TEXTURE AND STRUCTURE OF PRESSURE-SHIFT-FROZEN FOODS

YURI JIBU

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博士論文

圧力移動凍結した食品の物性と微細構造

治部　祐里

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CONTENTS

INTRODUCTION

Texture is a highly important quality of food gels. However, with foods of high water content (i.e. tofu, konnyaku, gel, steamed egg custard, egg, vegetables, or fruit), damage to structures through freezing is extensive and the texture after thawing becomes unacceptable. The rate of freezing is recognized as highly related to tissue damage. Slow freezing results in formation of large ice crystals and irreversible damage to structure and texture of food such as carrots (Fuchigami *et al*., 1994, 1995a, 1995b). When water is frozen at atmospheric pressure, the volume increases. This is one cause of tissue damage during slow freezing at -20°C at atmospheric pressure.

Under high pressure, a non-freezing region (liquid phase) below 0°C exists. When water is pressurized at 200 MPa at ca. -20°C, it dose not freeze (Fletcher, 1970). When reduced to atmospheric pressure after pressurizing at 200 MPa, -18°C (liquid phase), tofu froze rapidly. This method is called pressure-shift-freezing. In fact, it was found that the structure of pressure-shift-frozen tofu was better than tofu frozen by the air-blast method (Kanda *et al*., 1992, 1993).

The phase diagram for solid phases of water has been experimentally determined as shown in Fig. 1, with some extrapolations and estimates (Hobbs, 1974). When pressure was raised to 2400 MPa at -200°C ~ 80°C, several kinds of high pressure ices (ice I ~ ice IX) with different structures (Fig. 2) and properties were formed (Fletcher, 1970; Hobbs, 1974; Maeno, 1981; Franks, 1989). High-pressure forms of ice have included ices II and III, and this finding has continued in classic studies reporting ices V, VI, and VII and the metastable ice IV (Fletcher, 1970). Ice VIII and ice IX were reported by Whalley *et al*., (1966). Ice at atmospheric pressure is denoted as ice I (density, 0.92). From the co-ordination number of 4, the structure of ice I is very open with much empty space (Fletcher, 1970). It is the only ice that floats because it is less dense than liquid water. The density of high pressure ices is higher, and their crystal structure is very complex. The densities of ice II, ice III and ice V are 1.17, 1.14, and 1.23 g / cm^3, respectively. These high pressure ices have crystals with bent structures and they are much denser than ice I; the length (ice I: 2.76 Å, ice II: 2.75 Å ~ 2.84 Å) and angle (ice I: 109°, ice II: 80° ~ 129°) of hydrogen bonds grow, shrink, or bend (Fletcher, 1970; Hobbs, 1974; Maeno, 1981). Ice VI (1.31 g / cm^3) has a dual structure with 2 sets of crystals formed into one, and the density of ice VII and ice VIII are the highest (1.50 g / cm^3). When water is pressurized above 2200 MPa at temperatures above 82°C, hot ice is formed.

A region below 0°C (liquid phase) has enabled the non-freezing preservation of foods (Deuchi & Hayashi, 1990) when ice was pressurized to ca. 200 MPa, because pressure fusion (melting) resulted. Pressurization has been applied in high-pressure-thawing of frozen foods (Deuchi & Hayashi, 1991; Murakami *et al.*, 1994).

The purpose of this thesis is to show that the effects of high pressure on the quality (texture and structure) of different types of frozen foods.

Several research studies on boiled egg (Chapter I), egg custard gel (Chapter II and Chapter III), egg yolk (Chapter IV), agar gel (Chapter V) , carrageenan gel (Chapter VI and Chapter VII) will be performed and texture and structure will be determined. The effect of pressure-shift-freezing on improving the quality of frozen foods will be examined.

