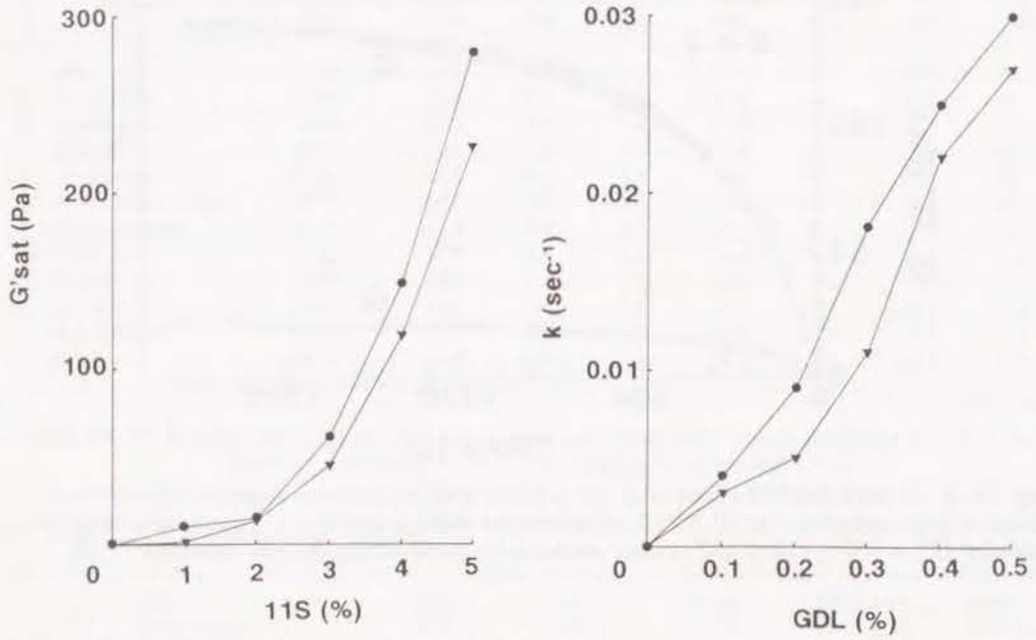


Fig. IV–3.(left) Dependence of saturated storage modulus G'_{sat} on the concentration of 11S globulin in the presence of 0.4 % GDL.
●, Enrei; ▼, Tsukuizairai.

Fig. IV–4.(right) Dependence of rate constant k on the concentration of GDL for a 5.0 % 11S globulin solution.
●, Enrei; ▼, Tsukuizairai.

IV–2. FACTORS AFFECTING THE GEL PROPERTIES

MATERIALS AND METHODS

Preparation of 11S globulin

Defatted soybean powder from the var. Enrei was dispersed in a 15–fold volume of water (pH 7.5) for 60 min at room temperature. Fibrous components were removed by filtration and centrifugation. Dry sodium bisulfite (SBS) was added to the supernatant to a final concentration of 10 mM and the pH was adjusted to 6.4. The mixture was allowed to stand at 5 °C overnight and then was centrifuged at 6500 g for 20 min. The precipitate was dialyzed against water and freeze–dried. It was confirmed by SDS–PAGE (Laemmli, 1970) that the precipitate was an 11S globulin fraction (11S) whose purity was 95 %.

Rheological measurements

11S was dissolved in deionized water and heated in boiling water for 10 min and then cooled to room temperature. GDL (reagent grade) was purchased from Wako Pure Chemicals Industries Ltd. (Osaka). It was dissolved in ice–cold water just before use. The 11S solution (~2.0 ml) was heated to the test temperature and 0.05 mL of GDL solution was added. Then, 1.6 mL of the solution was immediately injected into a cell of a Rheolograph Sol (Toyoseiki Seisakusho, Tokyo) which was heated to the same temperature beforehand. The surface of the sample was covered with silicone oil in order to prevent the evaporation of water. The sample solution was subjected to sinusoidal shear oscillations, and the storage and loss moduli were recorded as a function of time.

Analysis of the Gelation Process

The observed data were fitted to the fomula IV–2 as described in the section IV–1.

RESULTS AND DISSCUSSION

Saturated modulus and 11S Concentration

Fig. IV–5 shows the relationships between the saturated storage modulus of the gel, G'_{sat} and 11S concentration (C_p). Gelation of Tachisuzunari was studied at 60 °C (solid squares) and that of Enrei at 60 (open circles) and at 80 °C (solid circles); GDL concentration was 0.4 % in all cases. Gelation properties of Enrei at 60 °C were similar to those of Tachisuzunari. Gelation did not occur when C_p was lower than 1 %. The concentration dependence of G'_{sat} was more pronounced in the lower concentration range of C_p as in agarose (Watase et al., 1989), amylose (Clark et al., 1989), heat induced gels of soybean protein (Bikbov et al., 1979) and many other gels (Clark and Ross–Murphy, 1987).

Fig. IV–6 shows the double logarithmic plot of G'_{sat} and C_p. Gels, whose texture is within the range for ordinary kinugoshi–tofu, were obtained when the 11S concentration ranged from 2 to 6 %. In this concentration range, the exponent 3.4 in the power law relationship between the saturated modulus G'_{sat} and the polymer concentration C_p is rather larger than in

the case of the well-known square power law which has been observed for many biopolymer gels (Hirai, 1955; Fukada and Kaibara, 1973; Nishinari and Watase, 1983). However, the value 3.4 is smaller than 5 reported by Bikbov et al. (1979) for heat-induced soybean globulin gels of concentration of 7.5–58.4 %. Upon examination the relationship between G'_{sat} and C_p at higher concentrations, the concentration dependence of G'_{sat} would be less pronounced. However, since 11S globulin solutions of concentrations higher than 10 % unfortunately formed heat-induced gels during heating in boiling water for 5 min, it is impossible to examine them experimentally. The cubic power relationship did not depend greatly on the soybean cultivar or gelling temperature (60 and 80 °C). A similar tendency was also observed for the loss modulus. These findings will be useful to estimate the amount of water that should be added to soybeans in tofu processing.

The mechanical loss tangent (tanδ) decreased with heating time after addition of GDL. It suggests that the system was more liquid-like at the initial stage of gelation. Finally, a more solid-like gel was formed and tanδ became constant. Fig. IV-7 shows the relationship between 11S concentration, C_p, and tanδ at the final stage of gelation. Values of tanδ decreased with increasing protein concentration. This indicated that the gels with higher C_p were more solid-like. The three dimensional networks formed by the polymers become denser with increasing C_p. Values of tanδ in the presence of 0.2 % and 0.4 % GDL were not significantly different at the same 11S concentration. Since tanδ was around 0.1 even at higher C_p, this gel was more liquid-like than typical biopolymer gels whose tanδ values are about 0.01 (Ross-Murphy, 1984).

Galation Time and Gelation Rate

The plot of the gelation time against temperature is shown in Fig. IV-8. Since tofu-like gels were formed when 11S concentration was between 2 and 6 %, the middle concentration, 4 %, was chosen for this experiment. At low temperatures, gelation commerces slowly. Gelation began immediately after GDL was added to an 11S solution at 90 °C.

Fig. IV-9 shows the plot of rate constants against temperature. Contrary to the case of t_0, k increased with increasing temperature. In comparison with the temperature dependence of the gelation time (Fig. IV-8), the gelation rate seems to be estimated by the gelation time instead of the rate constant.

The rate constants at various temperatures were logarithmically plotted against the reciprocal of the absolute temperature (Fig. IV-10, solid circles). The rate constants lie on a straight line except for one measured at 50 °C. Because the gelation rate is very slow at such a low temperature, it is difficult to obtain saturated shear moduli from a rheological measurement. A large error in the k value may result. The other seven points lie on a straight line (upper solid line shown in Fig. IV-10). The activation energy for the gelation can be estimated from the slope. From the slope of this Arrhenius plot -4.08×10^3, the activation energy, E_a, was calculated as 1.5×10 kJ/mol. This value is rather small compared to 3.81×10^2 kJ/mol for the heat insolubilization of 11S globulin (Watanabe, 1988) or 1.13×10^2 kJ/mol for the heat denaturation of soybean 7S globulin kept at high temperatures (Iwabuchi et al., 1991). However, it is the same order as those reported for clotting of casein micelles by rennet [3.4×10 kJ/mol (Tuszynski, 1971), 4.2×10 kJ/mol (Tokita et al., 1982a), and 8.8×10 kJ/mol (Niki and Sasaki, 1987)]. The small activation energy results from the fact that the gelation process does not depend very much on temperature. GDL in the present system may play a similar role as an enzyme (rennet) does in the clotting of casein. The gelation process was not induced by heat. Richardson and Ross-Murphy (1981) plotted the logarithm of the gelation time (they call it gel time) against the reciprocal absolute temperature. A similar plot is shown in Fig. IV-10 with open circles. We omitted a gelation time obtained at 90 °C, because it was too short to calculate a log value. The other seven gelation times lie on a straight line (lower line in Fig. IV-10). The slope was 3.66×10^3 by least-squares method. It was close to the absolute value (4.08×10^3) of the slope from logarithm of k against the reciprocal of the absolute temperature. Therefore, the rate constant of this system would be inversely proportional to the latent time. If we assume that one slope of the Arrhenius plot represents one process (Richardson and Ross-Murphy, 1981), the gelation consists of a single process between 50 and 90 °C. Higher temperatures increase the gelation rate but would not change the gelation mechanism in this temperature range. The temperature at which a coagulant is added to soymilk does not greatly affect the properties of their final product, tofu, even though it changes the gelation rate. The thermal denaturation temperature of soybean 11S globulin in water was reported as 84.5 °C and the denaturation completed at around 93 °C by differential adiabatic scanning calorimetry (Bikbov et al., 1983). All 11S globulin molecules in our system should be denatured below 100 °C. Since the 11S globulin was heated in boiling water before GDL was added, the denaturation by heat was completed during heating. At 11S concentration lower than 7 %, the preheated protein solution did not form a gel without GDL below 90 °C. The gelation in the present system would not be induced by heat. The gelation is thus chemically induced by GDL.

Gelation Time and GDL Concentration

The plot of the gelation time against the concentration, C_g, of GDL is shown in Fig. IV-11. The test temperature was 60 °C to extend the gelation time. The gelation time became shorter with increasing concentration of GDL, and it became shorter with decreasing 11S concentration at the same GDL concentration.

If t_{sat} was taken as the time at which the gelation curve became saturated, t_{sat} also decreased with C_g. On the other hand, t_0 and t_{sat} increased with C_p at a constant C_g of 0.4 % when C_p were high enough to form gels as shown in Fig. IV-12.

Since the pH of the solution decreases on addition of GDL, the pH of the gel was measured. The results are shown in Fig. IV-13. The pH of the GDL solution did not depend

on GDL concentration. However, because of the buffering action of the protein, the pH value of the gel was higher than 3.9. The dependence of t_0 (Fig. IV–11) and the pH (Fig. IV–13) on C_g are similar. It suggests that the gelation of 11S is promoted by acidification, which is induced by the addition of GDL. GDL is partially cleaved into gluconic acid in water, and then some gluconic acid molecules dissociate. Each chemical species of GDL exists in chemical equilibrium, and the amount of each species is controlled by various conditions such as the temperature, the concentration of GDL, and the other component in the system. Since a lactone is chemically inactive, it should not react with 11S globulin. The lowered pH shows the existence of dissociated gluconic acid. It is clear that the acidic form of this coagulant is important to the gelation of 11S.

The gelation time decreased slightly with increasing concentration of GDL at high GDL concentration range, $C_g > 0.8$ % (Fig. IV–13). This might be attributed to a slight drop in pH, which in turn might cause a greater effect on the latent time. At higher concentrations of GDL, the pH seems to decrease only slightly with increasing concentration of GDL, although it is not clearly observed. Since it is difficult to measure the pH of gels accurately within the error of ± 0.1, this must be explored in the future.

In many cases, the gelation rate increases with increasing protein concentration as described below. This is reasonable since the density of reaction sites, which can form three dimensional networks, becomes higher with increasing polymer concentration. Nakamura et al. (1984) reported that the minimum time for thermal gelation of soybean 11S globulin decreased with protein concentration. A similar tendency was also observed for an enzymatically induced gelation, namely casein micelle clotting by rennet (Tokita et al., 1982b).

However, as mentioned above (Fig. IV–11), the gelation time for higher 11S concentrations was longer than that for lower 11S concentrations. GDL is partially cleaved into gluconic acid in the solution and the subsequent dissociation of gluconic acid molecules generates protons. Therefore, the pH of the system dropped. Fig. IV–14 shows the relationships between C_p and the pH for 11S solutions with and without GDL. Solutions of 11S without GDL ($C_g = 0$) showed pH values of around 6.8, independent of 11S concentration. Gels formed in the presence of the acidic coagulant GDL had a lower pH. However because of a buffering effect of the protein, the pH of the gels became higher with increasing protein concentration. The gelation of soybean protein in the presence of GDL is a gelation process of acidic coagulation. Since the higher protein concentration inhibits the tendency of the system to go to a lower pH, it slows down the gelation process, even though it increases the density of the reaction sites of 11S which form a three dimensional network. Therefore, the decrease in the pH by the addition of GDL greatly promotes the gelation kinetics.

At higher C_p, the drop in pH at any given GDL concentration was smaller than that at lower C_p (Fig. IV–13). Therefore, the increase in the gelation time with increasing C_p might be

related to pH. Gluconic acid produced by the hydrolysis of GDL generates protons to lower the pH to initiate coagulation of the proteins. The more protein that is present, the more acid is required to lower the pH to a given value. As the concentration of GDL is increased, more acid is generated in a given time, and hence the latent time for initiation of coagulation will be shorter.

The anomalous relationship between t_0 and C_p in the present system might be due to an insufficient amount of GDL for the 11S globulin. GDL was then added to the 11S solution so that the ratio of GDL to 11S became 0.1, because the ratio is near to the ratio of protein to GDL in practical conditions of tofu processing. The gelation time t_0 (open circles in Fig. IV–15) decreased with increasing concentration of 11S for the systems. Fukada and Kaibara (1973) studied the clotting kinetics of fibrinogen with a fixed ratio of thrombin to fibrinogen. The gelation rate increased with increasing concentration, C_p, of fibrinogen. The t_{sat} (open squares in Fig. IV–15) also decreased with increasing C_p, but it almost reached a constant value at higher concentrations of C_p. The value of pH gradually increased from 3.83 to 4.33 with increasing C_p from 1 to 8 % (closed hexagons in Fig. IV–15). The gelation time was not so much influenced by the pH change in these systems. Since the decreasing curve of t_{sat} against C_p was symmetric with the increasing curve of pH against C_p about the horizontal line, t_{sat} seems to be more influenced by the pH than t_0 did. On the contrary, in the presence of a fixed concentration of 11S, t_{sat} decreased with increasing C_g at high concentrations of C_g (Fig. IV–11). Since the systems in Fig. IV–15 contained a large amount of protein, protein inhibited the decrease of pH with increasing GDL. It seems that the pH governed the gelation kinetics by its effect on t_{sat} more than the gelation time. It suggests that a lower pH mainly affects the gelation rate.

Fig. IV–16 shows the relationships between the gelation time to 11S contentration at various ratios of GDL to 11S globulin. The gelation time increased with lowering the ratio. It was clearly shown that the lack of GDL amount made the gelation time longer.

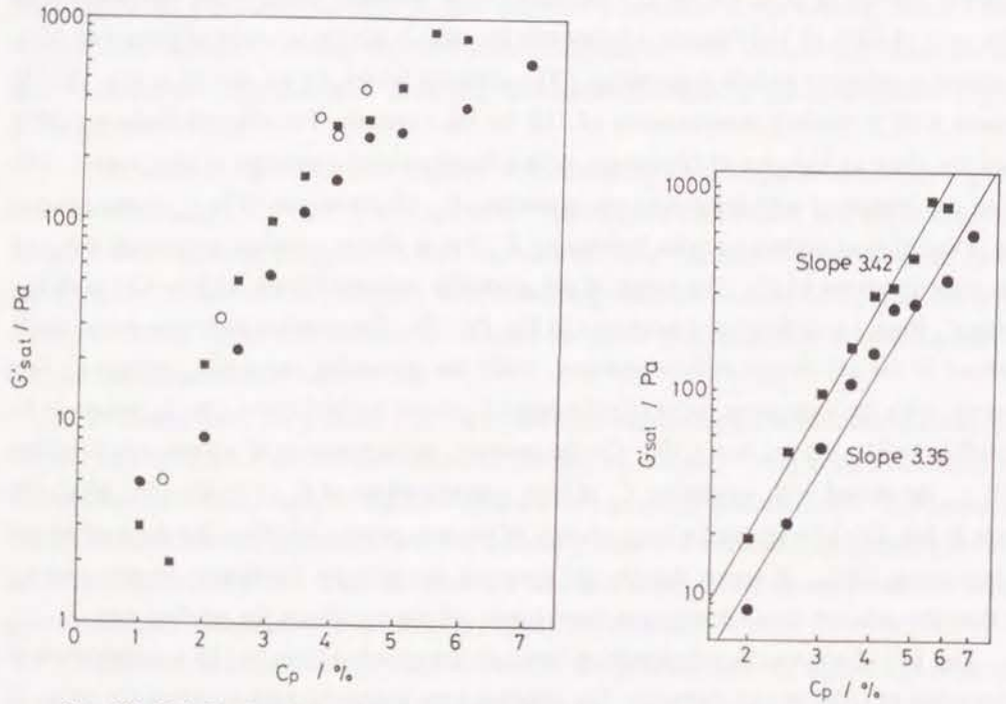


Fig. IV–5.(left) Semi–log plot of saturated storage modulus *versus* 11S concentration for 11S gel containing 0.4 % GDL.
Tachisuzunari at 60 °C (■); Enrei at 60 (○) and 80 °C (●).

Fig. IV–6.(right) The log–log plot of saturated storage modulus *versus* 11S concentration for 11S gels containing 0.4 % GDL.
Tachisuzunari at 60 °C (■); Enrei at 80 °C (●).

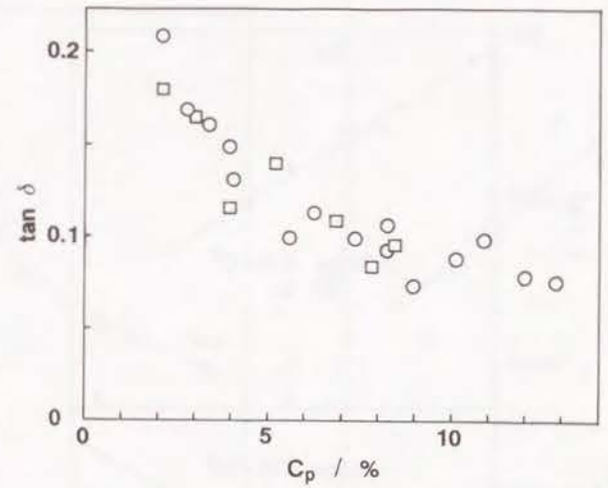
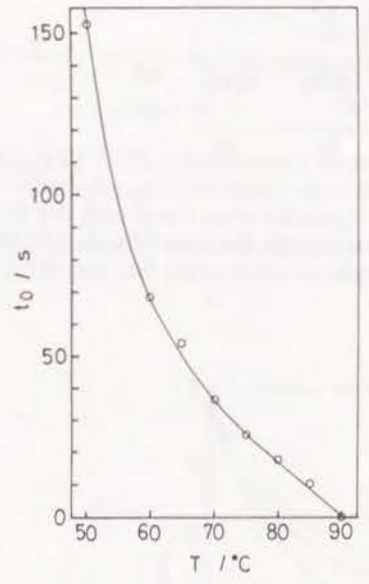


Fig. IV–7. 11S concentration dependence of the loss tangent (tanδ) at the final stage of gelation for 0.2 % (□) and 0.4 % (○) GDL concentration gels.



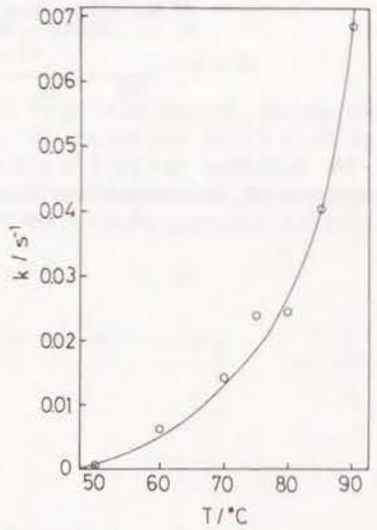


Fig. IV–8.(left) Temperature dependence of the gelation time.
Sample: 4 % 11S globulin separated from Enrei with 0.4 % GDL.

Fig. IV–9.(right) Temeperature dependence of the rate constant of gelation.
Sample: 4 % 11S globulin separated from Enrei with 0.4 % GDL.

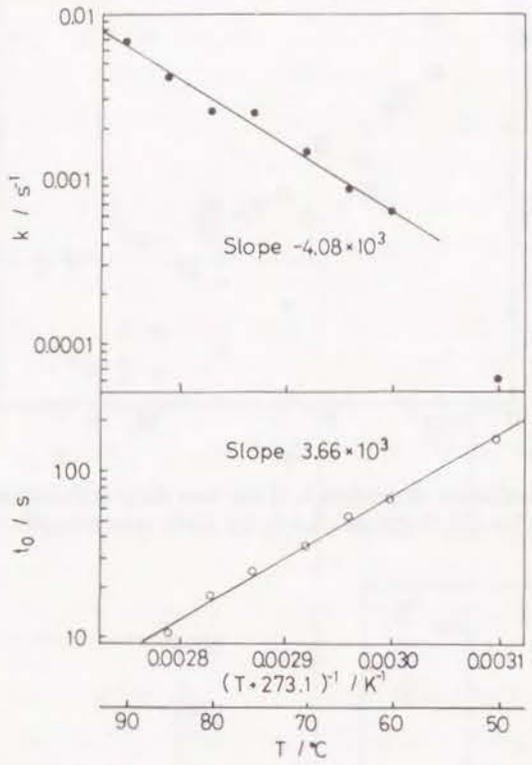


Fig. IV–10. Arrhenius plot for 4 % 11S globulin separated from Enrei with 0.4 % GDL. The rate constant (●) and the gelation time (○). Two straight lines are obtained by the least squares method.

Fig. IV–11.(left) GDL concentration dependence of the gelation time (t_0) and the time (t_{sat}) at which the gelation curve becomes sturated for the gel of 4 % 11S globulin.

Fig. IV–12.(right) 11S concentration dependence of the gelation time (t_0) and the time (t_{sat}) at which the gelation curve becomes saturated for the gel in the presence of 0.4 % GDL.

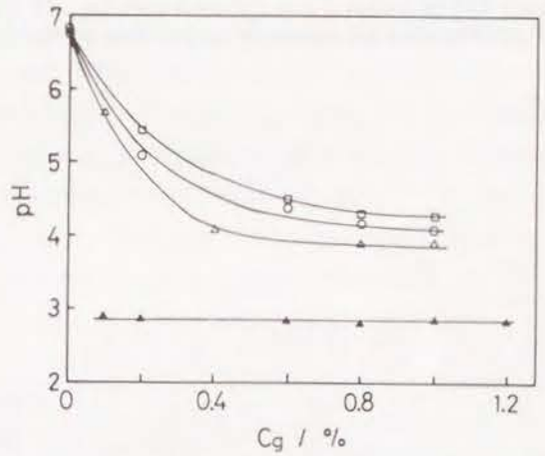


Fig. IV–13. The relationships between the pH of the gel and concentration of GDL. Tachisuzunari 11S: (▲) 0 %, (△) 2 %, (○) 4 %, (□) 6 %.

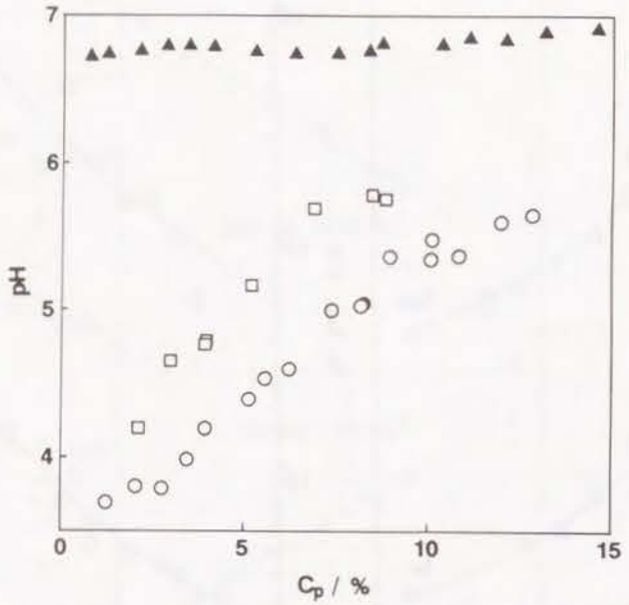


Fig. IV–14. The relationships between the pH and concentration of 11S globulin. GDL concentration: ▲, 0 % solutions; □, 0.2 % gels; ○, 0.4 % gels.

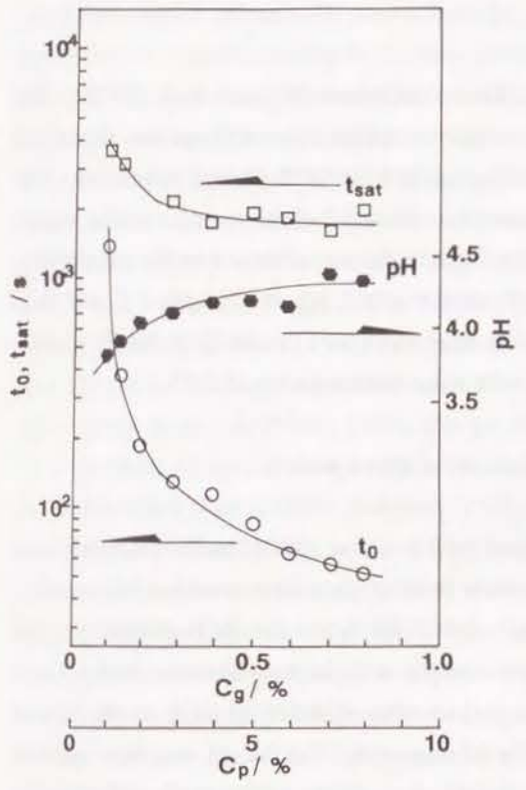


Fig. IV–15.(left) The gelation time (t_0), the time (t_{sat}) at which the gelation curve becomes saturated and pH of the gel as a function of 11S concentration at 60 °C. Each sample contains GDL and 11S at a fixed ratio of 0.1.

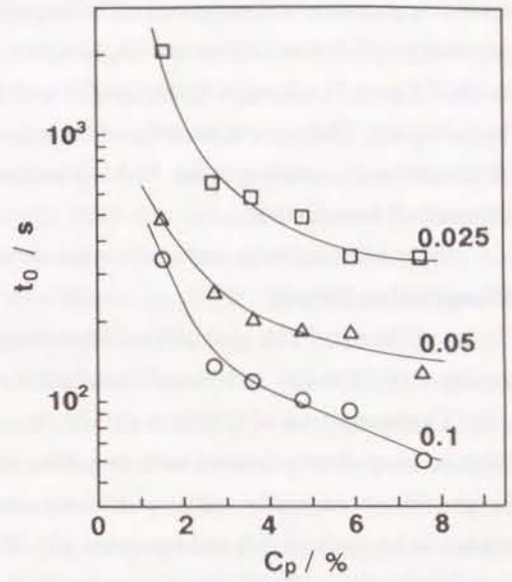


Fig. IV–16.(right) The gelation time (t_0) as a function of 11S concentration at 60 °C. Figures at the right of each curves represent the ratio of GDL to 11S globulin.

IV-3. DIFFERENCES BETWEEN THE 7S AND 11S GLOBULIN GELS

MATERIALS AND METHODS

Preparation of 7S and 11S Globulins

The details of the isolation method are published elsewhere (Nagano et al., 1992). The insoluble fraction of 11S globulin was separated as the precipitate by centrifugation, described in the preceding section. The supernatant was adjusted to 0.25 M NaCl and to pH 5.0. The insoluble fraction was removed. The supernatant was diluted 2-fold with ice-cold water, adjusted to pH 4.8 and then centrifuged again. The 7S globulin was obtained as the precipitate. Both 11S and 7S globulin fractions (7S and 11S) were washed, adjusted to pH 7.5 and then freeze-dried. The protein contents of these powders were 95 % for 11S and 92 % for 7S globulin determined according to the Kjeldahl method with a conversion factor of 6.25.

Dynamic Viscoelasticity

The measurement and analysis are described in the above section.

Compression Testing

A 7S or an 11S globulin solution preheated for 10 min at 100 °C in 20-mL vials was kept in a 60 °C bath. A 5 % volume of GDL solution freshly prepared was added to the solution. Concentrations of protein and GDL were adjusted to 4.0 % and 0.4 %, respectively. The mixture immediately poured into ring-shaped glass molds with 16 mm diameter and 10 mm height. They were allowed to stand for various periods after addition of GDL at 60 °C and cooled in ice bath (3 °C) and tap water (26 °C) for 60 min each. The the gel was then aged at room temperature (27 °C) for 60 min before the measurement. Compression testing of gels was carried out using a Rheoner RE-33005 (Yamaden Co. Ltd., Tokyo) attached a 2 kgf load cell. A cylindrical gel sample on the polyacetal stage was vertically compressed with a polyacetal flat plunger of 40-mm diameter at 27 °C. The compression rate was set to 1.0 mm/s. Breaking stress of gels was calculated from the load value at a breaking point divided by the initial cross-sectional area of the gel. Breaking strain was determined as the ratio of the deformation at a breaking point to the initial height. The Young's modulus was defined as the slope of each stress-strain curve at small strain range. The breaking energy was calculated from the area under the stress-strain curve, and it was normalized per unit volume (m^3). Mean value and standard deviation were calculated from four repeated experiments.

RESULTS AND DISCUSSION

True Gels Formed by 7S or 11S Globulin with GDL

Since both the frequency and amplitude applied to the sample affect storage moduli (Ferry, 1980), viscoelastic measurements were carried out at various frequencies and amplitudes of the sinusoidal strain. Frequency dependence of the saturated storage modulus (G'_{sat}) and the saturated loss modulus (G''_{sat}) at various amplitudes is shown in Figs. IV-17 (7S) and IV-18 (11S), respectively. Since the cell thickness was 1.0 mm, absolute strains made by amplitudes of 25, 50 and 100 μm became 2.5, 5.0 and 10.0 % respectively. The amplitude did not influence modulus values in the cases examined except for one: the maximum strain possible with this system, with an amplitude of 100 μm and a frequency of 3 Hz may destroy the gel structure, because the value of the storage modulus was smaller than that observed at smaller amplitudes for both the 7S and 11S gels. The decrease of storage modulus with increasing deformation shows that the applied strain was beyond a linear region. The storage modulus increased slightly with increasing frequency as observed, not only in various engineering polymeric materials (Ferry, 1980) but also in many other food gels such as agar (Nishinari, 1976), gelatin (Nijenhuis, 1991) and xanthan-carob mixture (Tonon et al., 1991) gels. The loss modulus at 0.1 Hz was larger than those at other frequencies. As are commonly observed for viscoelastic materials (Ferry, 1980), this gel showed liquid-like behavior at lower frequencies. The decrease of loss modulus with increasing frequency in a certain range of frequency had been described (Ferry, 1980; Nishinari, 1976; Richardson et al., 1981; Doublier, 1989) previously. This phenomenon is not seen when a viscoelastic liquid is very thin because the entanglement of polymers makes G' flat and sometimes makes G'' minimum at a certain frequency (Ferry, 1980). Oser and Marvin (1963), Chompff and Prins (1968) and Graessley (1971) proposed theoretical treatments to explain the dip of the loss modulus in the frequency spectrum, however, predictions from these treatments gave deeper dips than were observed. A theory which explains the dip quantitatively, taking into account the network structure, is needed. The lower 7S concentration of 2.0 % was also examined at a fixed amplitude (25 μm). The storage modulus increased slightly with increasing frequency (solid circles in Fig. IV-17).

Fig. IV-19 shows 7S concentration dependence of the saturated storage and loss moduli, G'_{sat} and G''_{sat}, for gels containing 0.4 % GDL. Heat-set gels were formed at 60 °C after heating in boiling water for 10 min without coagulant at 7S concentration higher than 6.2 %. Since the gelation mechanism of heat-induced gels is expected to be different from that of 7S-GDL gels, only the gelation process of 7S at concentrations lower than 6 % was studied. The dependence was more pronounced at lower concentrations of 7S, as observed in heat induced gels of soybean protein (Bikbov et al., 1979) and many other gels (Clark and Ross-Murphy, 1987). Values of tanδ at the final stage of the gelation decreased with increasing protein concentration C_p (solid triangles in Fig. IV-19). This indicated that the gels prepared from higher C_p were more solid-like. The three-dimensional networks formed by the polymers become denser with increasing C_p. Values of G'_{sat} and G''_{sat} for the 7S-GDL system were almost the same as observed in 11S-GDL system (data are not shown).

Since tanδ was larger than 0.1 even at higher C_p, the 7S or 11S -GDL gel was more liquid-like than other typical biopolymer gels such as agar (Nishinari, 1976; Nishinari et al., 1980), gelatin (Laurent et al., 1980; Nishinari et al., 1980), and ovalbumin (van Kleef, 1986).

However, van Kleef (1986) observed larger tanδ values for heat-induced soybean protein isolate and 11S globulin gels of 25 % protein. Many systems which have tanδ values of about 0.1 belong to the so-called "weak gels" or colloidal dispersions (Clark and Ross-Murphy, 1987). In weak gels, the storage and loss moduli as a function of frequency increase monotonously with increasing frequency, and the storage modulus decreases steeply with increasing shear strain because the structure is destroyed by the strain (Clark and Ross-Murphy, 1987). In comparison to weak gels, the typical "true gels" show a small frequency dependence of storage modulus, minimum loss modulus at a certain frequency, and little decrease in shear modulus even at large strain ranges (Clark and Ross-Murphy, 1987). The 7S and 11S gels were considered to be true gels for the following reasons: (i) it was thermally irreversible; (ii) its loss modulus decreased with increasing frequency from 0.1 to 1 Hz as mentioned above; (iii) its storage modulus did not decrease with increasing shear strain from 2.5 to 10 %. Richardson et al. (1981) observed that tanδ value increased with increasing disperse phase fraction for glass filled gelatin gels. Since both of 7S and 11S globulins are known as acid-precipitated proteins, the protein in the finally formed gel by the acidic coagulant GDL are considered to precipitate partially. A part of the protein could disperse as a filler in the gel network formed by the protein.

Protein Concentration Dependence of Saturated Shear Modulus

GDL was added to 7S globulin solution to adjust the ratio of 7S and GDL to 10:1. It was difficult to determine the saturated moduli experimentally for concentrations of 7S lower than 2.5 % because it took a long time (> 3 h) for saturation. Saturated storage and loss moduli increased with increasing 7S concentration; however, 7S concentration dependence of both moduli was less pronounced than that observed at a constant GDL condition (Fig. IV-19).

The shear modulus G' of polymer gels is often described by an exponential function of polymer concentraion C_p:

$$G' \approx K \cdot C_p^{\,m}, \qquad \text{(IV-3)}$$

where K and m are constant. In many cases, the exponent becomes 2 (Hirai, 1955; Fukada and Kaibara, 1973; Nishinari and Watase, 1983). However, the G'_{sat} of 11S gels containing 0.4 % GDL was proportional to 3.4th power of 11S concentration, when 11S concentration ranged from 2 to 6 % as described in the above section. The relationships between G'_{sat} and 7S concentration are examined in the same concentration range (2-6 %). The exponent m for 7S gels with 0.4 % GDL was 2.43 and that for gels containing C_p:C_g = 10:1 was 1.89, which was smaller than the former. Exponent values for 11S gels with 0.4 % GDL and 11S gels containing C_p:C_g = 10:1 are also calculated. In the former case, m became 3.52, which is not very different from that obtained (3.4) in section IV-2. In the latter case, m was also smaller (3.19) than the former value. The protein concentration dependence of G'_{sat} for both 7S and 11S was more pronounced in gels with 0.4 % GDL than in those containing a fixed ratio of GDL to

protein. This suggests that GDL decreases the saturated modulus slightly. Since m values for 7S were smaller than those for 11S, the saturated storage modulus of 7S gels was larger than that for 11S at lower C_p than 4-5 % and the inverse relationship was observed at higher C_p. Since the protein content of kinugoshi tofu is 5.0 % (Resources Council, Science and Technology Agency, 1982), tofu-like gels are made in the presence of GDL, when protein concentration is around 5 %. Both 7S and 11S gels containing about 5 % protein showed almost the same value for the saturated modulus.