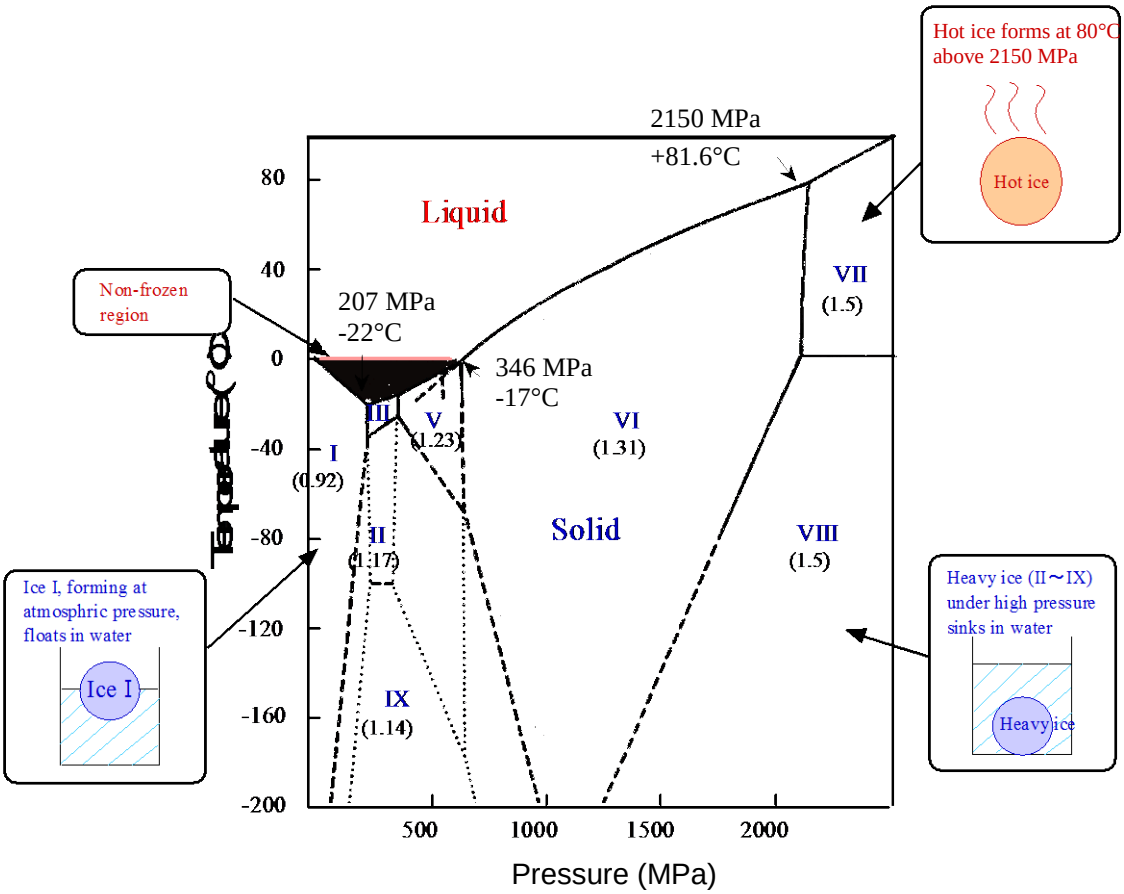


Fig. 1. Solid phase diagram of water

◀—— Triple points (Fletcher, 1970)

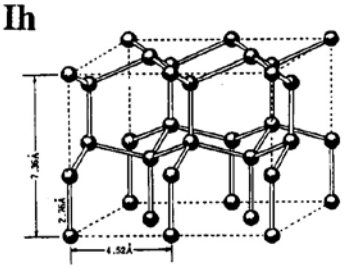
——— Measured stable lines

– – – Extrapolated or estimated lines

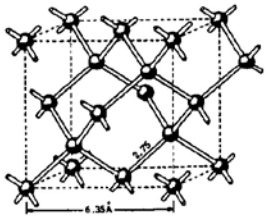
------- Extrapolated or estimated metastable lines (Hobbs, 1974)

( )

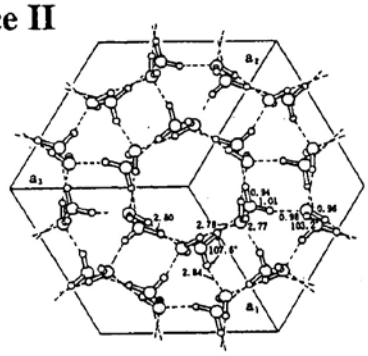
Ice Ih



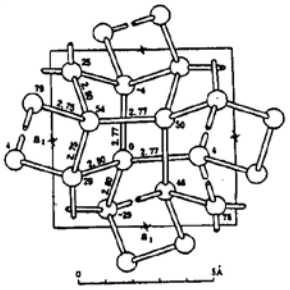
Ice Ic



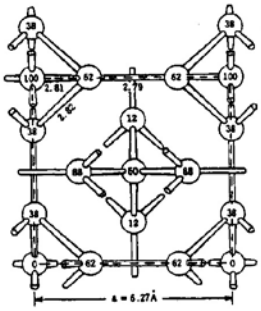
Ice II



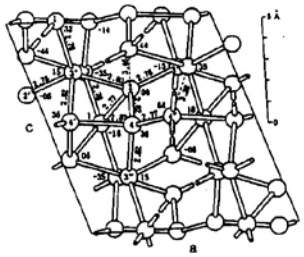
Ice III
Ice IX



Ice VI



Ice V



Ice VII
Ice VIII

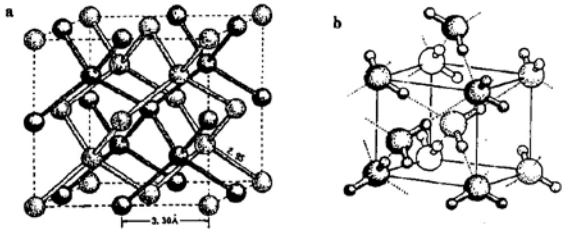


Fig. 2. Structures of high pressure ices

Chapter I

Structure and Texture of Pressure-shift-frozen Boiled Egg

INTRODUCTION

With food gels that have a high water content, damage to structures through freezing is extensive and texture after thawing becomes unacceptable. Such food gels frozen at atmospheric pressure (0.1 MPa) do not recover their gel phase when thawed. It has been already established that a non-freezing area (liquid phase) below 0°C exists under high pressure (Fletcher, 1970; Hobbs, 1974). In previous studies (Kanda *et al*., 1992), when tofu was pressurized at 200 MPa and -18°C, it did not freeze. However, when pressure was released, it froze quickly. This method was designated as "pressure-shift-freezing."

The effect of high pressure on the improvement in quality (texture and/or structure) of frozen food with protein has been discussed previously: tofu (Kanda *et al*., 1992; Fuchigami & Teramoto, 1997c; Fuchigami *et al*., 1998b; 2002; Teramoto & Fuchigami, 1999); pork (Martino *et al*., 1998); lobster (Chevalier *et al*., 2000); gelatin (Zhu *et al*., 2005); and egg custard gel (Teramoto *et al*., 2006). Also, pressure-shift-freezing effects on several kinds of food have been reviewed (Fuchigami, 1996, 2001, 2006; Fuchigami *et al*., 2008).

The results of these studies were as follows: pressure-shift-freezing at 200 ~ 400 MPa and -18°C ~ -20°C appeared to be effective in improving both the texture and/or histological structure of frozen food, because, when pressure-shift-frozen, nuclei formed during pressure release, and phase transition from liquid-to-ice I occurred quickly. Therefore, small ice crystals formed. Thus, this led to a beneficial effect on texture. However, the effect of high pressure on improving the quality of frozen food appeared to be related to the type of food.

When raw egg is frozen-thawed, the quality of albumen (egg white) is better than yolk (Kato, 1988). Conversely, the quality of albumen is worse than yolk when boiled egg (hard-cooked egg) is frozen. Hard-cooked egg yolks can be frozen successfully, but hard-cooked egg whites become tough and watery (The Betty Crocker Editors, 1989). Therefore, the objective of this study is to research the effect of pressure-shift-freezing on improving the quality of frozen boiled egg.