Gelation Rate

Typical gelation curves for 4.0 % 7S and 11S solutions in the presence of 0.4 % GDL at 60 °C are shown in Fig. IV-20. The saturated storage modulus for 4 % 7S seemed slightly larger than that for 4 % 11S, while the saturated loss modulus for 7S was almost the same as that of 11S. Therefore, the tanδ ($=G''_{sat}/G'_{sat}$) of completely gelled 7S were slightly smaller than that of 11S.

A large difference in the rate of gelation was observed between 7S and 11S as shown in Fig. IV-20. The gelation time t_0 of 7S was much longer than that of 11S at the same protein concentration C_p, GDL concentration C_g, and temperature. The gelation rate of 7S was also slower than that of 11S; therefore, it required a longer time before storage and loss moduli of 7S reached equilibrium values.

Fig. IV-21 shows the relationships between the rate constant of gelation k and C_g at a constant C_p (4.0 %) for both 7S and 11S globulins. Gelation proceeded faster in systems of higher GDL concentrations, and a similar tendency was observed for 11S systems. However, the value of k for 11S was 2-3 times larger than that for 7S.

The relationships between k and protein concentration at a constant C_g (0.4 %) are shown in Fig.IV-22. The gelation rate decreased with increasing protein concentrations for both 7S and 11S systems. A slower gelation rate was observed again for 7S than for 11S in the fixed GDL concentration systems.

Fig.IV-23 shows that the gelation time t_0 decreased with increasing GDL concentration at a constant 7S concentration (4.0 %) as observed for the 11S system (Fig. IV-11). Gelation was not observed at C_g lower than 0.2 %. If t_{sat} was taken as the time at which the gelation curve became saturated, t_{sat} also decreased with C_g as shown in Fig. IV-23. The gelation time for 7S was almost 10 times longer and t_{sat} for 7S was much longer than those of 11S-GDL gel (Fig. IV-11) when C_p, C_g and testing conditions were the same.

Comparison between Saturated Storage Modulus and Breaking Stress

Saio et al. (1969) reported that the hardness of 11S gels was several times larger than that of 7S gels from a fracture measurement using a texturometer. They used calcium sulfate as a coagulant and heated the mixture of the protein and the coagulant at 70 °C for 10 min. Hashizume et al. (1975) also reported a similar tendency, although they used GDL as a coagu-

lant and heated samples at 60 °C for 90 min. However, our findings from the dynamic viscoelasticity measurement suggest that both 7S and 11S globulins form true gels, and the saturated storage modulus for 7S–GDL gel was almost the same as that of 11S. We also studied fracture phenomena of 4.0 % protein gels with 0.4 % GDL by a uniaxial compression testing. From the results of dynamic viscoelasticity, it appears that 7S globulin requires longer heating time to form a gel than 11S. The compression testing was then carried out on gels made after various heating periods at 60 °C. The breaking stress and the breaking strain, Young's modulus E and the breaking energy for 7S and 11S gels as a function of heating time are shown in Figs. IV–24–27 respectively. Mean values and standard deviations were plotted against heating time in each figure. All four parameters increased with time and then seemed to level off. However, the breaking strain for 7S gels slightly increased even after being heated for 240 min at 60 °C. Faster gelation in 11S than 7S was clearly shown again in these data. Curve shapes for the breaking stress, and also Young's modulus and the breaking energy were similar to the storage or loss modulus curve (Fig. IV–20) for both 7S and 11S globulins. Even though both 7S and 11S globulins show such a relationship individually, the ratio of the saturated storage modulus to the breaking stress was not the same.

It appears that covalent bonds, including disulfide bonding, contributed to the higher breaking stress and strain of 11S–GDL gels. However, hydrogen bonds play an important role in gel formation of 7S and 11S in the presence of GDL, and it affects small deformation properties, such as Young's modulus, storage and loss moduli. If 7S forms a gel only by secondary bonds such as hydrogen bonds and hydrophobic interactions and 11S makes a true gel with three dimensional networks with covalent bonds such as disulfide bonds, breaking properties of 11S would become higher than those of 7S as suggested by Saio et al. (1969). This consideration appears plausible since 7S does not contain any sulfhydryl groups while 11S does (Peng et al., 1984). Utsumi and Kinsella (1985) reported that electrostatic interactions and disulfide bonds were involved in gelation of 11S by heat, while hydrogen bonding was mainly responsible for formation of heat–induced 7S gel.

Utsumi and Kinsella (1985) observed that the breaking force of heat–induced 7S gels was larger than that of 11S gels. It is well–established that the denaturation temperature of 7S globulin is lower than that of 11S (German et al., 1982; Varfolomeyeva et al., 1986). Therefore, 11S globulin requires a higher heating temperature to form a gel than 7S globulin. The heating temperature (80 °C) adopted by Utsumi and Kinsella (1985) may be too low for 11S to form a gel with consistency and resulted in stronger 7S and weaker 11S gels. The observation by Nakamura et al. (1986) that a shorter heating time at 100 °C made 7S gels with larger breaking force than 11S gels also may be attributed to less thermal stability of 7S globulin.

As shown in Figs. IV–20–22 and also Figs. IV–24–27, the gelation time for 7S was much longer than that for 11S and the gelation rate for 7S was from a half to one–third of that

for 11S. Therefore, the time required to saturate mechanical properties for 7S gels became longer from several to 10 times than that for 11S. When enough time had passed and mechanical properties were almost saturated, the 11S gel showed larger breaking stress, breaking strain and Young's modulus than did 7S gels. Breaking stress and breaking energy of 11S gels after 60 min heating were more than twice as large of those of 7S after 240 min heating. Typical stress–strain curves for fully gelled 7S and 11S are shown in Fig. IV–28. Stress values of both gels were almost the same at small strain, but 11S gels showed larger stress than 7S when a large strain was applied. 7S gels were more brittle than 11S gels, even though 7S gels had a Young's modulus similar to that of the 11S gels as observed in the dynamic viscoelasticity measurement. The result that 11S gels showed larger breaking stress than 7S is the same as previous studies (Saio et al., 1969; Hashizume et al., 1975). However, they observed larger differences, whereas the difference between both protein gels was at most 2–3 times in the present study. That may be caused by the different time elapsed before measurement. Previous researchers adopted a short processing time, while 7S needs a longer time for gelation. The same 7S system heated longer would show higher breaking stress, and the difference would become smaller. The differences in coagulant materials and/or in the amount of coagulant may also cause different results; we will confirm this in the future.

Relationships between pH and Gelation Rate

Fig. IV–29 shows the relationships between C_p and pH for 7S and 11S solutions with 0.4 % GDL and without GDL. Both 7S and 11S solutions without GDL ($C_g = 0$) showed a constant pH around 6.8, which was independent of protein concentration. Gels formed in the presence of the acidic coagulant GDL showed lower values of pH. 7S gels showed a smaller pH depression than 11S gels containing the same concentrations of protein and GDL. Because of a buffering effect of protein, pH of the gels remained higher with increasing protein concentration in both cases.

It is well–established that isoelectric point of 7S globulin (4.5–5.0) is lower than that of 11S (6.3–7.0) (Brooks and Morr, 1985). Therefore, the 7S globulin contains more negatively charged groups ($-COO^-$) in excess of positively charged groups at pH higher than the isoelectric point (4.8). However, anionic groups in 11S are balanced with cationic groups at the isoelectric point (6.4). The buffering effect against protons is stronger in 7S than in 11S in the pH range from 4 to 5. When the same amount of protons was added to both 7S and 11S solutions, pH of 7S systems remained higher than that of 11S. The slower gelation in 7S may be attributed to the smaller pH depression as shown in Fig. IV–29. Since the higher protein concentration inhibits the tendency of the system to go to a lower pH, it slows down the gelation process, even though it increases the density of reaction sites in protein. Therefore, a decrease in pH by the addition of GDL greatly promotes the gelation kinetics. The gelation of soybean 7S globuln in the presence of GDL was also a process of acidic coagulation as observed in 11S

systems.

The gelation time decreased with coagulant concentration as shown in Fig. IV–23. Globulin molecules have already been denatured and unfolded during heating in boiling water for 10 min before the addition of GDL (Morr, 1990). As shown in Fig. IV–29, GDL decreases pH and promotes aggregation of the unfolded protein by increasing hydrophobicity and insolubilization. The phenomenon is similar to the aggregation of casein micelles by chymosin, which cleaves κ–casein from the micelle and makes the surface of casein more hydrophobic (Dalgleish, 1986). It is known that 7S globulin precipitates at a pH range from 4.5 to 5.0 while 11S does at pH range from 6.3 to 7.0 (Brooks and Morr, 1985). Thanh and Shibasaki (1976) utilized the solubility difference in Tris buffer to isolate 7S and 11S globulins. The present method (Nagano et al., 1992) is also based on the different solubilities of 11S and 7S globulins at pH 6.4 and 4.8. It suggests that more GDL is required to increase hydrophobicity and insolubilize 7S than 11S if the system contains the same amount of protein. Therefore, the gelation of 11S–GDL is much faster than that of 7S as clearly shown in Figs. IV–20–22 and Figs. IV–24–27.

Critical Concentration of Proteins

Assuming that the reciprocal of the gelation time was proportional to the storage modulus, Ross–Murphy (1991) proposed the following equation for the gelation time as a function of the polymer concentration C_p:

$$t_0 \approx K/[\{(C_p/C_0)^n-1\}^p] \qquad \text{(IV–4)}$$

where K is a proportionality constant, C_0 is the critical polymer concentration for gel formation, n is the number of cross–linking loci which form a junction zone, and p (>0) is the critical exponent. According to this equation, an increase in polymer concentration shortens the gelation time.

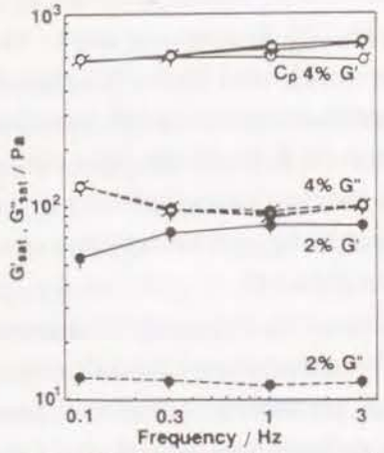
This equation describes fairly well the gelation kinetics for heat–induced bovine serum albumin (BSA) gels (Ross–Murphy, 1991). As is predicted by his proposal, the gelation time decreased with increasing polymer concentration in this system. If the protein concentration was very low, a gel should not be formed and the gelation time should be infinite. Ross–Murphy's explanation appears to be plausible because the gelation time becomes shorter with increasing C_p. A similar tendency that the gelation time was shortened with increasing polymer concentration was also observed for carrageenan and furcellaran (Oakenfull and Morris, 1987) gels, clotting of fibrinogen by thrombin (Fukada and Kaibara, 1973) and casein by rennet (Tokita et al, 1982a; Niki and Sasaki, 1987; Niki et al., 1991). However, the gelation of 7S and 11S globulins by GDL showed the opposite relation between t_0 and C_p. When the protein concentration was very low, a gel should not be formed and the gelation time would tend toward infinity. Since no gelation was observed experimentally for the sample containing 1.0 % 11S, t_0 should become a minimum at some C_p.

The relationship between 7S or 11S globulin concentration C_p and the gelation time t_0 was fitted to equation IV–4 by the least squares method. Fig. IV–30 shows the result. The calculated critical concentration of 7S was 0.479 % and that of 11S was 1.03 %. This value is considered to be close to the real critical concentration because the mixture which contained 1.00 % 11S and 0.1 % GDL exhibited fluidity after standing at 60 °C for 90 min. This critical value for 7S is lower than that of 11S. This is contrary to the observation for heat–induced gelation of 7S and 11S globulins by Nakamura et al. (1986), who reported the minimum concentration of protein for the gelation was 7.5 % for 7S and 2.5 % for 11S.

The calculated n value became 0.019 for 7S and 2.6 for 11S. The value 2.6 suggests that the junction zones of the 11S–GDL system are formed by aggregation of 2.6 single chains. From the definition of n, n chains make a junction zone of the gel, therefore a small value such as 0.019 indicates that the system can not make a gel or a three–dimensional network. It is contrary to the observation that the system actually did form gels.

The critical exponent p values were 0.346 for the 7S and 0.341 for the 11S gels. The theoretical value for p is 3 in the classical theory of percolation on a Bethe lattice (Gordon and Ross–Murphy, 1975) and around 1.8 for percolation on a cubic lattice (Stauffer et al., 1982). A value of 0.346 or 0.341 seems to be implausible. The gelation time for a fixed ratio of the protein and GDL system did not decrease as much at higher concentrations of C_p. If the gelation kinetics was governed by the structural change of polymers such as unfolding, the p value would be unity because the gelation reaction should be written by a linear function of the polymer concentration. The gelation rate in the system with excess polymers to coagulant might be determined by the amount of coagulant as observed in the enzymatic clotting of casein micelles by chymosin (Tokita et al., 1982a, b). In such a case, p would tend to be zero. However, polymers would not be excessive relative to GDL in the system examined because it contained the protein and GDL in a fixed ratio. Therefore, the gelation of soybean protein by GDL can not be treated by using equation IV–4.

Currently, we cannot explain the entire mechanism of the gelation process. Further structural studies are required to understand the gelation process.



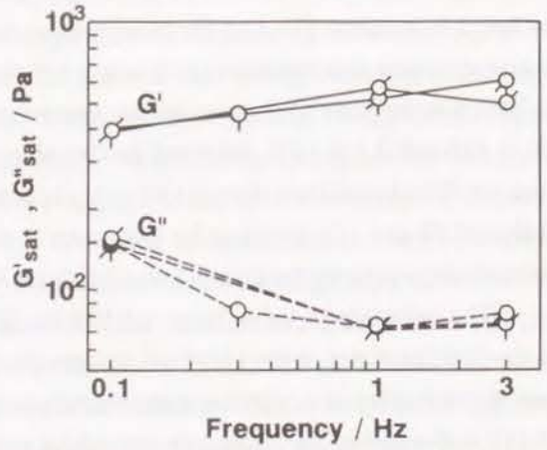


Fig. IV–17.(left) The saturated storage (G'_{sat}) and loss (G''_{sat}) moduli measured at various frequencies and amplitudes of 7S globulin separated from Enrei in the presence of 0.4 % GDL.
7S globulin concentration C_p:(●) 2.0 %, (○) 4.0 %. Temperature: 60 °C.
Amplitude:(♀) 25 μm, (♀) 50 μm, (♂) 100 μm.

Fig. IV–18.(right) The saturated storage (G'_{sat}) and loss (G''_{sat}) moduli measured at various frequencies and amplitudes.
Sample: 11S globulin separated from Enrei, 4 %, with 0.4 % GDL.
Measuring temperature: 60 °C. Amplitude: (♀) 25 μm, (♀) 50 μm, (♂) 100 μm.

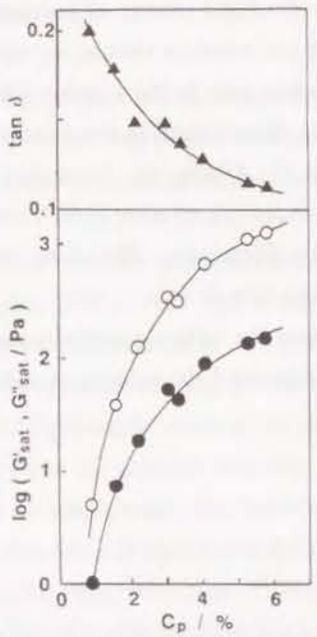
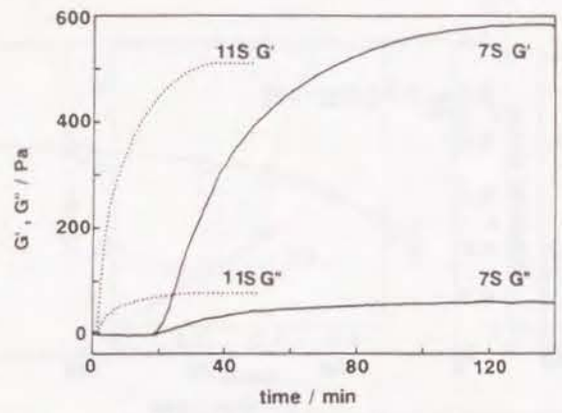


Fig. IV–19. Semi–log plot of saturated storage (○) and loss (●) moduli versus 7S concentration for 7S gels containing 0.4 % GDL at 60 °C.
The values of mechanical loss tangent at the final stage of gelation are also shown in closed triangles.



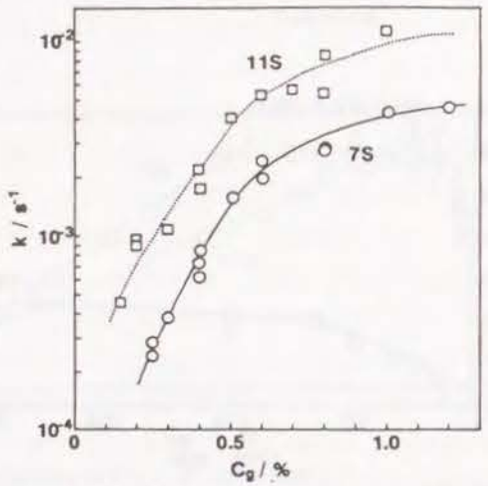
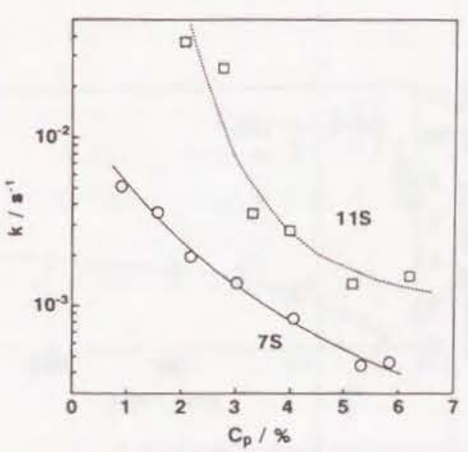


Fig. IV–20.(left) Typical gelation curves for a 7S (solid lines) and an 11S (dotted lines) globulins in the presence of 0.4 % GDL.
Protein concentration: 4.0 %. Temperature: 60 °C.

Fig. IV–21.(right) GDL concentration dependence of the rate of gelation k for 4.0 % 7S (○and solid line) and 11S (□and dotted line) globulins at 60 °C.



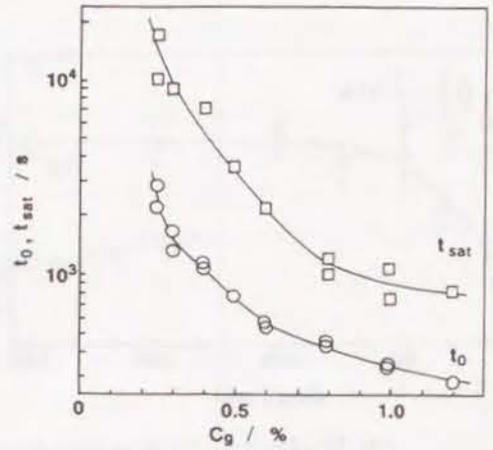


Fig. IV–22.(left) Protein concentration dependence of the rate of gelation k for 7S (○and solid line) and 11S (□and dotted line) globulins in the presence of 0.4 % GDL at 60 °C.

Fig. IV–23.(right) GDL concentration dependence of the gelation time (t_0, open circles), and the time (t_{sat}, open squares) at which the gelation curve becomes saturated for the gel of 4.0 % 7S globulin.

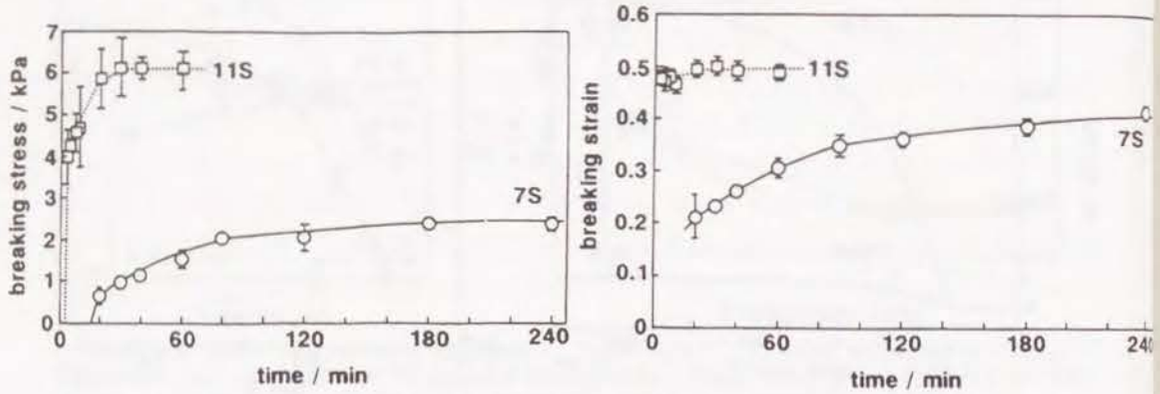


Fig. IV–24.(left) Heating time dependence of the breaking stress for 7S (open circles and solid line) and 11S (open squares and dotted line) globulin gels.
Concentrations of protein and GDL are 4.0 % and 0.4 % respectively. Heating temperature: 60 °C. Sample size: 16 mm dia. x 10 mm. Compression rate: 1.0 mm/s. Test temperature: 27 °C. Polyacetal stage and plunger are used. Bars attached symbols represent the standard deviation.

Fig. IV–25.(right) Heating time dependence of the breaking strain for 4.0 % 7S and 11S globulin gels in the presence of 0.4 % GDL. Symbols and test conditions are the same as Fig. IV–24.

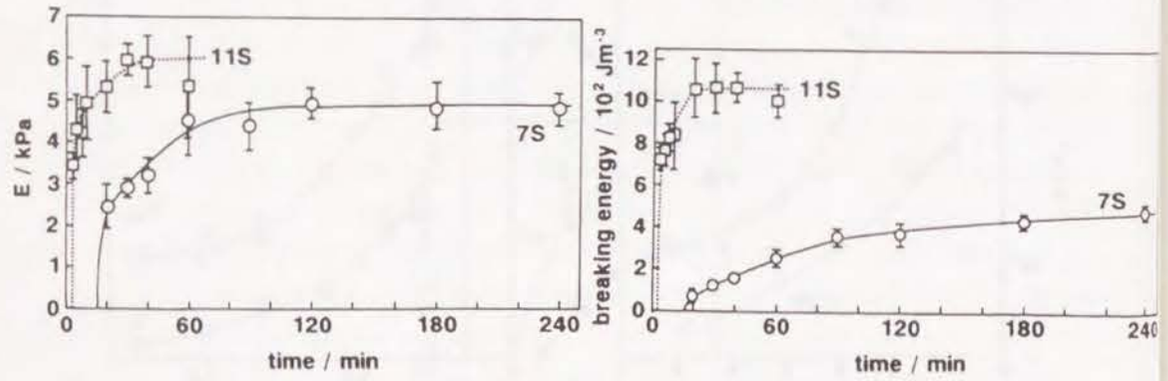
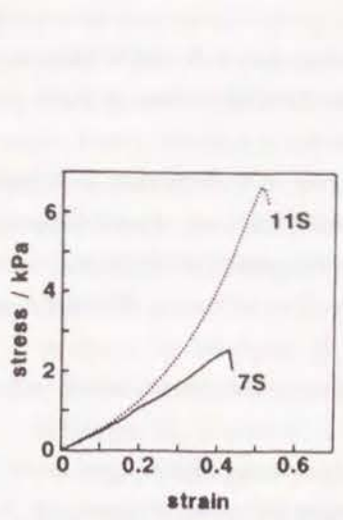


Fig. IV–26.(left) Heating time dependence of the Young's modulus E for 4.0 % 7S and 11S globulin gels in the presence of 0.4 % GDL. Symbols and test conditions are the same as Fig. IV–24.

Fig. IV–27.(right) Heating time dependence of the breaking energy for 4.0 % 7S and 11S globulin gels in the presence of 0.4 % GDL. Symbols and test conditions are the same as Fig. IV–24.



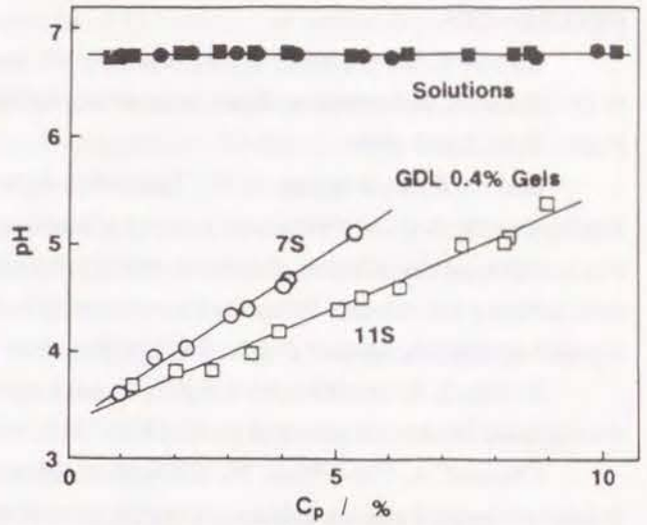


Fig. IV–28.(left) Typical stress–strain curves for a 7S (solid line) and an 11S (dotted line) globulin gels in the presence of 0.4 % GDL. Protein concentration: 4.0 %. Heated at 60 °C for 240 min in 7S, 60 min in 11S.

Fig. IV–29.(right) The relationships between pH and concentration of protein. (●) 7S solutions without GDL, (■) 11S solutions without GDL, (○) 7S gels with 0.4 % GDL, and (□) 11S gels with 0.4% GDL.

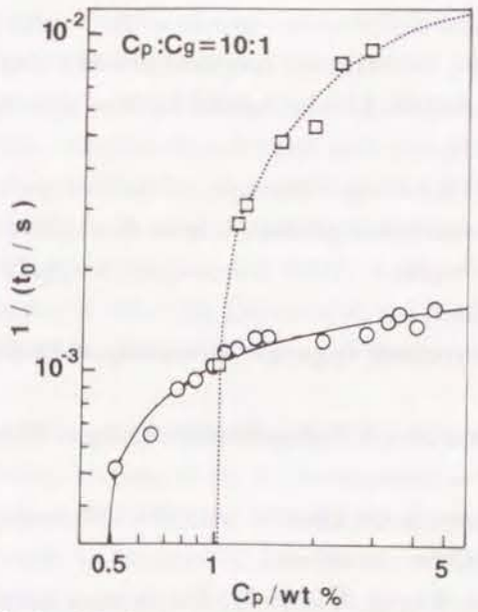


Fig. IV–30. Double–logarithmic plot of reciprocal gelation time t_0 against protein concentration C_p.
The ratio of GDL to protein was 0.1 in both cases. Experimental data for 7S globulin (open circles) and for 11S globulin (open squares). Caluculated curve for 7S using equation IV–4 with n=0.0192, p=0.346, C_0=0.479 and K=221 (solid line) and that for 11S with n=2.61, p=0.341, C_0=1.03 and K=271 (dotted line), respectively.

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Chapter V

KONJAC GLUCOMANNAN WITH DIFFERENT MOLECULAR WEIGHTS

Konjac glucomannan (KGM) is a major component of the tuber of *Amorphophallus konjac* K. Koch. Its main chain is composed of β-1,4-linked D-mannose and D-glucose at a ratio of 1:1.6 (Kato and Matsuda, 1969) with some branching points at C3 (Maeda et al., 1980). Upon dissolving in water, it forms a highly viscous solution.

KGM has been utilized as a raw material for both the food and polymer industries in Japan since ancient times. The sol of about 2 % is made into Japanese traditional food "kon-nyaku" under alkaline conditions (pH 11-12) by heating. Maekaji proposed that the gelation was initiated by elimination of carbonyl groups from the glucomannan molecules in the presence of alkali reagents (Maekaji, 1974, 1978). However, since refined KGM has little or no acetyl groups, the mechanism of the gelation remains enigmatic in some respects. Recently many researchers have taken an interest in this material, since the texture of the gel is unique and glucomannan is known as a dietary fiber. It has also been used as a food additive recently in Western countries. KGM interacts with other hydrocolloids (Nishinari et al., 1992) and most studies in the Western literature have been devoted to a binary system, such as a mixture of KGM with carrageenan (Cairns et al., 1988, 1991) or xanthan gum (Brownsey et al., 1988; Williams et al., 1991; Shatwell et al., 1991).

The physicochemical properties of KGM have not been fully elucidated, mainly be-cause of difficulty in obtaining well-fractionated konjac glucomannan samples (Nishinari et al., 1987; Mitsuyuki et al., 1987). Rheological properties of gels generally depend on the molecu-lar structure of the gelling agent. It is known that the number of ionic groups or the degree of substitution in polysaccharides such as agar-agar (Nishinari and Watase, 1983), carrageenan (Watase and Nishinari, 1981, 1987b) or pectin (Powell et al., 1982) markedly affects gel properties. Relationships between rigidity of gels and molecular weight, which is indexed by the intrinsic viscosity (Mitchell, 1979) have been studied for gelatin (Ferry, 1948; Saunders and Ward, 1955), alginate (Mitchell, 1979), carrageenan (Ainsworth and Blanshard, 1979; Rochas et al., 1990) and agarose (Watase and Nishinari, 1983). In mixed systems, the gel properties were also affected by the structural change of one component (Dea et al., 1977; Ainsworth and Blanshard, 1980; Fernandes et al., 1991; Turquois et al., 1992). Mitchell (1980) summarized the molecular weight dependence of the rheological properties of gels: the elastic modulus is independent of molecular weight above a certain limiting value, while the rupture strength continues to rise with increasing molecular weight. Since KGM is virtually a neutral polysac-charide and has few acetyl groups, ionic effects of KGM molecules may be small. The molecu

lar weight is expected to affect the gel properties; however, research works about dependence of molecular weight of one component in a synergistic gel system have been scant (Ainsworth and Blanshard, 1980; Turquois et al., 1992).

In the first part of this chapter, molecular weight distribution of KGM is discussed. The next part deals with the solution properties, and in the last, properties of synergistic gels with κ-carrageenan are described.

V-1. MOLECULAR WEIGHT DISTRIBUTION

MATERIALS AND METHODS

Materials

Powdered KGM from konjac tuber (cv. Harunakuro) and four fractions of KGM with different molecular weights, prepared by an enzymatic degradation, were kindly supplied by Shimizu Chemical Co. (Hiroshima). The native KGM (ND) was treated with an enzyme (SP-249, Novo Nordisk A/S, Copenhagen, Denmark) for different reaction times at ambient temperature, and LM4, LM3, LM2 and LM1 fractions with low molecular weights were obtained.

All chemicals used in this study were of reagent grade. The moisture contents of CAR, degraded KGMs, and ND KGM were 2.2, 4.0, and 6.3 w/w%, respectively.

Chemical Analysis

The ratio of mannose (Man) to glucose (Glc) for KGM samples was determined by high performance liquid chromatography (HPLC). KGM (2 g) was hydrolyzed in 2 % hydrochloric acid (100 mL) at 100 °C for 2 h. After neutralization, the solution was diluted to 200 mL and filtered. The filtrate (20 mL) was again diluted with water up to 50 mL. An aliquot of the solution (20 μL) was applied to the HPLC columns of SUGAR SP1010 and Ionpak KS801 (8 mmID X 30 cm, each) (Showa Denko, Tokyo) heated at 80 °C. The mobile phase was water at a flow rate of 0.8 mL/min. Man/Glc ratio was calculated from the peak areas of mannose and glucose detected with a refractive index detector Shodex R1 SE-51 (Showa Denko, Tokyo). Analysis of metal ions was carried out using an inductively coupled argon plasma emission spectrophotometer (ICP-AES) (ICAP-575 Mark II, Nippon Jarrell-Ash, Kyoto).

Intrinsic Viscosity

KGM samples were dissolved in cadoxen (Henly, 1961) with stirring for 12 h at room temperature. The cadoxen contained 28 w/w% ethylenediamine, 0.35 M sodium hydroxide, and 1.83 w/w% cadmium by atomic absorption spectrophotometry (AA-781, Nippon Jarrell-Ash, Kyoto). Both the KGM solutions and the cadoxen solvent were diluted with the same volume of water and filtered through a glass filter before measurement. Intrinsic viscosities were measured at 25.0 °C using an Ubbelohde viscometer. The flow time for the solvent, diluted cadoxen, was 259 s.

Gel Permeation Chromatography (GPC)

Molecular weight of each KGM fraction was determined by GPC at room temperature (23 °C). The GPC system consisted of a computer controlled pump CCPM, an automatic sampler AS-8000, a system controller SC-8020, and a refractive index detector RI-8012 (Tosoh, Tokyo). Two GPC columns of TSKgel $GMPW_{XL}$ (7.8 mmID X 30 cm) in series were used. The mobile phase of the diluted cadoxen was pumped at 1.0 mL/min. KGM samples were dispersed in cadoxen and then stirred for 12 h at room temperature until they were completely dissolved. The KGM solution and the cadoxen solvent was diluted by the same volume

of water and filtered through a polytetrafluoroethylene membrane with pore size of 0.5 μm. 200 μL of the sample solution (about 0.2 % concentration) was supplied to the analysis. Eight pullulan samples with a narrow molecular weight (Nishinari et al., 1991) distribution were used for calibration.

RESULTS AND DISCUSSION

Chemical Analysis

Chemical analysis data are shown in Table V–1. The ratio of mannose to glucose was about 2.0 for all the four KGM fractions, which was higher than the 1.6 reported previously (Kato and Matuda, 1969; Shimahara et al., 1975; Maeda et al., 1980; Chanzy et al., 1982). It was rather close to the ratio 2.1 for glucomannan from Scotch pine (Chanzy et al., 1982). However, it did not differ significantly before and after the enzyme treatment. This supports the observation that KGM does not have any block structures of mannose or glucose (Kato and Matsuda, 1969; Shimahara et al., 1975), because it is expected that glucose and mannose residues in glucomannan exibit differences in enzyme reactivity.

All fractions of KGM were similar in chemical composition. Since there might be a specific structure easily attacked by the enzyme, it may be possible that the higher molecular weight fractions have a different sugar sequence from the lower molecular weight fractions. A chemical study before and after the enzyme treatment would be necessary to confirm this. However, it would be very difficult to show that the chemical structures for KGM with different molecular weights are completely the same.

Molecular Weight of KGM

Information about the molecular weight is shown in Table V–2. It is clear that all five fractions have different molecular weights. KGM lost solubility in water by strong hydrogen bonds like cellulose once it has been purified or dried. Therefore, it was very difficult to make aqueous solutions of pure KGM. We tried to dissolve the KGM in water, but failed. Though it was partially dissolved, the residue was considered to include the KGM with higher molecular weight more than the KGM dissolved in the solution. Because, generally speaking, polymers which have lower molecular weights exhibit higher solubilities. Since we wanted to discuss effects of molecular weight, the molecular weight needs to be determined correctly. We concluded that water was not an appropriate solvent, and dissolved KGM in cadoxen. The solvent is known to dissolve cellulose and to make a clear, colorless and stable solution (Henley, 1961). Schwald and Bobleter (1988) reported GPC study for cellulose using cadoxen as the solvent and the mobile phase. A colorless solution allows the use of a refractive index detector (Schwald and Bobleter, 1988), and a much lower viscosity of the cadoxen solution than that of aqueous solution is a great advantage for GPC analysis.

As shown in the third column of Table V–2, reasonable values (0.3–0.5) of Huggins' coefficient were obtained. Intrinsic viscosity increased in the order of LM1, LM2, LM4, and non–degraded sample (ND). The highest value 3.91 dL/g the intrinsic viscosity in cadoxen at 25 °C was much smaller than that reported 4.6–18.8 dL/g for methyl KGM (Kishida et al., 1978) and 7.3–7.7 dL/g for sonicated KGM (Clegg et al., 1990) in water at 30 °C.