MATERIALS AND METHODS

1. Sample preparation

One-day-old White Leghorn hen eggs (large size, 66.88 ± 1.94g) were purchased from a poultry farm. Seven eggs were placed in saucepan, 800 ml water was added to come at least 1cm above eggs, and heated rapidly to boiling over a medium flame, stirring constantly. It took 4.5 min from 23°C to 100°C, and eggs were boiled for 12 min over low heat, and then put into tap water to prevent further cooking. Eggshell was first removed, then each egg was vacuum packed in a heat-sealed poly-flex bag (Hiryu, Asahi Kasei Pax Co., Tokyo).

2. Method of pressure-shift-freezing

A sample was pressure-shift-frozen using a Dr. Chef high pressure food processor (Kobe Steel Ltd., Kobe) (Kato *et al*., 1997) (Fig. I-1). Weston brine PS (66% propylene glycol, CCI Ltd., Gifu) was used for the pressure medium. The pressure medium was first placed in a pressure vessel (6 cm inside diameter and 20 cm high) and kept at -18°C by a cooler, then removed. Next, after the two samples were placed in a pressure vessel, the thermocouple (k-type) was inserted in the center of one sample. Then, the pressure medium (-18°C) was added to the pressure vessel. The samples were immediately pressurized for 60 min at 200 MPa. After reduction of pressure, the samples were stored for 1 day at -30°C then thawed at 20°C in a low temperature incubator.

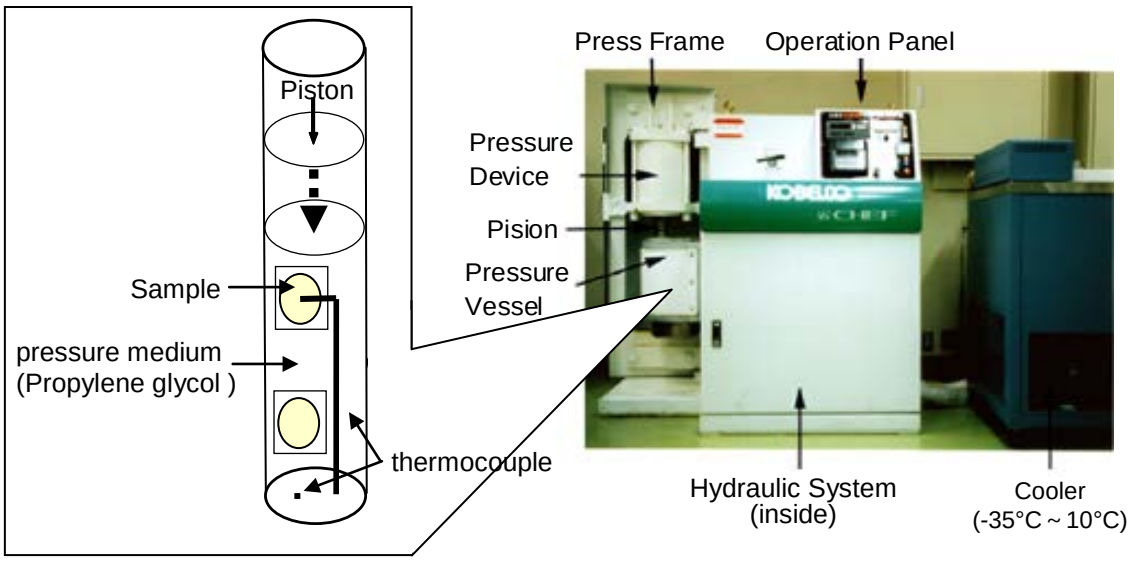


Fig. I-1. Dr. Chef, high pressure food processor (Kobe Steel Ltd., Kobe)

The operation was fully automated and both pressure and temperature of one sample (upper section of the pressure vessel) and pressure medium (lower section) were recorded in intervals of 5 sec using Thermodac E/Ef (Eto-denki Ltd., Tokyo). This experiment was repeated 3 times.

3. Method of freezing at atmospheric pressure

These samples were also frozen at atmospheric pressure (0.1 MPa) in a pressure vessel at -18°C or in freezers (-18°C, -30°C or -80°C). The temperature of the sample was measured at intervals of 30 sec using a data collector (AM-7002 K, Anritsu-keiki Ltd., Osaka).

4. Texture measurement

After the amount of drip was measured, a photograph of the outside appearance of frozen-thawed boiled egg was taken. Then, boiled egg was sliced into pieces 1 cm thick using an ultrasonic sample cutter (USC-3305, Yamaden Ltd., Tokyo). After taking a photograph and a monochrome copy of frozen-thawed boiled egg, the rupture stress and strain of albumen and yolk were measured by a creepmeter (Rheoner, RE-33005, Yamaden Ltd., Tokyo). Thickness of samples was measured using a sample-height counter (HC-3305, Yamaden Ltd., Tokyo) then punctured by using a plunger (cylindrical shape, 22 mm long) at 1 mm/sec stopping at 99% of the thickness using a loadcell of 200 g. Plungers of 2 mm and 5 mm dia were used for albumen and egg yolk, respectively. Rupture stress and rupture strain were indicated.

5. Structure measurement

Structure of the samples was observed with a cryo-scanning electron microscope (S-4500, Hitachi Ltd., Tokyo) (Fuchigami *et al*., 1995). Samples were cut into 6 mm × 1 mm × 1 mm. The specimen was contained in a metal holder and quickly frozen by immersing in LN_2, transferred to the cold stage of a cryo-system for scanning electron microscopy and then cut with a knife (-150°C). After etching at -85°C, the surface was coated with gold then observed at -120°C under low acceleration voltage (1kV). The magnifications used to observe ice crystals and micro-structure were ×100, ×400, ×1,000 and ×10,000, respectively. Micrographs of ×100 and ×10,000 were indicated.

Texture and structure of pressure-shift-frozen boiled egg were compared with those of the non-frozen boiled egg, boiled eggs frozen in freezers (-18°C, -30°C or -80°C) or boiled eggs placed in a pressure vessel at 0.1 MPa and -18°C.

RESULTS AND DISCUSSION

1. Changes in temperature of samples during freezing

Changes in the pressure and temperatures of boiled egg and pressure medium during freezing are shown in Fig. I-2. Within 1 min, the defined pressure was reached and was maintained for 60 min then reduced to atmospheric pressure within about 20 sec.