The peak for the highest molecular weight fraction (8.53×10^5) of pullulan appeared at 11.73 min and that for the lowest one (5.80×10^3) did at 15.07 min. The standard pullulan samples gave a good linear calibration curve (data not shown). The logarithm of molecular weight calculated from a regression analysis is shown in Fig. V–1 (lower horizontal axis).

Weight average molecular weight (M_w, the fourth column of Table V–2) for five KGMs decreased in order of ND, LM4, LM3, LM2 and LM1, and was the same order of the reaction time with the enzyme. The molecular weight distribution was not narrow according to the chromatograms as shown in Fig. V–1, and the ratio of M_w to number average molecular weight (M_n) and that of z–average molecular weight (M_z) to M_w are shown in the fourth and fifth column of Table V–2, respectively. The elution curve for ND in Fig. V–1 shows the second peak in the smaller molecular weight region. Sample did not homogeneously flow through the columns, probably due to high viscosity. The behavior is typically observed in a highly viscous solution, and the true average molecular weight may be higher. Even though in cadoxen, which forms a KGM solution with far lower viscosity than in water, it was difficult to prepare a suitable solution for GPC analysis especially in the case of high molecular weight fraction. Native KGM is considered as a gum with very high viscosity (Nishinari et al., 1992), it makes it difficult to analyze the molecular weight of native KGM by GPC. As shown in Fig. V–1, ND sample contains molecules with higher molecular weights more than 1.0×10^6, but no molecular weight fraction lower than 1.0×10^4. A similar wide distribution pattern of molecular weight was also reported for aqueous konjac mannan (Clegg et al., 1990). It ranged from 4×10^4 to more than 1×10^6, and was almost the whole range separable for their GPC gels. The enzyme treatment increased the ratio of lower molecular weight polymers, however, the fraction of higher molecular weight than 1×10^6 still remained even in LM1. The exclusion limit for the GPC column was assumed to be 5×10^7, because there are no standard polymers with higher molecular weight than 1×10^6. It is somewhat irresponsible and difficult to assign a molecular weight for the earlier eluted fraction. High accuracy for the molecular weight obtained from GPC, therefore, should not be expected. However, it is certain that the KGM fractions have different molecular weights.

The average molecular weight value 6.89×10^5 for the ND sample was smaller than that for methyl KGM (usually higher than 1,000,000) (Kishida et al., 1978), however it was larger than 4.9×10^5 for two konjac flours determined recently (Clegg et al., 1990). Therefore, the KGM sample was considered not to be damaged significantly by the strong alkalinity of cadoxen.

Four points are not sufficient to derive a relationship between M_w and intrinsic viscosity [η], however, a double logarithmic plot of M_w against [η] can confirm whether cadoxen dissolves KGM or not. A linear relationship was obtained as shown in Fig. V–2. The Mark–Houwink relationship, $[\eta] = K \cdot M_w^{\alpha}$, was found to be valid, and two parameters K and α were 3.55×10^{-4} dL/g and 0.69, respectively (r=0.991). The α value 0.69 was between 0.5 for Flory θ–solvent and 0.8 for a thermodynamically good solvent (Sperling, 1992), and was little bit smaller than 0.74 reported for aqueous methyl KGM by Kishida et al. (1978). The measured K value was far smaller than that of 6.37×10^{-4} dL/g reported by Kishida et al. (1978). The Mark–Houwink parameters were near those obtained for guar gum (galactomannan) in water ($K = 3.8 \times 10^{-4}$ dL/g, and $\alpha = 0.723$) as reported by Robinson et al. (1982). The KGM in cadoxen appears to be more flexible than methyl KGM in water. Clegg et al. (1990) found that the viscosity average molecular mass (M_v), calculated using the K and α values of Kishida et al. (1978) for two KGM samples, became lower than those experimentally obtained by GPC and laser light scattering. Shatwell et al. (1991) argued that methylation may have modified the KGM chain causing extention and a smaller K value such as 3.8×10^{-4} dL/g for guar gum gave a better M_v for native KGM.

Table V–1. Analytical data for various KGM samples.

Sample	Man/Glc	Na^+	K^+	Ca^{2+}	Fe^{2+}	Mg^{2+}
		(μg/g)				
LM1	2.04	8	275	538	---	30
LM2	2.09	6	407	606	---	35
LM3	2.02	7	412	530	---	38
LM4	2.04	7	420	510	---	48
ND	1.95	6	1185	480	12	50

Table V–2. Information of molecular weight for various KGM samples.

Sample	$[\eta]^a$(dL/g)	k'^a	$M_w{}^b$	M_w/M_n	M_z/M_w
LM1	1.98	0.38	2.56×10^5	6.50	5.04
LM2	2.63	0.31	4.38	7.72	5.06
LM3	----	----	4.44	7.09	4.30
LM4	3.50	0.41	5.96	5.68	3.85
ND	3.91	0.48	6.89	8.02	4.44

a) by Ubbelohde viscometer at 25 °C in cadoxen.
b) by GPC at room temperature in cadoxen.

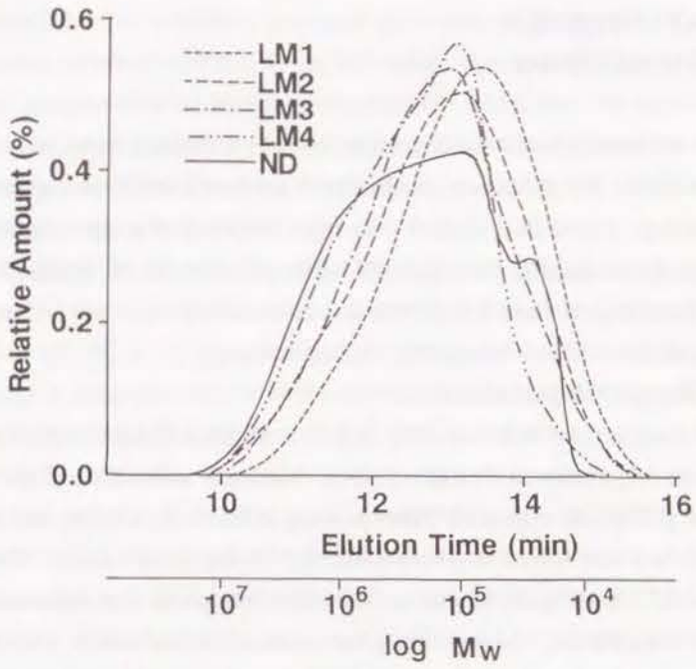


Fig. V–1. Elution curves for various KGM solutions in cadoxen.
The lower horizontal axis shows the logarithm of molecular weight calibrated by 8 pullulan samples. A relative amount was calculated as the ratio of the RI intensity at each time to the accumulative value.

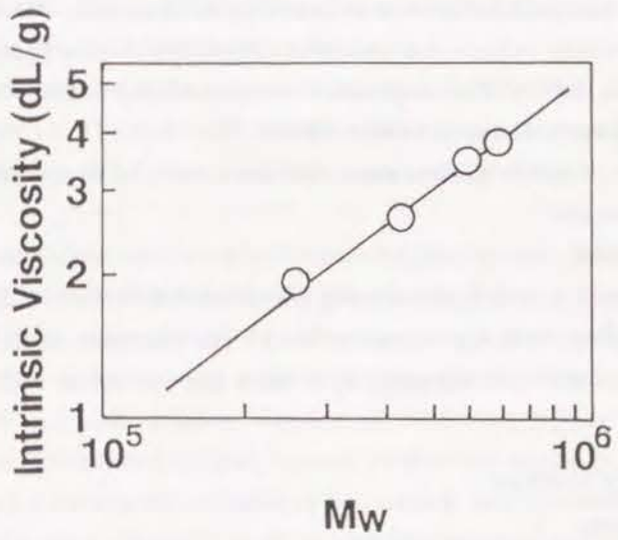


Fig. V–2. Double logarithmic plot for the intrinsic viscosity vs. molecular weight for KGM samples measured in cadoxen at 25 °C.

V-2. SOLUTION PROPERTIES

MATERIALS AND METHODS

Materials

Konjac flour was isolated from konjac tuber (cv. Zairai) harvested at Agatsuma, Gunma, Japan, in 1987. The flour was washed with methanol until the supernatant became clear soon after stirring. It was then washed with ether and dried at room temperature. Methanol-washed konjac glucomannan (Maekaji, 1974) supplied by Dr. K. Maekaji of Hiroshima Prefectural Food Techological Research Center was also used in this study. Chemical reagents were special grade and were used without further purification.

Fractionation of Konjac Glucomannan

10 g of the konjac flour was dissolved in 5 L of distilled water containing 50 g of urea. The insoluble matter was removed by a glass filter. Methanol was added to the filtrate until a cloud-like, fibrous precipitate appeared. After heating at 50 °C on a water bath to dissolve the precipitate, the solution was stored in a cold room at 5 °C for several days. The solution was then centrifuged at 8.27×10^3 g for 40 min at 5 °C. The precipitate was separated, dissolved in 2 M of potassium thiocyanate, and then dialyzed against distilled water. After dialysis was complete, the dialysis tubes were stored in cold 0.02 % sodium azide solution until further use to prevent bacterial degradation. This fractionation process was repeated several times. Four fractions were obtained for analysis.

Specific Volume Measurement

The apparent specific volumes were evaluated by the densimetry of solutions. Densities of solution were measured using a density meter (DMA20D, Anton-Paar, Kärntnerstrasse, Austria) (Kratky et al., 1973). The temperature was controlled by a circulated water system (RMS6, Lauda, Pfarrstrasse, Germany) within ± 0.005 °C.

Concentration of konjac glucomannan was determined by dry-weight analysis of the samples after measurement.

Viscosity Measurement

Intrinsic viscosity was determined using Ubbelohde viscometers at 25 ± 0.02 °C. The flow times for the solvent were approximately 240 s. The viscosities of dilute konjac glucomannan solutions were also observed using a low shear rate viscometer at 25 °C (Kaibara and Fukada, 1976).

RESULTS AND DISCUSSION

Partial Specific Volume

The partial specific volumes in sodium azide solution agreed with those obtained in water to within 0.01 cm^3/g when the measurement was carried out just after the preparation of the solution.

Because of the low solubility of konjac glucomannan in the neutral region, the maximum concentration obtained was 0.6 %. In this region, the density of the solution was a linear function of the concentration of konjac glucomannan. Therefore, the partial specific volume was independent of concentration of glucomannan, and could be determined without extrapolation to zero concentration. Studies were therefore performed for one concentration (~0.2 %) at various temperatures and pHs thereafter.

Fig. V-3 shows the dependence of the apparent partial specific volume on temperature. The apparent partial specific volume increased with increasing temperature from 5 °C to 40 °C and then levelled off. Since the specific volume υ decreased when cooled from 55 °C, the large value of υ at higher temperatures cannot be attributed to the degradation of konjac glucomannan molecules by heating. The apparent partial specific volume of konjac glucomannan at 25 °C was 0.576 cm^3/g is smaller than that obtained for other neutral polysaccharides dextran (Gekko, 1971) and pullulan (Kawahara et al., 1984; Nishinari et al., 1991) which gave almost the same value of 0.61 cm^3/g for partial specific volume in water.

The dependence of the apparent partial specific volume on pH is shown in Fig. V-4. The specific volume was almost constant between pH 3 and 11 and then increased steeply. This phenomenon depends solely upon pH. Results shown in Fig. V-5 suggest that any alkali hydroxide increases the apparent partial specific volume in this pH region. The change of the specific volume is independent of the size or valency of the cation. Since the gelation of konjac glucomannan occurs between pH 11.3 and 12.6, the change of the specific volume at this pH range is very important. This could be attributed to a change in molecular structure which may be necessary for the gelation of konjac glucomannan.

The apparent partial specific volume decreased reversibly when pH of the solution was neutralized from pH 12. The molecules of konjac glucomannan were not decomposed by alkali hydroxides but probably suffer some reversible conformational change.

Viscosity

Fig. V-6 shows that the viscosity decreased gradually with increasing pH up to 11.7. There is no specific pH at which the viscosity changes drastically. It suggests that no gelation or association of konjac glucomannan molecules occurs at this pH and concentration range. Probably the decrease of the viscosity might be attributed to the degradation of konjac glucomannan molecules by alkaline reagent. The intrinsic viscosities [η] and Huggins coefficients (Huggins, 1942) k' caluculated by least squares method are shown in Table V-3. Konjac glucomannan solution showed almost the same [η], while k' decreased with increasing pH from 11.7 to 12.8. If the cross-linking is made so high that microgel particles begin to form, k' decreases (Cragg and Manson, 1952). The decrease of k' at pH = 12.8 suggests that the microaggregation is formed even at low concentrations.

The plots of the reduced viscosity against concentration in water and 4 M urea solution

are shown in Figs. V–7 and V–8 respectively. Fractions of higher molecular weight showed a deviation from linearity in the plot of reduced viscosity against concentration at lower concentrations in both solvents than fractions of lower molecular weight. Table V–4 shows [η] and k' evaluated from the linear part of each curve in Figs. V–7 and V–8. Each fraction in 4 M urea had a value for the intrinsic viscosity 1.1–1.2 times larger than that in water. Urea exhibits two effects on polysaccharide solutions: (i) breaking hydrogen bonds between hydroxyl groups in polysaccharide molecules and (ii) increasing solubility of polysaccharides through the disruption of water structure (Watase and Nishinari, 1986a). The konjac glucomannan solution became more viscous in the presence of urea. The value of k' is about 0.5 in an ideal solvent (Sakai, 1968). If the solvent becomes poorer, k' value increases (Cragg and Manson, 1952; Sakai, 1968). A large k' value and a low solubility in water of konjac glucomannan suggest that hydrophilic characteristics of this molecule are very weak. It appears that considerably strong hydrogen bonding occurs between konjac glucomannan molecules. Values of k' shown in Table V–4 show that urea makes the solvent slightly poorer for konjac glucomannan and the solvent becomes poorer for higher molecular weight fraction. Since k' increases with increasing degree of cross–linking (Sakai, 1968), highly branched polymer has a larger value of k' than linear chain molecules. The degree of branching may be higher for higher molecular weight fractions.

The relationships between the logarithm of the zero shear specific viscosity and the logarithm of the concentration, c, of the konjac glucomannan are shown in Fig. V–9. Log η_{sp} increased linearly with increasing log c when the value of log η_{sp} was less than about 1. The double logarithmic plots of the zero shear specific viscosity η_{sp} against the coil overlap parameter $c[\eta]$ are shown in Fig. V–10. For dilute solutions, slopes of the plots were close to 1.4 for all fractions as has been observed for many polysaccharide solutions (Morris et al., 1981). The inflection point of the curves in Fig. V–10 is not clear because the low solubility of konjac glucomannan hindered the preparation of solutions which had large $c[\eta]$ values. However the slope of the curves increased gradually with increasing the logarithm of $c[\eta]$. This suggests that significant coil overlap and interpenetration had already commenced when $c[\eta]$ is larger than unity. Konjac glucomannan molecules begin coil overlap at lower concentrations than other polysaccharide (Morris et al, 1981).

As is often the case with proteins (Kauzmann, 1959), the partial specific volume of polysaccharide molecules in solution may depend on several factors: (i) the constitutive atomic volume, (ii) the void volume due to imperfect atomic packing, (iii) the volume of charged groups which are strongly solvated by the solvent and (iv) structural changes in the solvent in the vicinity of non–polar groups. Since the constitutive atomic volume should be constant at normal pressure, the increase in specific volume is influenced by the other three factors. Since the hydroxyl groups of glucomannan molecules are attracted by hydroxide ions at high pH, the

molecule is largely extended in strong alkaline solution. This could be one main factor in explaining the increase of the specific volume of konjac glucomannan at high pH.

At present, the author can only speculate why the specific volume increases in alkaline condition. Further investigations are required in order to clarify this point.

Table V–3. Dependence of intrinsic viscosity [η] and Huggins' coefficient k' of konjac glucomannan on pH.

pH	[η] ($10^2 cm^3 g^{-1}$)	k'
7.4	8.904	0.681
10.2	8.621	0.599
11.1	8.231	0.631
11.7	7.244	0.592
12.8	7.298	0.318

Table V–4. Intrinsic viscosity [η] and Huggins' coefficient k' of konjac glucomannan fractions in water and 4 M urea at 25.4 °C.

sample	in water		in 4 M urea	
	[η] ($10^2 cm^3 g^{-1}$)	k'	[η] ($10^2 cm^3 g^{-1}$)	k'
unfractionated	13.634	1.006	15.237	1.006
fraction 1	19.245	1.350	21.782	1.436
fraction 2	18.636	1.152	20.408	1.182
fraction 3	13.555	1.137	16.382	1.102
fraction 4	6.579	0.929	8.049	1.023

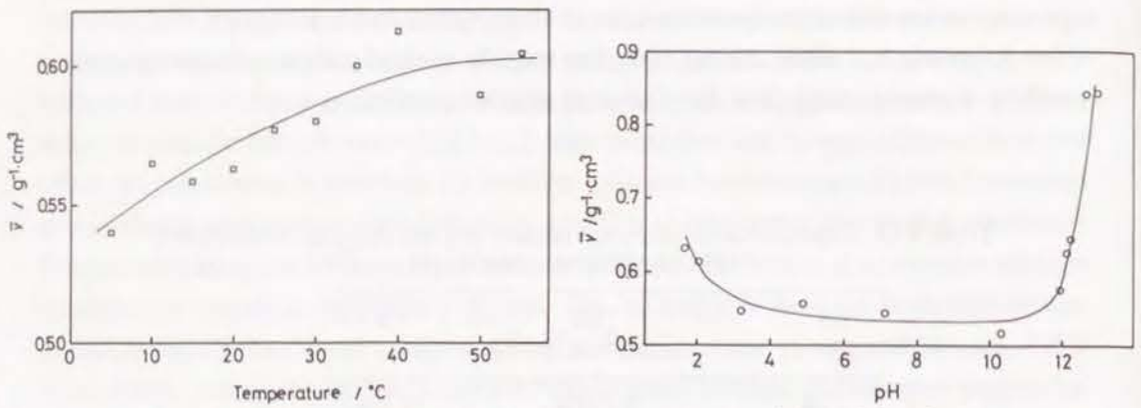


Fig. V–3.(left) Temperature dependence of apparent partial specific volume.
Concentration of konjac glucomannan: 0.205 w/w%.