The initial temperature of samples was about 25°C. When the boiled egg was pressurized at 200 MPa and -18°C, it was cooled to about -18°C, and an exothermic peak was not detected during pressurization (Fig. I-2a). However, when pressure was released, the temperature of the sample rose quickly to -2°C, maintained about -2°C for 9 min then decreased to about -18°C. Therefore, it partially froze during depressurization. This indicates that the boiled egg must have frozen through pressure-shift-freezing. When boiled egg was frozen in a pressure vessel at atmospheric pressure and about -18°C, the temperature of sample decreased quickly to 0°C and took approximately 24 min to decrease from 0°C to -2°C (freezing plateau) (Fig. I-2b). The average freezing time (freezing plateau at 0°C ~ -2°C), shown, is the average of three measurements.

When boiled egg was frozen at atmospheric pressure in freezers, the average freezing time was 125 min at -18°C, 27 min at -30°C, and 11 min at -80°C, respectively (Fig. I-3). Freezing time increased according to the following order; pressure-shift-frozen at 200 MPa and -18°C < frozen in a -80°C freezer < placed in a pressure vessel at 0.1 MPa and -18°C < -30°C freezer < -18°C freezer, respectively. Freezing time for pressure-shift-freezing at -18°C was shortest and the immersion method in a pressure vessel (-18°C) was shorter than the air blast method in a freezer (-18°C).

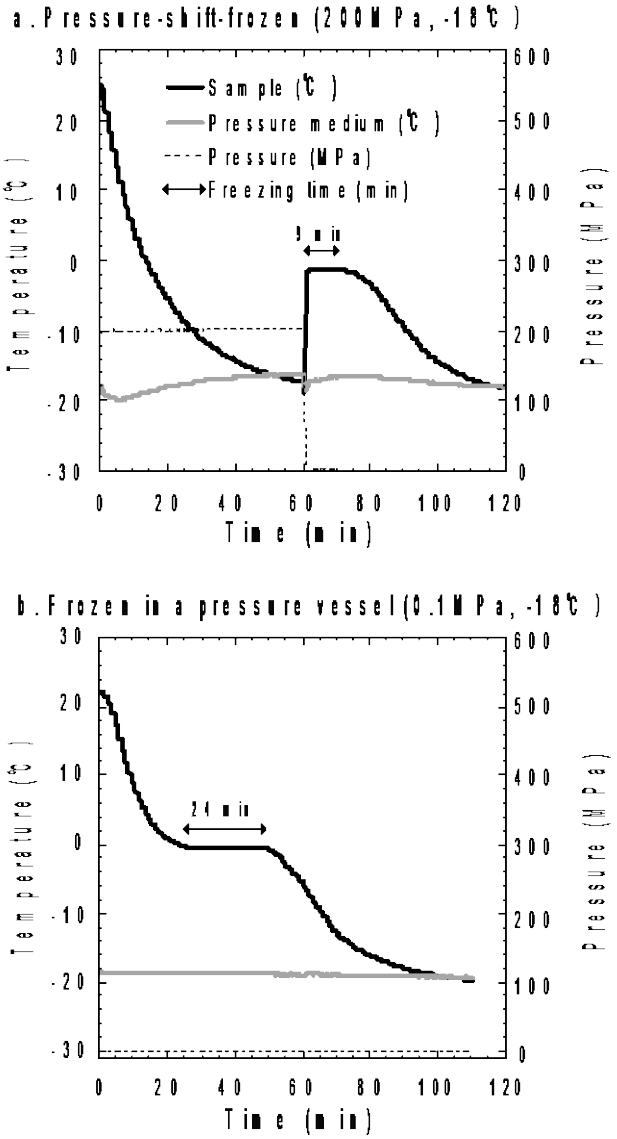


Fig. I-2. Changes in pressure and temperature of boiled egg and the pressure medium in a pressure vessel at -18°C

a. pressure-shift-freezing at 200 MPa and -18°C,
b. frozen in a pressure vessel at atmospheric pressure and -18°C

The average freezing time (freezing plateau at 0°C ~ -2°C), shown, is the average of three measurements.

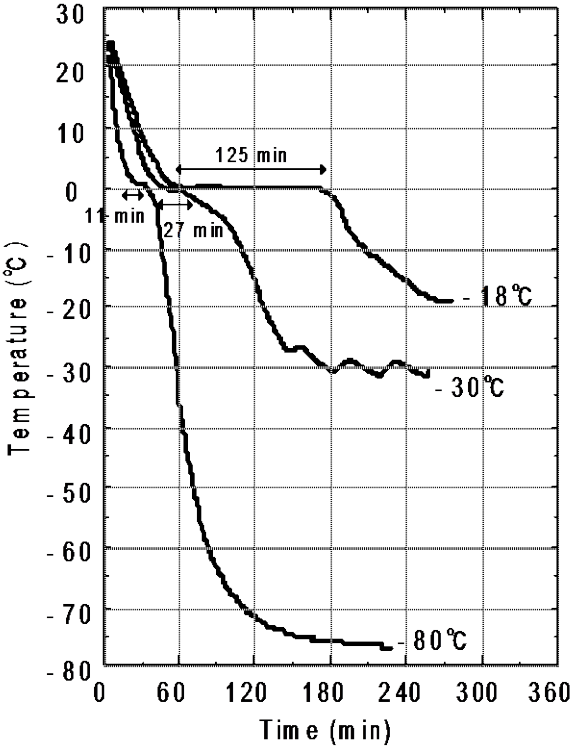


Fig. I-3. Changes in temperature of boiled egg frozen in freezers; frozen at -18°C, frozen at -30°C, and frozen at -80°C

The average freezing time (freezing plateau at 0°C ~ -2°C), shown, is the average of three measurements.

2. Visual appearances of frozen-thawed boiled egg

Visual appearances of frozen-thawed boiled eggs (both whole and sliced) were compared (Fig. I-4, Fig. I-5). The surface appearance of the control and pressure-shift-frozen eggs was smooth. However, surface of eggs frozen in freezers was pockmarked and marks increased when freezer temperature was higher -80°C < -30°C < -18°C, respectively. Also, the surface became transparent and the egg yolk could be seen. As ice crystals melted, sliced albumen, when frozen at -18°C, became cracked and pulled away from the outer layer. The egg yolk also cracked but became more watery at a higher temperature (-18°C).

The total appearance of boiled eggs (especially albumen) frozen in a freezer at -18°C differed greatly from non-frozen boiled egg (control) due to drip. The degree of damage to structures of albumen through freezing escalated according to the following order; pressure-shift-frozen at 200 MPa and -18°C < frozen in a -80°C freezer < placed in a pressure vessel at 0.1 MPa and -18°C < -30°C freezer < -18°C freezer, respectively. The ratio of area of cross section of albumen and egg yolk differed between non-frozen and frozen eggs; the area of albumen decreased by freezing-thawing. In fact, appearance of pressure-shift-frozen boiled egg was the similar as control.

3. Drip from frozen-thawed boiled egg

The amount of drip is shown in Table I-1. The amount of drip was least to greatest when the sample was pressure-shift-frozen at 200 MPa and -18°C < frozen in a -80°C freezer < placed in a pressure vessel at 0.1 MPa and -18°C < -30°C freezer < -18°C freezer, respectively. There were significant differences between the amount of drip from eggs frozen in -18°C and -30°C freezers and frozen in a pressure vessel at 0.1 MPa paired to drip from pressure-shift-frozen egg.

Table I-1. Amount of drip from frozen-thawed boiled eggs

	Temperature (℃)	Pressure (MPa)	Drip (%)
Frozen in a pressure vessel	-18	0.1	6.8 ± 2.2 *
	-18	200	4.0 ± 2.0
Frozen in freezers	-18	0.1	14 ± 3.7 **
	-30	0.1	9.7 ± 3.5 **
	-80	0.1	5.5 ± 1.0

* $p < 0.05$, ** $p < 0.01$: significant differences by T-test
(pair to "Frozen in a pressure vessel at 200 MPa and -18°C ")

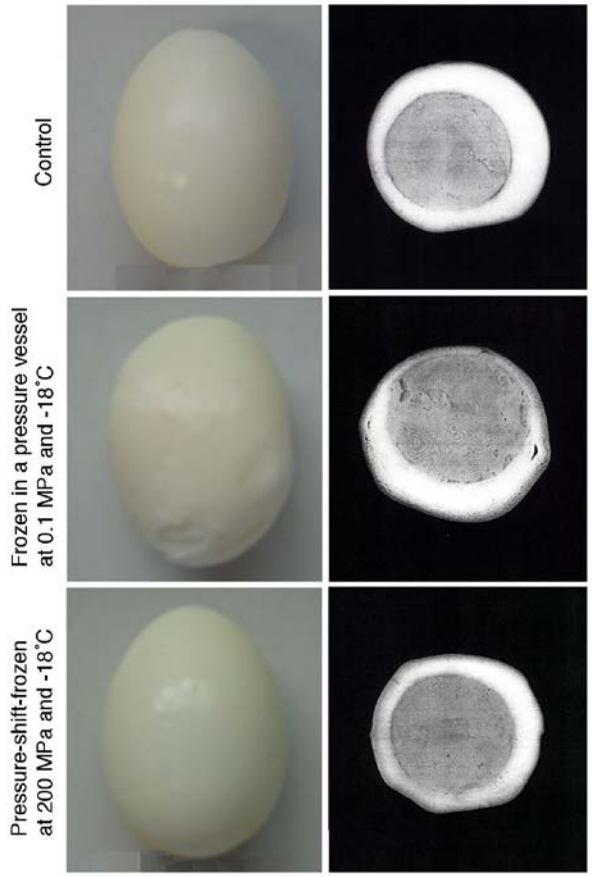


Fig. I-4. Visual appearances of boiled eggs frozen in a pressure vessel (Non-frozen control, pressure-shift-frozen at 200 MPa and -18°C and frozen in a pressure vessel at 0.1 MPa and -18°C.)

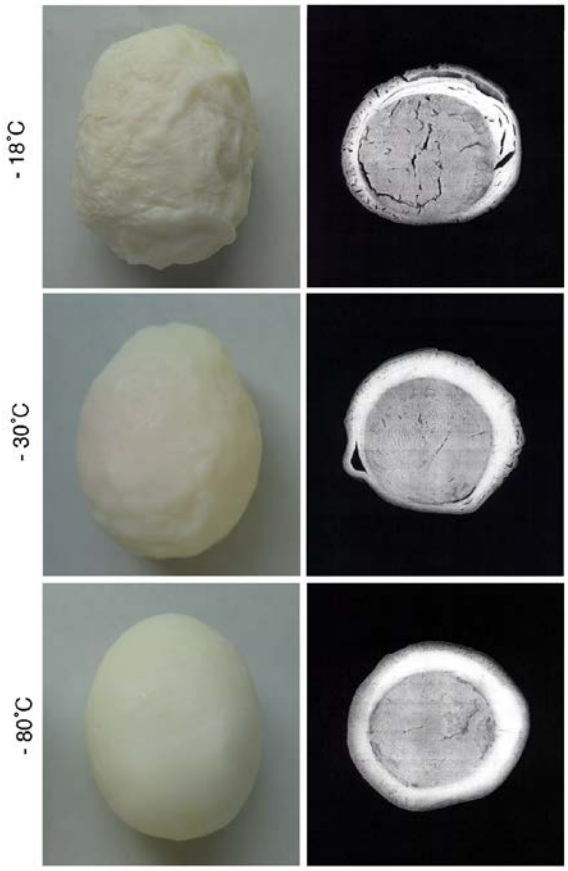


Fig. I-5. Visual appearance of boiled eggs frozen in freezers

4. Texture of frozen-thawed boiled egg

Typical stress-strain curves of egg yolk and albumen of non-frozen boiled egg are compared in Fig. I-6 and the average values of rupture stress and rupture strain are compared in Table I-2. Rupture stress of non-frozen egg yolk was smaller than that of albumen, although rupture strain of egg yolk was greater than that of albumen. This indicated that albumen was more brittle than egg yolk.