Fig. V–4.(right) Apparent partial specific volume of konjac glucomannan as a function of pH at 25.0 °C.
pH was adjusted by addition of HCl or NaOH.
Concentration of konjac glucomannan: 0.221 w/w%.

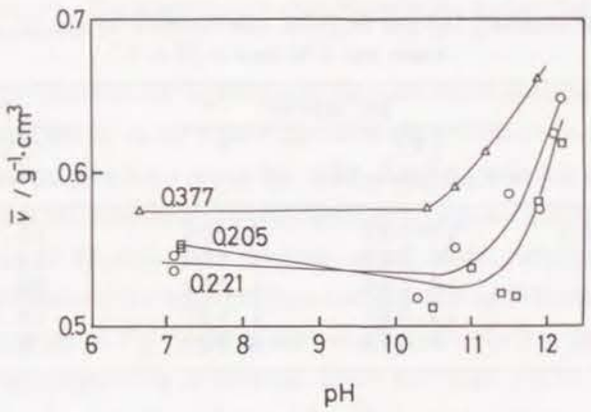


Fig. V–5. Dependence of apparent partial specific volume on pH at 25.0 °C.
pH was adjusted by several alkali reagents: ○, NaOH; □, KOH; △, $Ca(OH)_2$.
The numbers beside each curve represent the concentration of konjac glucomannan in w/w%.

Legends to figures on next page

Fig. V–7.(left bottom) Reduced viscosity as a function of the concentration of konjac glucomannan in water at 25.4 °C.

Fig. V–8.(right bottom) Reduced viscosity as a function of the concentration of konjac glucomannan in 4M urea solution at 25.4 °C.

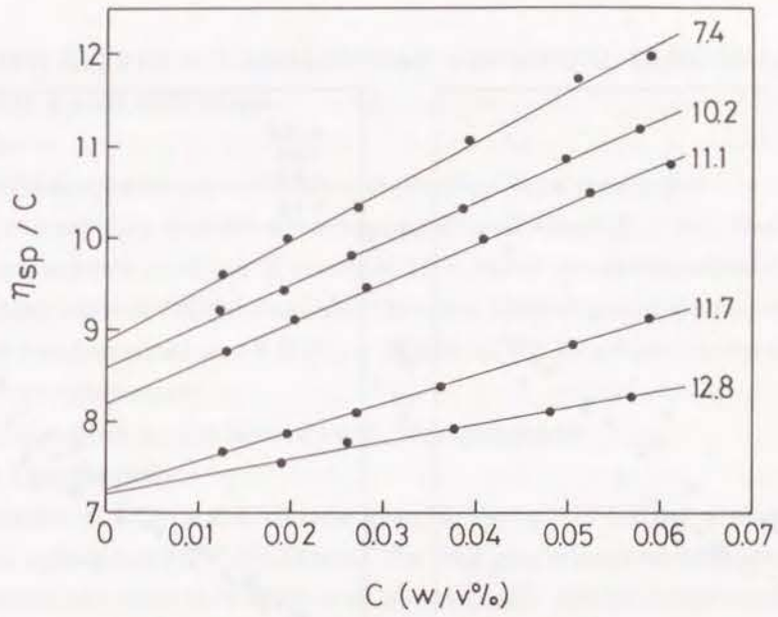
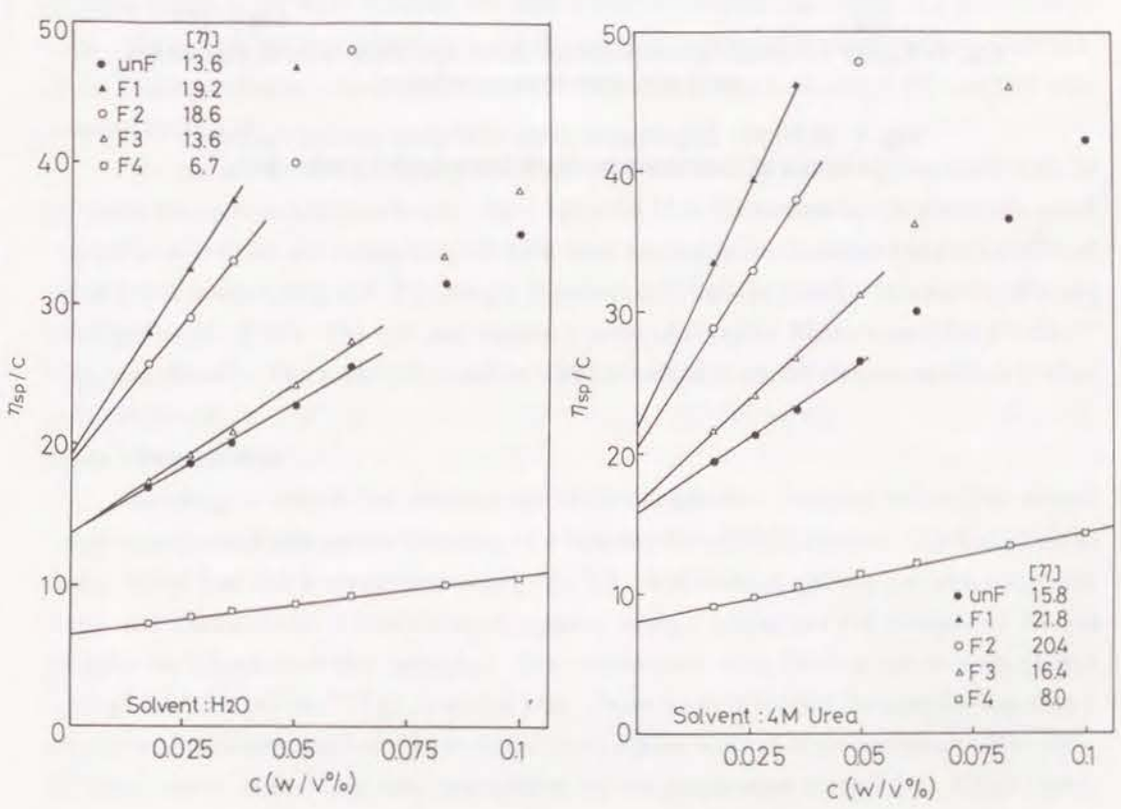


Fig. V–6. Reduced viscosity as a function of the concentration of konjac glucomannan at 25.0 °C.
The numbers at right side of each line represent the pH value.
Solvent contains 0.02 % of sodium azide.



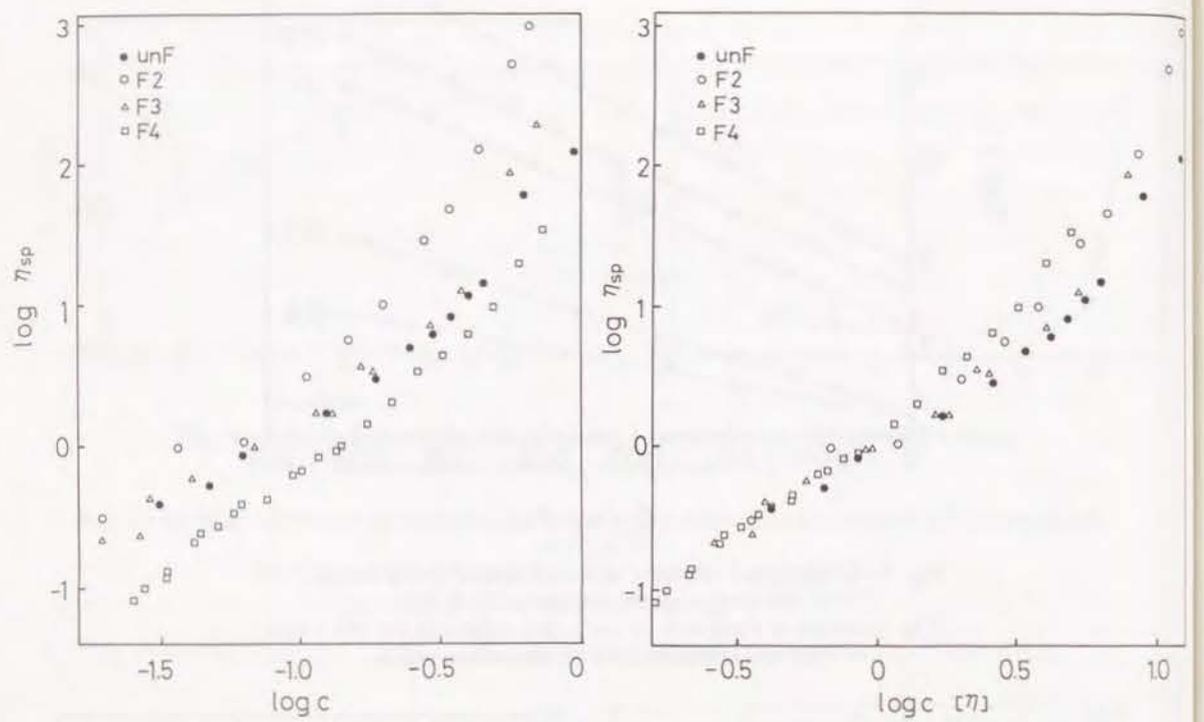


Fig. V–9.(left) Concentration dependence of the zero shear specific viscosity η_{sp} for konjac glucomannan solution.

Fig. V–10.(right) Dependence of the zero shear specific viscosity of konjac glucomannan on the coil overlap parameter $c[\eta]$.

V–3. MIXED GELS OF K–CARRAGEENAN AND KONJAC GLUCOMANNAN

MATERIALS AND METHODS

Materials

KGM samples used were the same as described in the section V–1.

A commercially available κ–carrageenan (San–Ei Gen F. F. I., Inc., Osaka) was washed with ethanol and then air–dried. It contained 22 % sulfate groups determined by a gravimetric method (Association of Official Analytical Chemists, 1990) after acid–decomposition. Molecular weight was determined as 4.3 X 10^5 in 50 mM $NaNO_3$ by gel permeation chromatography (GPC) at room temperature.

All chemicals used in this study were of reagent grade.

Dynamic Viscoelasticity

Powders of KGM and CAR were mixed in the ratio of 1:1 and dispersed in deionized water with agitation at 85 °C for 30 min. The total gum content was adjusted to 1.5 w/w%. CAR solutions of 1.5 and 0.75 w/w% were also prepared. The hot mixture was injected into a cell of a Rheolograph Sol (Toyoseiki Seisakusho, Tokyo) (Kaibara and Fukada, 1976), which had been heated beforehand to 75 °C. The surface of the sample was covered with silicone oil in order to prevent the drying. The temperature was lowered from 75 to 20 °C at 1.0 °C/min and then heated to 60 °C at the same rate with a thermo–module controlled by a micro–computer. The sample sol was subjected to 1.0 Hz sinusoidal shear oscillations with an amplitude 25 μm corresponding to a shear strain of 0.025. The storage and loss moduli (G' and G'') were recorded every 1 °C.

The hot sol of CAR 1.5 % or CAR–KGM mixture was poured into glass molds with 20 mm inner diameter and 30 mm height. After aging for 15 h, the sinusoidal vibrations of a small amplitude of 100 μm and a frequency of 3 Hz were applied to the lower end of the cylindrical gel at room temperature (20 °C) using a Rheolograph Gel (Toyoseiki Seisakusho, Tokyo) (Nishinari et al., 1980). The real and imaginary parts of complex Young's modulus E' and E'' were read directly. The mean value and standard deviation from the measurements of 8 trials were calculated.

Static Viscoelasticity

Sample gels with 20 mm diameter and 20 mm height were prepared as described above. Creep measurement was carried out using of a Rheoner RE–33005 (Yamaden Co. Ltd., Tokyo) with a 200 gf load cell at room temperature (20 °C). A cylindrical gel was put on a polyacetal stage, and a constant load was uniaxialy applied using a polyacetal flat plunger of 40 mm diameter for 1.0 min and then unloaded. From preliminary tests, the load values were chosen as 30 gf for CAR gels and 10 gf for mixed gels. These loads deformed the samples less than 1 mm (several percent strain) which was in the linear region between stress and strain. The vertical displacement of the stage was measured to the last micrometer every 0.1 s. Creep curves

were analyzed using a Burgers' four-element model consisting of a Maxwell body and a Voigt body connected in series, and five parameters, elastic modulus of a Maxwell body (Hookean) (E_0), the retarding elastic modulus and viscosity of a Voigt body (E_1 and η_1), the retardation time associated with the Voigt body (τ_1), and viscosity of a Maxwell body (Newtonian viscosity) (η_0) were obtained. Mean and standard deviation values were taken from more than 10 repeated measurements.

Tensile Testing

Hot mixtures of KGM and CAR prepared as above were poured into molds of depth d (= 11 mm) and then covered with glass plates. They were allowed to stand at 25 °C for 15 h. The gel was then cut into rings which had an inner diameter R_i of 20 mm and the outer diameter R_o of 30 mm using coaxial cylinders made of stainless steel. Tensile testing of gels was carried out using a Rheoner RE-33005 (Yamaden Co. Ltd., Tokyo) with a 2 kgf load cell at 25 °C. A ring-shaped gel sample was hung on two polyacetal bars of 7 mm diameter arranged vertically. The gel ring was initially deformed under its own weight and then vertically elongated at the rate of 5.0 mm/sec. Breaking stress τ_B of gels was calculated from the load value F_B at a breaking point divided by the initial cross-sectional area A_0, = $(R_o-R_i)d$ (110 mm^2) of the gel. Breaking strain γ_B was determined as the ratio of the elongation ($2\Delta L$) at a breaking point, to the initial effective circumference C_e (Tschoegl et al., 1970):

$$C_e = \pi(R_b+R_a)/2 + 2L_0 \qquad (V-1)$$

where R_b is the diameter of the bar (7 mm), R_a is the outer diameter of the hung gel, = $R_o-R_i+R_b$ (17 mm), and L_0 is the initial distance of two bars (depending on the samples). The dimensions of a sample gel are illustrated in Fig. V-11. Young's modulus was defined as the slope of each stress-strain curve at small strain range ($\gamma < 0.05$). The measurement was repeated more than 20 times, and the mean value and standard deviation were determined.

Differential Scanning Calorimetry (DSC)

Cooling and heating DSC measurements were carried out by use of a Seiko SSC5200H thermal analysis system with a DSC 120 module, an automatic cooling unit CA-5, and an X-Y plotter SP-520 (Seiko Instruments Inc., Tokyo). Approximately 60 mg of the sample sol was put into a silver cell of 70 μL and was hermetically sealed. Water was used as the reference. The sample cell kept at 85 °C for 30 min was cooled down to 4 °C with a scanning rate of 1.0 °C/min and kept there for 30 min, and then heated up to 85 °C at the same rate.

RESULTS AND DISCUSSION

Dynamic Viscoelasticity

Cooling curves and heating curves of dynamic viscoelasticity measurements for CAR and mixtures of CAR and KGM are shown in Figs. V-12 and V-13, respectively. At higher temperatures, all the samples tested were sols and the loss modulus was a little bit larger than

the storage modulus. Both storage and loss moduli for the sol at 60 °C containing higher molecular weight KGM were highest. Both moduli increased gradually with lowering the temperature until a certain temperature, and then increased steeply at around 30 °C. KGM alone showed monotonous change in viscoelasticity with temperature, and did not gel even at 20 °C (Williams et al., 1992). Gelling, or sol-to-gel transition temperature, (T_{gel}) was determined as the temperature at which the storage modulus began to deviate from the baseline during cooling. Usually the value for the storage modulus was about 2 Pa at T_{gel} as observed in a 1 % mixture of CAR and galactomannan (Fernandes et al., 1992). Below this temperature, samples lost fluidity and the storage modulus became far larger than the loss modulus for all the samples at 25 °C as shown in Fig. V-12.

The storage and loss moduli for 1.5 % CAR gel changed abruptly at about 29 °C during cooling. The change could not be attributed to the structure breakdown but should be attributed to the slip induced by syneresis (Stainsby et al., 1984). Since no such change in shear modulus was observed for mixed systems of CAR and KGM, KGM prevented the syneresis of CAR gel. Even though syneresis occurred, 1.5 % CAR gel exhibited much larger shear moduli than any mixed systems.

When the gel state mixture was heated, the storage and loss moduli decreased with temperature (Fig. V-13); however the values were larger than those observed during the cooling at the same temperature in the temperature range 20-60 °C. The melting temperature or gel-to-sol transition temperature (T_{sol}) was determined as the temperature at which the storage modulus in the heating process reached the same level (baseline) as observed in the cooling process. Therefore, samples showed similar values of storage and loss moduli both in heating and cooling processes at a temperature above T_{sol}. All mixed systems had T_{gel} and T_{sol} between those of 1.5 % and 0.75 % CAR as shown in Table V-5. The difference in molecular weight of KGM had little observable effect on both T_{gel} and T_{sol}. T_{sol} was about 20 °C higher than T_{gel} for all the samples. CAR showed thermal hysteresis in temperature scanning measurements of calorimetry (Watase and Nishinari, 1986b, 1987b; Gekko et al., 1987), optical rotation (Dea et al., 1972; Plashchina, 1975; Morris et al., 1980; Roshas and Rinaudo, 1980, 1984; Norton et al., 1984), viscosity (Rochas and Rinaudo, 1984), conductivity (Rochas and Rinaudo, 1984), and Cotton-Mouton constant in magnetic birefringence (Rochas and Rinaudo, 1984). The observed hysteresis in gel formation of the mixed system with KGM was influenced by those characteristics of CAR. A similar hysteresis was also reported for CAR-galactomannan mixed system (Fernandes et al., 1992). Higher T_{sol} than T_{gel} is commonly observed for many thermoreversible gels such as agarose (Hayashi et al., 1975; Indovina et al, 1979; Watase and Nishinari, 1987a; Watase et al., 1989) and gelatin (Djabourov et al., 1988). In the curve for mixed systems, storage modulus decreased more slowly at around 40 °C than at lower temperatures.

Observed complex Young's moduli for mixed gels are shown in Table V-6. The real

(E') and imaginary (E'') part of the Young's modulus for CAR gel were much larger than those for mixed gels of CAR and KGM. Among the mixed gels, both E' and E'' became larger with increasing molecular weight of KGM in the mixture. Mechanical loss tangent (E''/E') decreased with increasing molecular weight of KGM. It suggests that KGM with higher molecular weights made the mixed gel more elastic and solid-like. Both E' and E'' were about ten times larger than shear moduli (G' and G'') observed in a Rheolograph Sol apparatus at 20 °C, respectively (Fig. V-12). The aging time of the gel affected greatly those values, *i.e.*, G' and G'' were measured immediately after the gelation, while E' and E'' were measured after aging for 15 h at 20 °C. Assuming the Poisson's ratio is 0.5, Young's modulus becomes three times of shear modulus (Ross-Murphy, 1984). It is expected that E' and E'' become almost three times larger than G' and G'' if the samples aged similarly. All properties observed for the mixture with highest molecular weight of KGM (ND) are rather small compered to those for CAR gels.

Static Viscoelasticity

As shown in Table V-7, a similar behavior was also observed in the four viscoelastic parameters (E_0, E_1, η_1, and η_n) of Burgers' model obtained from the static viscoelasticity measurement. These parameters increased with increasing molecular weight of KGM in the mixture, but they were much smaller than those for CAR 1.5%. The retardation time τ_1 was slightly longer in mixed gels than in CAR alone, and increased with increasing molecular weight of KGM in the mixture. Ainsworth and Blanshard (1980) measured creep compliance for mixed gels containing 0.5 % kappa-carrageenan and 0.5 % carob gums (galactomannan) and compared them with 1.0 % carrageenan gels. They fitted the creep curves to a model consisting of a Maxwell body in series with one or two Voigt bodies. The mixed gels showed smaller instantaneous modulus, but larger retarding modulus and Newtonian viscosity than gels of carrageenan alone. Decomposition of carrageenan by heat processing decreased both the instantaneous and retarding moduli, but strengthened the Newtonian viscosity. In the present case, gel properties for CAR-KGM differed from those in their system. KGM showed stronger effect in reducing viscoelasticities and increasing deformability of CAR gels.

Large-deformation Properties

Force-deformation curves and tensile characteristics for gels are shown in Fig. V-14 and Table V-8 respectively. CAR gels are firm and brittle as shown by a large Young's modulus and small breaking strain. The addition of KGM to CAR made the breaking strain larger. Two systems containing KGM of lower molecular weights (LM1 and LM2) showed almost the same behaviors during tensile testing. The breaking stress and the breaking strain for mixed gels of higher molecular weight KGM were larger. Therefore, it is considered that KGM of higher molecular weight contributes to formation of a strong network structure in the mixed system. Polymers with high molecular weight can form a network whose junction zones are connected by molecular chains with long contour length. Such a network has enough flexibility and is considered to endure against a strong force or a large deformation. KGM molecules with higher molecular weight have long chains and produce rubber-like properties in the mixed gel.

The Young's modulus for CAR with non-degraded KGM (ND) became smaller than that of the other mixed gels, which is consistent with the results of the dynamic viscoelasticity at 25 °C. Probably, it is influenced by air bubbles in the gel. Since the sol of ND sample showed an extremely large viscosity, it was difficult to remove small air bubbles in the mixed sol formed with CAR by suction or centrifugation. The effect of large molecular weight KGM on breaking stress and breaking strain was very strong, the breaking properties became larger than those for other mixtures even though air bubbles decreased these values. Except the CAR-ND gels, the dynamic viscoelasticity and the Young's modulus in the tensile testing increased with molecular weight of KGM among three mixed gels of CAR and KGM. The difference was smaller than that observed in the breaking properties. The breaking stress greatly increased with increasing molecular weight of KGM in CAR-KGM mixed gels.

Thermal Properties

Typical DSC curves for the mixed system are shown with those for 0.75 % and 1.5 % CAR in Fig. V-15. As widely reported in the literature (Watase and Nishinari, 1986b, 1986c, 1987b; Nishinari et al., 1990a; Williams et al., 1992; Iida et al., 1992), an exothermic peak in cooling and an endothermic peak in heating DSC curves were observed for CAR alone systems. Both peaks shifted to higher temperatures with increasing CAR concentrations (Watase and Nishinari, 1986b, 1986c, 1987b; Nishinari et al., 1990a). 1.5 % KGM did not show any peak in both the cooling and the heating processes as previously reported (data not shown) (Williams et al., 1992).

All the mixed systems exhibited similar DSC curves indicating the sol-to-gel transition is independent on molecular weight of KGM. Williams et al. (1992) reported that mixed systems of kappa-carrageenan and konjac mannan in KCl solutions (0.6 % total gum content) exhibited an exothermic peak in cooling curves of DSC, while excess carrageenan, *i.e.*, > 0.7, showed the second peak at a lower temperature than the first peak temperature. In the present case, CAR content was 0.5 so that only one peak was observed.

The peak temperature (T_c) and the enthalpy for the transition (ΔH_c) determined by the area of exothermic curve, whose example is shown by the shaded area in curve A in Fig. V-15, divided by the gum content in mg are tabulated in Table V-8. T_c for the mixed systems was higher than that for 0.75 % CAR alone, but lower than that for 1.5 % CAR as observed in the dynamic viscoelasticity. T_c values were lower than T_{gel}, about several degrees, were determined from the cooling measurement of dynamic viscoelasticity. The incipient point of each exothermic peak was rather close to T_{gel}. A similar behavior was observed for a mixture of 0.15 % carrageenan and 0.05 % konjac mannan in 25 mM KCl (Williams et al., 1992). When

the network structure begins to form, the system loses fluidity and viscoelasticity begins to rise. However, the transition has occurred only partially.

In the heating process, the width of the endothermic peaks was broader than that observed during cooling, and the transition temperature was much higher than that for sol-to-gel transition as previously reported (Williams et al., 1992). Two endothermic peaks were observed for the mixed systems, however gels of CAR alone showed only one endothermic peak which is related the gel–to–sol transition as mentioned above. The multiple endothermic peaks in heating DSC curves sometimes appeared in the melting of polymorphic crystals and they seemed to be induced by the reorganization of the CAR crystalline region during heating (Watase and Nishinari, 1986b). However, reheating or a rapid heating rate gave an endothermic peak (Watase and Nishinari, 1986b). In the present case, the endothermic peak was observed in the reheating process and it appeared similarly in further repeating measurements. The first peak temperature (T_{h1}) was about 10 °C lower than the second one (T_{h2}). As well as in cooling curves, the peak temperatures were independent of the molecular weight of KGM. T_{h1} was lower than the endothermic peak temperature for 0.75 % CAR and T_{h2} was between the endothermic peak temperatures for 0.75 and 1.5 % CAR samples. Comparing the results with those from the dynamic viscoelasticity, T_{h2} considered to be concerned with the melting of network structure made by CAR chains. It was reported that sol–to–gel or gel–to–sol transition for 1:1 mixture of carrageenan and konjac mannan with total gum content of 0.6 % occurred at higher temperatures than 0.6 % carrageenan alone in the presence of KCl (Williams et al., 1992). Our observation is completely the opposite, however our results in DSC is consistent with the rheological observation. Moreover, we tried DSC measurement for 2 % mixture of CAR and KGM with different CAR/KGM ratios, the peak temperatures increased with increasing CAR in the mixture (Iida et al., 1992), the results will be reported in a separate paper. T_{h2} for all systems was also lower than T_{sol} as observed in cooling process. The final point of each endothermic peak was close to T_{sol} obtained in the rheological measurement. When little part of the network remains, the system shows gel–like behavior rheologically, even though the shear modulus has already begun to decrease. Since the border between the two peaks was not clear as shown in Fig. V–15 (heating curve C), the enthalpy for melting the gel (ΔH_h) was determined from each heating DSC curves without separation.

The gap between T_c and T_{gel} and T_{h2} and T_{sol} was larger in the mixed system of CAR and KGM with higher molecular weights and smaller in 0.75 % CAR. That might be reflected by a low accuracy with respect to the rheological determination of the gelling temperature. As shown in Figs. V–12 and V–13, higher G' values observed in the sol state mixture including higher molecular weight of KGM, make difficult to observe T_{sol} or T_{gel} accurately. T_c for all mixed systems was between those for 0.75 and 1.5 % CAR as observed in the rheological measurement.

The accuracy in the enthalpy changes (ΔH_c and ΔH_h) during the transitions unfortunately seemed to be low, because of comparatively low concentration of CAR for the DSC sensitivity. In all cases, ΔH_c did not agree with ΔH_h. Values of ΔH_c and ΔH_h might be similar, since the changes of sol–to–gel and gel–to–sol are completely reversible by thermal treatment. Both ΔH_c and ΔH_h for 1.5 % CAR were slightly larger than those for CAR of 0.75 %. Enthalpy values in heating curve of CAR gels of concentration ranging from 1 to 6 % were reported by Nishinari et al. (1990). Since they calculated it as 1 mg gel weight, we recalculated them to compare as the enthalpy per 1 mg carrageenan. Derived values were 48, 58, 87, 77 and 55 mJ/mg gum for 1, 2, 3, 4, and 6 % kappa–carrageenan gels, respectively. The large variance suggests that the enthalpy might depend on the concentration, however, it was difficult to confirm this in sensitivity with our apparatus.

ΔH_c and ΔH_h for mixed systems seem to be approximately one–half of those of CAR alone. It supports the proposal that only CAR component contributes to make the gel networks (Cairns et al., 1988, 1991). KGM in the mixture shifted the sol–to–gel and gel–to–sol transition temperatures higher than those for 0.75 % CAR. KGM in the system interrupts the free movement of CAR sol by absorbing the medium, water, thus allowing the CAR in the mixture to easily form an ordered structure. The effect of chain length in KGM on the gel structure seems to be negligeble, since molecular weight dependence was not observed in the thermal properties.

Explanation of the Transition Temperatures Using a Zipper Model

Recently, a zipper model approach has been proposed to explain the thermo–reversible gel–to–sol transition (Nishinari et al., 1990b). The gel–to–sol transition was treated as an opening process of molecular zippers which make junction zones. According to this approach, the heat capacity of gels is expressed as a function of the number of zippers N, the number of parallel links N in a single zipper, the rotational freedom G of a link, and the energy ε required to open a link (see equation II–2 in section II–1). As described in section II–1, gel–to–sol transition temperature is determined by a certain average $G_{av,g}$ of G for gel state and sol–to–gel transition temperature is determined by an average $G_{av,s}$ of G for sol state. Since $G_{av,g}$ is smaller than $G_{av,s}$, T_{sol} or T_{h2} is expected to be higher than T_{gel} or T_c, respectively.

The zipper model could imply that the transition is sharper in cooling than in heating as previously observed (Nishinari et al., 1990b; see section II–1). This explanation corresponds well to the experimental data: (i) the storage modulus G' decreased gradually with increasing temperature (Fig. V–13), while G' began to rise steeply at a narrow temperature region with lowering temperature (Fig. V–12). (ii) the peak width for the heating DSC curve was broader than that for cooling (Fig. V–15).

Network Structure of CAR–KGM

Cairns et al. (1988) observed no interaction between CAR and KGM from X–ray fiber

diffraction patterns for 1:1 mixture. They proposed a model involving CAR network containing the KGM within the CAR gel for the mixed gel (Cairns et al., 1988, 1991). According to this model, junction zones made from the interaction between CAR and KGM did not exist. Junction zones in the mixed gels were formed only by CAR molecules.

In the present work, the structure of a junction zone was independent of the molecular weight of KGM, since gel-to-sol and sol-to-gel transition of the systems occurred at almost the same temperature. As shown in Tables V-5, V-8 and Fig. V-15, sol-to-gel and gel-to-sol transition temperatures for mixed systems were higher than those for the 0.75 % CAR. Since mixed systems contained 0.75 % CAR, the gel structure was stabilized by the addition of KGM. However, those temperatures for the mixtures were lower than those for 1.5 % CAR. Therefore, the interaction between KGM and CAR is weaker than that between CAR and CAR, but strong enough to produce another elastically active chains (Treloar, 1975). In other words, KGM creates weak junction zones which contribute to rheological characteristics but not to thermal stability.

It is well established that gelatin gels formed with higher molecular weights melt at higher temperature (Eldridge and Ferry, 1954). Molecular weight dependence of melting temperature for methyl cellulose gels was not remarkable even though the molecular weight ranged from 5.1×10^4 to 1.1×10^6 (Kato et al., 1978).

Since a KGM molecule has few branching points (Maeda et al., 1980), it is essentially a string, like cellulose. Therefore, the higher the molecular weight, the longer the KGM chains. The number of junction zones N is considered to increase with increasing molecular weight of KGM because Young's modulus and G' at 25 °C for mixed gels increased with increasing molecular weight of KGM. If the molecular weight of KGM increases, the number N of parallel links in a zipper or the contour length of flexible chains which connect zippers will increase. Since the gel-to-sol and sol-to-gel transition temperatures were almost independent of molecular weight of KGM, the binding energy ε and the rotational freedom G of a parallel link are considered to be also independent of the molecular weight of KGM. The contour length of flexible chains which connect junction zones is considered to increase because breaking stress and breaking strain increased with increasing molecular weight of KGM. In conclusion, the increase in molecular weight of KGM increases the number of junction zones and makes the contour length of flexible chains which connect junction zones longer. As is seen from zipper model approach, T_{gel} or T_{sol} does not shift to higher temperatures with increasing number of junction zones (zippers). The contour length of flexible chains does not affect T_{gel} or T_{sol} because these temperature are determined mainly by the structure of junction zones. The effect of molecular weight increase of KGM on the gel network is schematically shown in Fig. V-16.

The two fractions of KGM with lower molecular weights showed almost the same breaking stress. Since elastically effective chains are the flexible chains between two (weak)

junction zones, those two KGM molecules were probably too short to form enough weak junction zones.

The intermediate segment between the two peaks is considered to be related to the temperature at which the decrease of dynamic viscoelasticity in heating curves (Fig. V-13) of the mixed systems became slower at around 40 °C. Some part of CAR melted at lower temperatures by effect of KGM. That may be formed by CAR alone, but should interact with KGM. It is difficult to explain this behavior by the model of Cairns et al. (1988, 1991). According to Williams et al. (1992), the setting and melting peaks for 1:1 mixture of carrageenan and konjac mannan indicated a specific interaction between the two polymers. They also showed the interaction by electron spin resonance. They observed the gelation of carrageenan alone in the mixture containing higher carrageenan content than 0.7. Our observation suggests the existance of two types of junction zones in the 1:1 mixture: one is made by CAR molecules and other is a weak junction which involves some interaction between CAR and KGM.

This conclusion contradicts results of other workers (Cairns et al., 1988, 1991; Williams et al., 1992). The difference in gum concentration and/or solvent system might cause these different results. Further study is urgently necessary to clearly elucidate the interaction between CAR and KGM.

Table V-5. Sol to gel (T_{gel}) and gel to sol (T_{sol}) transition temperatures obtained from rheological method for CAR and KGM mixtures.

Sample	T_{gel} (°C)	T_{sol} (°C)
CAR 1.5 %	33.0±0.8	51.0±0.8
CAR 0.75%	23.0±0.0	38.0±0.0
CAR+LM1	29.5±0.5	49.0±0.0
CAR+LM2	29.5±1.5	49.0±0.0
CAR+LM3	28.0±0.0	47.0±0.0
CAR+LM4	30.5±0.5	49.5±0.5
CAR+ND	30.3±0.9	50.5±1.1

Scanning rate: 1.0 °C/min.

Table V–6. Complex Young's modulus for mixed gels of CAR and various KGM at 20 °C.
Total gum content: 1.5 %.

Sample	E' (kPa)	E'' (kPa)	E''/E'
CAR	57.1 ± 2.6	4.19 ± 0.44	0.073
CAR+LM1	4.8 ± 0.2	0.30 ± 0.00	0.063
CAR+LM2	5.0 ± 0.2	0.30 ± 0.00	0.060
CAR+LM3	5.9 ± 0.3	0.33 ± 0.04	0.055
CAR+LM4	6.9 ± 0.2	0.40 ± 0.00	0.058
CAR+ND	7.7 ± 0.3	0.36 ± 0.07	0.047

Table V–7. Creep viscoelasticity for mixed gels of CAR and various KGM at 20 °C.
Total gum content: 1.5 %.

Sample	E_0 (kPa)	E_1 (kPa)	η_1 (MPas)	τ_1 (s)	η_n (MPas)
CAR	35.77±3.75	469.8±22.6	3.246±0.533	6.90±1.07	41.00±3.91
CAR+LM1	4.17±0.25	20.3± 2.8	0.172±0.040	8.38±1.02	2.29±0.03
CAR+LM2	4.49±0.30	27.6± 1.3	0.203±0.076	8.37±0.68	2.83±0.18
CAR+LM3	4.62±0.67	26.6± 2.8	0.241±0.027	8.98±0.31	2.56±0.18
CAR+LM4	6.74±0.63	40.5± 3.5	0.400±0.038	9.77±0.31	3.38±0.22
CAR+ND	8.01±0.91	58.5± 8.3	0.539±0.050	9.84±0.86	4.85±0.50

Table V–8. Tensile characteristics for mixed gels of CAR and various KGM samples.
Total saccharide content: 1.5 %.

Sample	Breaking stress (kPa)	Breaking strain (cm/cm)	Young's modulus (kPa)
CAR	16.47 ± 2.54	0.210 ± 0.025	60.64 ± 2.04
CAR+LM1	8.57 ± 0.97	0.388 ± 0.043	15.15 ± 1.43
CAR+LM2	9.23 ± 0.84	0.369 ± 0.030	15.46 ± 1.24
CAR+LM4	37.36 ± 1.18	0.870 ± 0.048	20.76 ± 1.09
CAR+ND	74.66 ± 4.55	1.958 ± 0.103	9.97 ± 0.96

Table V–9. Peak temperatures and enthalpy in DSC measurements for mixed systems of CAR and various KGM.
Total gum content: 1.5 %.