When boiled eggs were frozen-thawed, there was not a great difference in the patterns of typical stress-strain curves of egg yolk and albumen. Therefore, only the average values are shown in Table I-2. Both rupture stress and rupture strain of egg yolk decreased significantly when frozen in -18°C and -30°C freezers ($p<0.01$) and -80°C

freezer ($p<0.05$) and frozen in a pressure vessel at 0.1 MPa and -18°C ($p<0.05$). The damage to texture (rupture stress and rupture strain) was escalated according to the following order; when the sample was pressure-shift-frozen at 200 MPa and -18°C < frozen in a -80°C freezer ≒ being placed in a pressure vessel at 0.1 MPa and -18°C < -30°C freezer < -18°C freezer, respectively.

On the other hand, there was no significant difference in rupture stress of albumen between non-frozen and frozen-thawed boiled eggs. Rupture strain of pressure-shift-frozen albumen was the same as control. However, the rupture strain of albumen frozen by the other freezing methods increased. Thus, texture of pressure-shift-frozen boiled egg was better than that of the other treated boiled eggs.

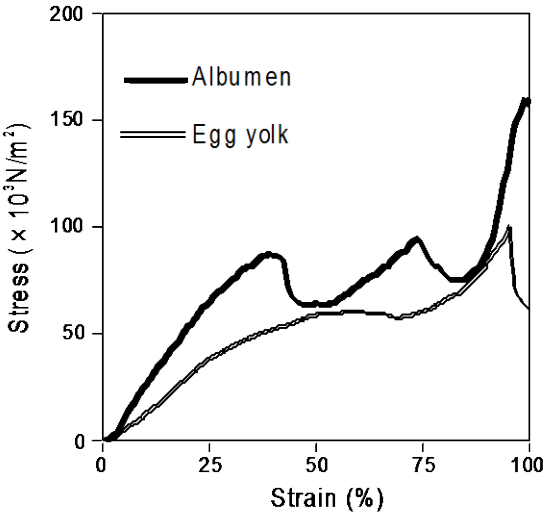


Fig. I-6. Typical stress-strain curves of non-frozen boiled egg

Table I-2. Average of rupture stress and rupture strain of frozen-thawed boiled eggs

	Temperature (°C)	Pressure (MPa)	Rupture stress (× 10^3N/m^2)		Rupture strain (%)	
			Egg yolk	Albumen	Egg yolk	Albumen
Control			62 ± 9	88 ± 15	56 ± 8	35 ± 6
Frozen in a pressure vessel	-18	0.1	41 ± 10*	103 ± 35	34 ± 9*	48 ± 11
	-18	200	51 ± 11	107 ± 22	41 ± 11	34 ± 5
Frozen in freezers	-18	0.1	26 ± 6**	88 ± 54	26 ± 6**	42 ± 20
	-30	0.1	31 ± 7**	99 ± 33	27 ± 7**	44 ± 15
	-80	0.1	39 ± 9*	90 ± 24	35 ± 11*	44 ± 11

* $p < 0.05$, ** $p < 0.01$: significant differences by T-test (pair to control)

5. Structure of frozen-thawed boiled egg

Cryo-scanning electron micrographs of frozen-thawed boiled egg are compared (egg yolk in Fig. I-7 and albumen in Fig. I-8). Ice crystal traces appear dark in the micrographs. The yolk sphere was a polyhedron. As freezing temperature increased, the space that surrounded the yolk spheres became large due to formation of ice crystals (especially frozen in -18°C and -30°C freezers). However, the space did not change greatly when frozen in a pressure vessel or pressure-shift-frozen (Fig. I-7).

Conversely, there were great differences in size of ice crystal traces of frozen-thawed albumen. Large amounts of round and small ice crystals formed in the albumen which was pressure-shift-frozen at 200 MPa. This indicated that ice formation was instantaneous. Ice crystal traces were smaller and more homogeneous in the pressure-shift-frozen albumen than in the other treated albumen.

When boiled egg was frozen in freezers at atmospheric pressure, ice crystals in albumen frozen at -18°C were larger than in albumen frozen at -30°C > -80°C. Ice crystals were also larger in albumen frozen at -18°C in a freezer than albumen frozen in a pressure vessel at 0.1 MPa and -18°C (Fig. I-8).

Ice at atmospheric pressure is denoted as ice I (density, 0.92). It is the only ice less dense than liquid water and thus floats (Fletcher, 1970; Hobbs, 1974). When boiled egg was frozen at 0.1 MPa (in a pressure vessel or freezers) and -18°C or pressure-shift-frozen at 200 MPa, ice I (Fuchigami and Teramoto, 1997c) formed in all eggs. However, size of ice crystals differed greatly. The initial freezing temperatures and also freezing time (freezing rate) appeared to affect the size of ice I; consequently, pressure-shift-freezing appeared to be more effective due to rapid freezing. The pressure-shift frozen albumen was supercooled to about -18°C. These results indicated that as the initial freezing temperature (super cooling temperature) became lower, the ice-nuclei numbers increased and growth of ice crystals was prevented (Watanabe, 1995); consequently, ice crystals became smaller. As ice crystals in egg yolk were larger, the amount of drip increased; consequently, frozen-thawed egg yolk became more watery, therefore rupture stress and rupture strain decreased.

Also, the textural quality of pressure-shift-frozen-thawed boiled egg was good. It appeared that the size of ice crystals affected the amount of drip and rupture strain of the thawed samples. Because ice crystals were larger, drip promoted shrinkage of the albumen gel and rupture strain increased.

There was not a great difference in gel structures of egg yolk and albumen when observed at magnifications of ×10,000.

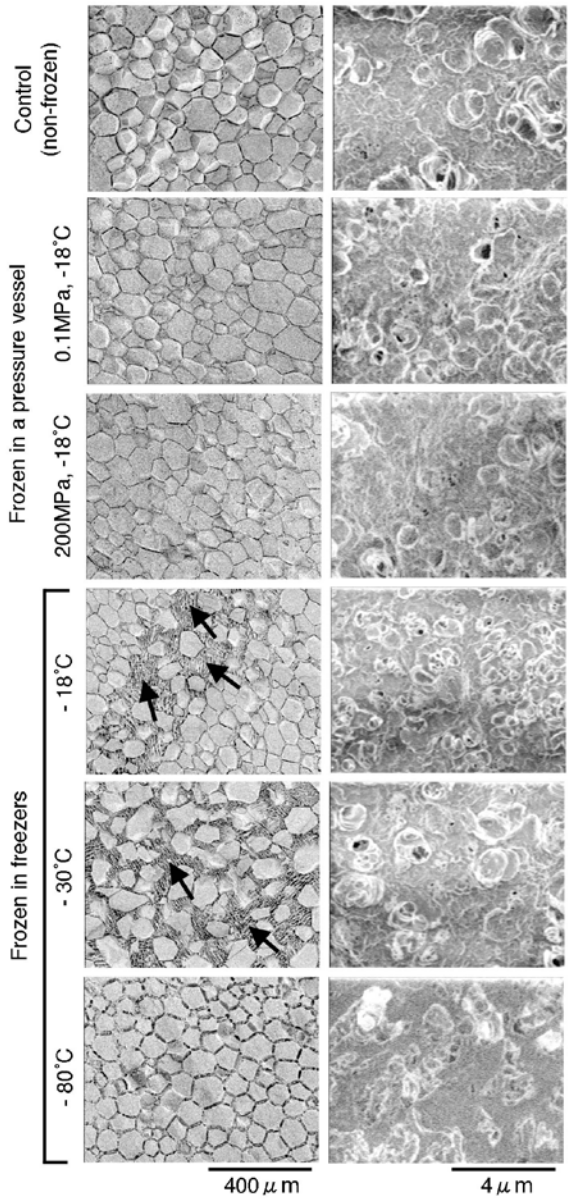


Fig. I-7. Cryo-scanning electron micrographs of frozen-thawed egg yolk

Arrow head: a trace of ice.

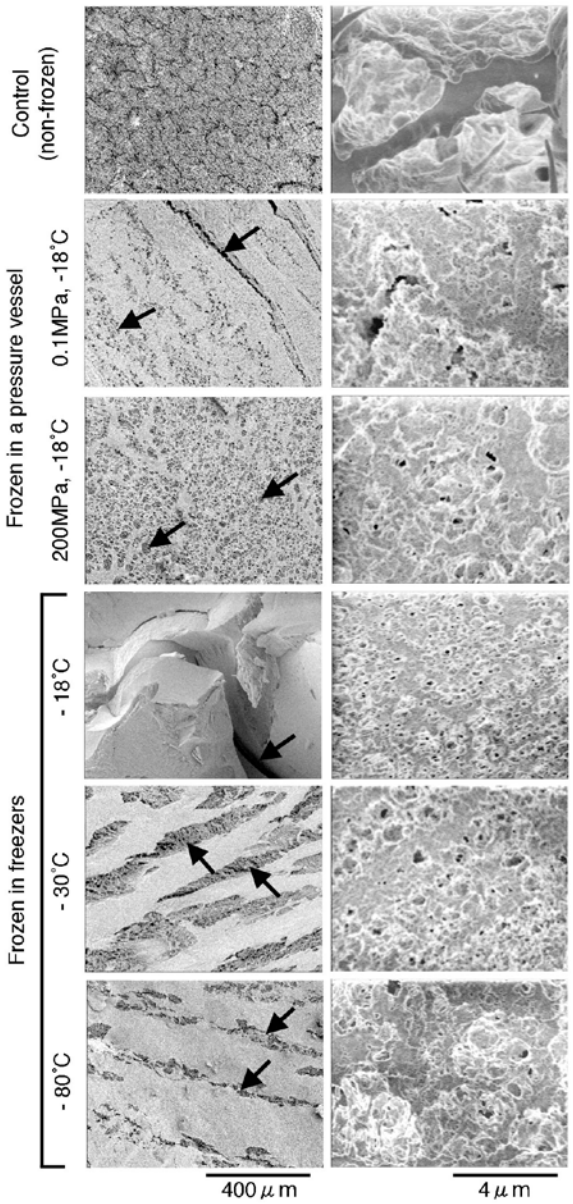


Fig. I-8. Cryo-scanning electron micrographs of frozen-thawed albumen

Arrow head: a trace of ice.

SUMMARY

To determine the effect of pressure-shift-freezing, boiled egg was pressurized at 200 MPa and -18°C. The structure, texture and the amount of drip of this egg were compared to those of eggs frozen at atmospheric pressure in a pressure vessel at -18°C and frozen in freezers (-18°C, -30°C or -80°C). Freezing time was shortest to longest with amount of drip least to greatest when the sample was pressure-shift-frozen at 200 MPa and -18°C < frozen in a -80°C freezer < placed in a pressure vessel at 0.1 MPa and -18°C < -30°C freezer < -18°C freezer, respectively. Ice crystals were smaller in the pressure-shift-frozen albumen than in the other treated albumen. As freezing temperature increased, the space that surrounded the yolk spheres became large due to formation of ice crystals. However, the rupture stress and strain of pressure-shift-frozen gel did not change greatly. Thus, pressure-shift-freezing was effective in improving the quality of frozen boiled egg.

Chapter II

Structural and Textural Quality of Pressure-shift-frozen Egg Custard Gel as Affected by Glucose, Trehalose or Sucrose

INTRODUCTION

Egg custard gel is formed by heat-induced aggregation of egg protein molecules. Heating induces denaturation of proteins. As a result of the conformational changes, non-polar and reactive thiol groups are exposed which can cause aggregation of proteins via non-covalent interaction and covalent disulphide bonds.

A smooth taste is highly important in egg custard gels such as chawan-mushi (egg custard soup with shrimp, chicken, and shitake mushroom), tamago-tofu (egg custard), and custard pudding. The egg custard gel is slow frozen at atmospheric pressure (0.1 MPa), its texture become spongy and unsuitable for consumption. The volume of water increases when it is frozen at 0.1 MPa. Ice I, formed at 0.1 MPa, is the only ice less dense than liquid water. This seems to cause tissue damage during freezing. Conversely, under high pressure, several kinds of ice (ice II ~ IX), each with a different structure and physical properties, are formed. The density of this high pressure ice is greater than that of water. Also, a non-freezing region (liquid phase) below 0°C exists under high pressure (Bridgman, 1912; Fletcher, 1970; Hobbs, 1974; Maeno, 1981), shown in Fig. II-1. When water is pressurized at 200 MPa at approximately -20°C, it does not freeze. However, when the pressure is released, water freezes quickly by pressure-shift-freezing. If this is done to a frozen gel, damage to the gel may be reduced.