Sample	cooling DSC		heating DSC		
	T_c (°C)	ΔH_c (mJ/mg)	T_{h1} (°C)	T_{h2} (°C)	ΔH_h (mJ/mg)
CAR 1.5 %	27.5±0.1	-39.5±2.0	---	46.7±0.3	36.9±0.7
CAR 0.75%	19.9±0.1	-33.7±1.0	---	35.0±0.2	32.8±0.3
CAR+LM1	22.0±0.2	-14.3±0.6	31.5±0.1	41.1±0.1	17.2±0.2
CAR+LM2	21.7±0.0	-12.9±0.2	31.7±0.1	41.8±0.1	13.7±0.7
CAR+LM3	22.4±0.0	-15.8±0.8	31.0±0.0	42.2±0.2	13.8±0.2
CAR+LM4	22.0±0.0	-13.9±0.7	32.2±0.1	42.0±0.1	12.2±1.0
CAR+ND	22.5±0.1	-14.2±0.2	33.7±0.5	43.3±0.0	10.3±0.1

Scanning rate: 1.0 °C/min.

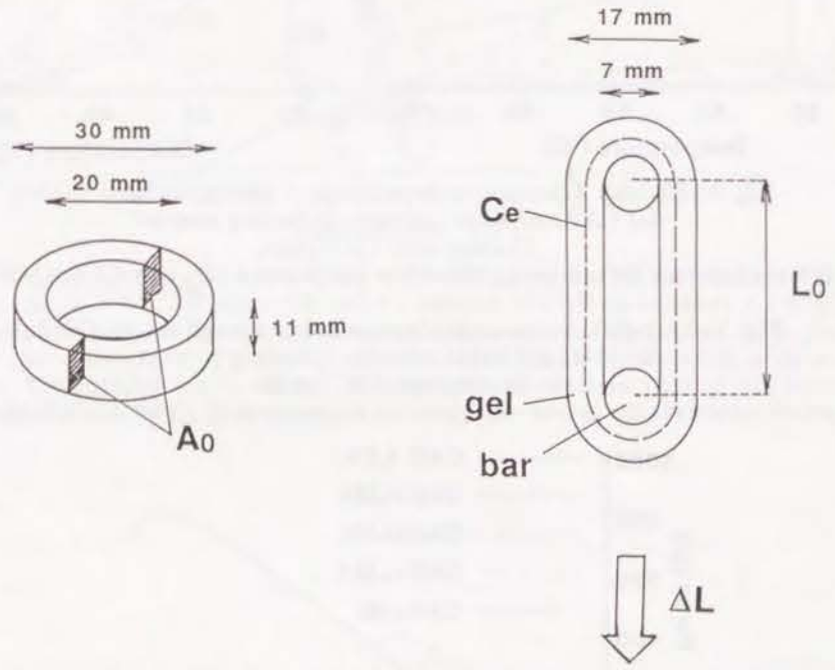


Fig. V–11. Shape and dimension of the gel ring for the tensile testing.

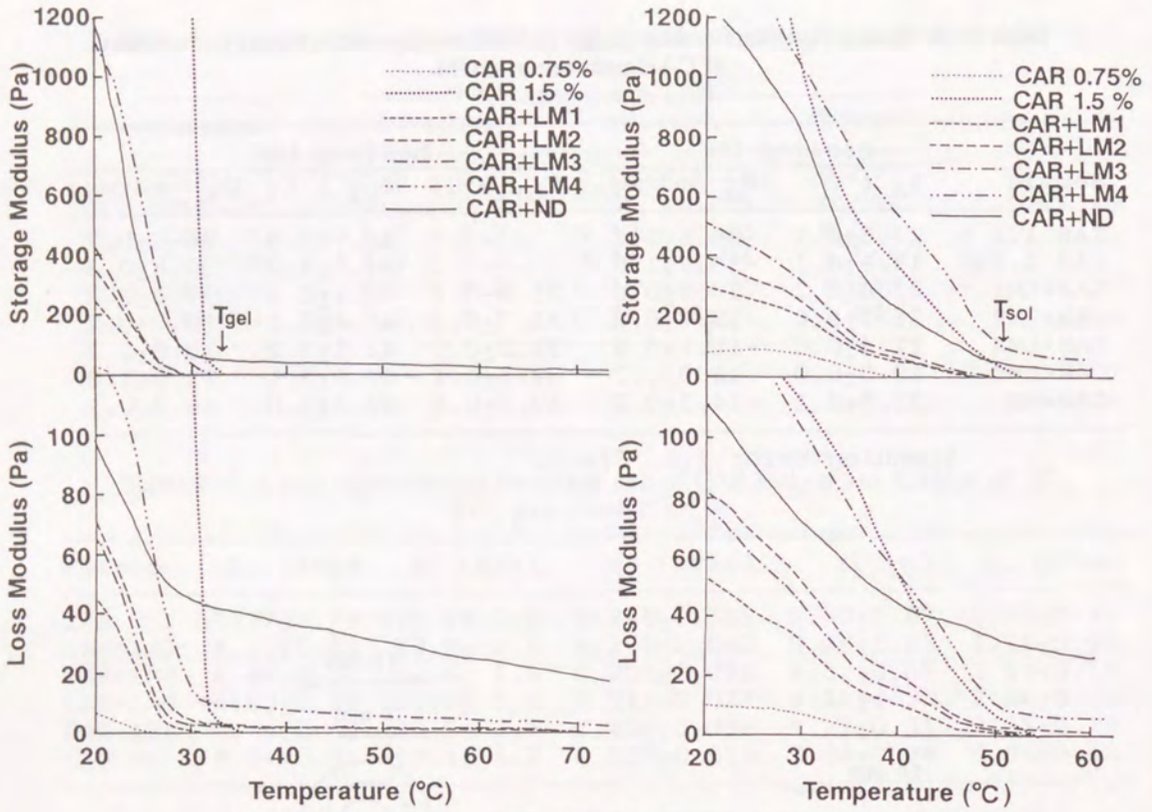


Fig. V–12.(left) Temperature dependence of storage and loss moduli for CAR and KGM mixtures in cooling process.
Cooling rate: 1.0 °C/min.
The arrow represents the sol–to–gel transition temperature (T_{gel}) for 1.5 % CAR sample.

Fig. V–13.(right) Temperature dependence of storage and loss moduli for CAR and KGM mixtures in heating process.
Heating rate: 1.0 °C/min.
The arrow represents the gel–to–sol transition temperature (T_{sol}) for 1.5 % CAR sample.

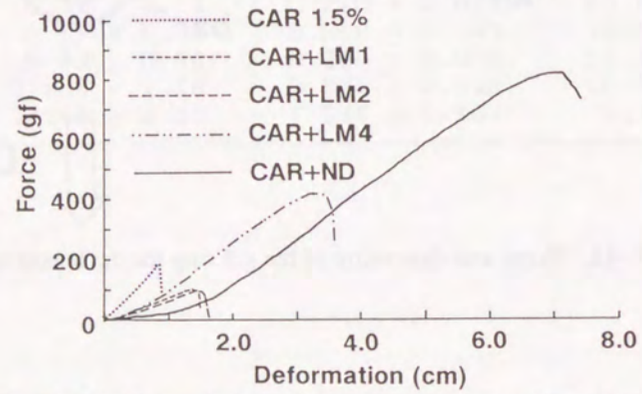


Fig. V–14. Force–deformation curves in the tensile testing for CAR–KGM mixed gels of 1.5 %.

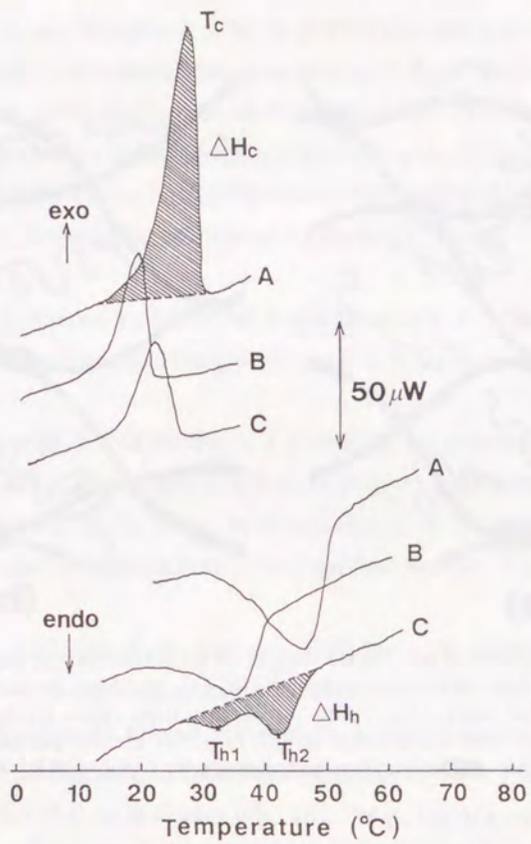


Fig. V–15. Typical cooling (the upper three) and heating (lower) DSC curves for 1.5 % CAR alone (A), 0.75 % CAR alone (B), and 1:1 mixture of CAR and LM4 of 1.5 % (C).
Scanning rate: 1.0 °C/min. The upward direction is the exotherm. Examples of the peak temperatures are drawn by T_c, T_{h1} and T_{h2}. The heat required for the transition is shown by the shaded area. The enthalpy for the sol–to–gel or gel–to–sol transition (ΔH_c or ΔH_h) is calculated by dividing the area by the gum amount.

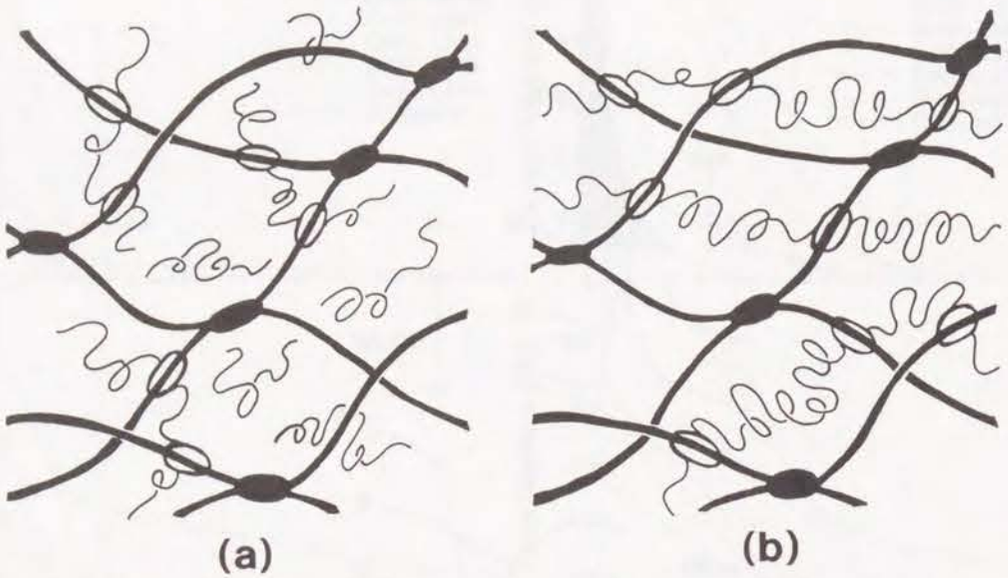


Fig. V–16. The effect of molecular weight of KGM on the gel network structure. Mixed gels of CAR and low molecular weight KGM (a), and high molecular weight KGM (b). Thick lines represent CAR molecular chains whilst thin lines show KGM chains. The number of elastically active chains and the contour length of active chains increase with increasing molecular weight of KGM. ●: junction zones made by CAR–CAR, ⬭ : junction zones made by CAR–KGM.

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SUMMARY

Chapter II

The melting point of thermo-reversible gels was measured automatically. This method retained the merits of the falling-ball method, *i.e.*, it was mechanically simple, reproducible and accurate. The vertical displacement of a ball put on a gel was converted into a voltage signal by this system. The temperature of the gel was raised at a constant rate by use of a micro-computer. The displacement of the ball and the temperature of the gel were fed into a recorder simultaneously. The digital camera avoided the need for tedious observation of the displacement of a ball by a cathetometer.

A mechanical property is good indicator of the gelation. A dynamic viscoelasticity measurement was carried out to detect nonzero elastic modulus indicating the sol-to-gel transition (gelation) point. A rotational viscometer at a low shear rate and a damped oscillation rheometer were also tried to study the increase in viscosity at the initial stage of gelation. All three methods could indicate the initiation of the gelation, however the transition points indicated differed from each other.

Breaking force in an empirical apparatus is often used in food industry. Unfortunately, a number of reports by empirical measurements have given insufficient information to compare each other's data. Puncture and compression tests of tofu were carried out and discussed along with the experimental conditions. Apparent breaking stress in the puncture test depended on plunger diameter, but in the compression test it was independent of sample diameter. A tensile or compression testing was better than a puncture test to descrive properties of gels objectively. Some methods categorized in small deformation rheology were also discussed.

Chapter III

The effects of physically or chemically modified celluloses on gelatinization and retrogradation of sweet potato starch were examined by rheological measurements and differential scanning calorimetry (DSC). The mixture of sweet potato starch and four modified celluloses (powdered microcrystalline cellulose, alkaline soluble fibrous cellulose, carboxymethylcellulose and methylcellulose) in the ratio of 9 to 1 was used as a model, because this ratio of starch to cellulose is approximately the same for sweet potato root. Cellulose derivatives generally did not show important effects on gelatinization temperature and enthalpy of gelatinization. Cellulose derivatives which are not water-soluble increased starch retrogradation, while water-soluble methylcellulose unexpectedly prevented retrogradation.

The effect of water soluble sugars contained in sweet potato on gelatinization and retrogradation of the starch was studied by DSC. Gelatinization peak temperature T_p and gelatinization enthalpy increased with increasing sugar concentration. The shift of T_p to higher temperatures was attributed to the stabilization of crystalline region in starch and the immobilization of water by sugar molecules. The effect was larger in this order: sucrose, glucose, fructose. All these sugars retarded retrogradation of sweet potato starch paste and the order of effectiveness was the same as in the case of increaseing gelatinization temperature. The effect of sucrose concentration on T_p was explained by a zipper model approach.

Chapter IV

A new method to evaluate soybeans for making tofu (soybean curd) was proposed. Dynamic viscoelasticity measurements were carried out to examine the gelation process of soymilk, storage and loss moduli being observed as a function of time after adding glucono-δ-lactone (GDL). The gelation curves fitted well with first-order reaction kinetics. The saturated value of the storage modulus correlated well with the gel hardness by a curdmeter.

The saturated storage modulus depended mainly by the concentration of soybean 11S globulin (11S). The rate constant of the gelation increased with increasing gelling temperature and was mainly governed by the concentration of GDL. The activation energy of the gelation was calculated to be 1.5×10^1 kJ/mol from an Arrhenius plot of the rate constants. The gelation time at which the shear modulus began to deviate from the base-line became shorter with increasing concentration of GDL at a constant 11S concentration (4 %). However, the gelation time was not shortened by an increase in protein concentration, in contrast to previous findings for many other protein gels. The mechanical loss tangent at the final stage of the gelation decreased with increasing protein concentration, but was independent of the GDL concentration. The gelation time of systems which consist of 11S and GDL at a fixed ratio, decreased with increasing 11S concentration. However, the time at which the gelation curve reached saturation did not decrease very much with increasing GDL concentration at higher concentrations of the protein. It was found that the decrease of the pH by addition of GDL greatly contributes to the gelation time.

Both storage and loss moduli at the final stage of the gelation for soybean 7S globulin (7S)-GDL gels showed almost the same value as those of 11S gels when concentrations of protein and GDL were the same. However, parameters obtained in the compression testing, *i.e.*, breaking stress and breaking strain of fully gelled 7S were smaller than those of 11S gels. The rate of gelation for 7S was much slower and the gelation time was longer than for 11S. The gelation rate increased and the gelation time decreased with increasing GDL concentration at a constant 7S concentration as observed for 11S-GDL systems. The gelation time of systems which consist of the protein and GDL at a fixed ratio of 10:1 decreased with increasing protein concentration. The minimum concentration of 7S protein for the gelation in the presence of GDL was lower than that of 11S-GDL system.

Chapter V

Several fractions of konjac glucomannan (KGM) with different molecular weights were prepared. The molecular weights were indexed by the intrinsic viscosity, and molecular weight distribution was analyzed by gel permeation chromatography using cadoxen as the solvent and the mobile phase.

Solution properties of KGM were examined by measuring the viscosity and specific volume as a function of temperature, concentration and pH. At lower concentrations the high molecular weight fraction deviated from linearity even at lower concentrations in comparison to lower molecular weight fraction in the plot of reduced viscosity against concentration. At a low concentration range of glucomannan, specific volume was independent of concentration of glucomannan. Apparent partial specific volume as a function of temperature and pH; increased with increasing temperature up to 40 °C where it then leveled off and on increasing the pH remained constant up to pH 11 after which a sharp increase was observed. It was suggested that the change of molecular structure is necessary for gelation of KGM. The intrinsic viscosities of four fractions of KGM in 4 M urea solution were larger than those in water. The slope of double logarithmic plots of the zero shear specific viscosity against the coil overlap parameter were about 1.4 for all fractions.

Physicochemical properties for 1:1 mixed gels of KGM with different molecular weights and κ-carrageenan (CAR) were studied. Shear modulus for sol state mixtures containing higher molecular weight of KGM was higher. Dynamic and static viscoelastic moduli for the mixed gels were much smaller than CAR gel and became larger with increasing the molecular weight of KGM. Two systems containing KGM of lower molecular weights showed almost the same stress-strain curves in the tensile testing. The breaking stress and the breaking strain for mixed gels became larger with increasing molecular weight of KGM, and the difference was larger than that observed in the small deformation measurements. Similar gel-to-sol and sol-to-gel transition temperatures for all mixed systems were observed in both dynamic viscoelastic measurement as a function of temperature and differential scanning calorimetry (DSC). Gel-to-sol transition for mixed systems occurred at the temperature higher than the temperature for 0.75 % CAR alone and lower than that for 1.5 % CAR. A similar relationship was observed in sol-to-gel transition temperature. The difference in the transition temperatures was explained by a zipper model approach. One exothermic peak was shown in cooling DSC curves, while two endothermic peaks were observed in heating DSC curves for mixed systems. It suggested that the mixed gels involved two junction zones: some were made by CAR molecules and others were formed by the interaction between CAR and KGM.

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