In previous studies, in food with a high water content such as tofu (Kanda *et al*., 1992, 1993; Fuchigami and Teramoto, 1997c; Fuchigami *et al*, 1998b; Teramoto *et al*,. 1999), carrot (Fuchigami *et al*., 1997a, 1997b), Chinese cabbage (Fuchigami *et al*,. 1998a), eggplant (Otero *et al*., 1998), potato (Knorr *et al*., 1998), emulsion (Levy *et al*., 1999), peach, mango (Otero *et al*., 2000), lobster (Chevalier *et al*., 2000), gellan gum gel (Fuchigami and Teramoto, 2003a) and agar gel (Fuchigami and Teramoto, 2003b, 2004), the damage to the texture and structure was reduced by pressure-shift-freezing at 200 ~ 400 MPa. However, it has been ineffective in improving the quality of frozen konnyaku (Teramoto and Fuchigami, 2000). The coarse gel network observed in unfrozen konnyaku was compressed by freezing due to formation of ice crystals. The

rupture stress increased and strain decreased in all frozen konnyaku.

Several kinds of sugar have been used to prevent the loss of quality in frozen food (MacDonald and Lanier, 1991). Sucrose and glucose are usually used in food processing. To determine the effects of high-pressure-freezing, agar gel with 0, 5, 10 or 20% sucrose was frozen at 0.1 ~ 686 MPa and -20°C. Exothermic peaks were detected at 0.1, 100, 500 ~ 686 MPa during pressurization. This shows that a phase transition from liquid-to-ice occurred during pressurization (freezing). However, at 200 ~ 400 MPa, the gel did not freeze but froze during the pressure release. Thus, the structure of gel frozen at 200 ~ 400 MPa was better than that of other samples due to quick freezing. The phase transition from high-pressure-ices to ice I at -20°C might have promoted the growth of ice crystals. With the addition of sucrose, the initial freezing temperature decreased and the structural quality improved (Fuchigami and Teramoto, 2003b). When 5% or 10% sucrose was added to egg custard gel, the texture and structure of frozen-thawed gels improved with increases in the rate of freezing and the amount of sucrose (Teramoto *et al.*, 2001).

Trehalose is a non-toxic disaccharide of glucose; α-D-glucopyranosyl(1→1)-α-D-glucopyranoside ($C_{11}H_{22}O_{11}$, Mw 342.30). Trehalose is not particularly sweet (45% of the sweetness of sucrose), and it does not change the flavor of food. Also, trehalose can be produced from starch at low cost. Furthermore, trehalose has attracted attention because of its protective effect on the structure and function of the following; dried or frozen food (Tuley, 1990; Roser, 1991; Fuchigami *et al.*, 2002), enzymes (Apostolova *et al.*, 1992; Colaço *et al.*, 1992; Lippert and Galinski, 1992), cells and microorganisms (Bhandal *et al.*, 1985; Rudolph and Crowe, 1985; Honadel and Killian, 1988; Antoni *et al.*, 1989). These reports indicate that trehalose has a remarkable ability to protect organisms from damage when they are dried or frozen. It has also been suggested that trehalose protects gels from damage by freezing. When 2.5% trehalose was added to tofu, the smooth mouthfeel of tofu frozen at 400 MPa was more like that of the non-frozen control in sensory evaluation (Fuchigami *et al.*, 2002).

Therefore, the objective of this chapter was to determine the effects of high pressure with the addition of several kinds of sugar (glucose, sucrose or trehalose) on the improvement in texture and structure of frozen egg custard gel.

MATERIALS AND METHODS

1. Sample preparation

Egg whites and yolks were separated and stirred lightly so they would not bubble. The egg whites and yolks were then remixed in a ratio of 2:1 (w/w), and strained. 50% water and 5% sugar (glucose: extra-pure reagent, Ishizu Seiyaku Ltd., Osaka; sucrose: saccharose, extra-pure reagent, Ishizu Seiyaku Ltd., Osaka or trehalose: trehaose, provided from Hayashibara Ltd., Okayama) of the final weight were added to the egg mixture and stirred. The mixture was preheated for 20 min at 60°C for degassing (Tomie and Okubo, 1982). The mixture (20 g), was vacuum packed in heat-sealed polyethylene bags (25 mm in width and 150 mm in length), then heated for 15 min at 90°C in a water bath. After the egg mixture was coagulated, the egg custard gel was stored at 5~10°C within 2 days.

2. Method of freezing at high pressure

High hydrostatic pressure treatments were carried out using a high pressure food processor Dr. Chef (Kobe Steel Ltd., Kobe) (Kato *et al.,* 1997; Teramoto and Fuchigami, 2000). Propylene glycol (pressure medium), put in a pressure vessel (6 cm inside diameter, 20 cm high, surrounded with a jacket) previously kept at about -20°C by a circulation-type cooler (-35°C ~ 10°C), was removed. Three packs of egg custard gel were placed in a pressure vessel with a thermocouple inserted in the center of one sample and the pressure medium (-20°C) then poured into the pressure vessel. Samples were immediately pressurized at 100 ~ 686 MPa for 90 min (Fig. II-1). The operation was fully automated, and the pressure and temperatures of both the sample (the upper part) and the pressure medium (the lower part) were recorded. After depressurization, the gels were left in the pressure medium for 20 min to ensure complete freezing then stored for 1 day at –30°C. The frozen gel was then thawed at 20°C and 0.1 MPa in a low temperature incubator. This was compared with non-frozen gel (control), gel frozen at 0.1 MPa in a pressure vessel (-20°C) or in freezers (-20°C, -30°C or -80°C). The temperature of the samples put in freezers was monitored at 1min intervals by a thermodac E (Eto Denki Co., Tokyo).

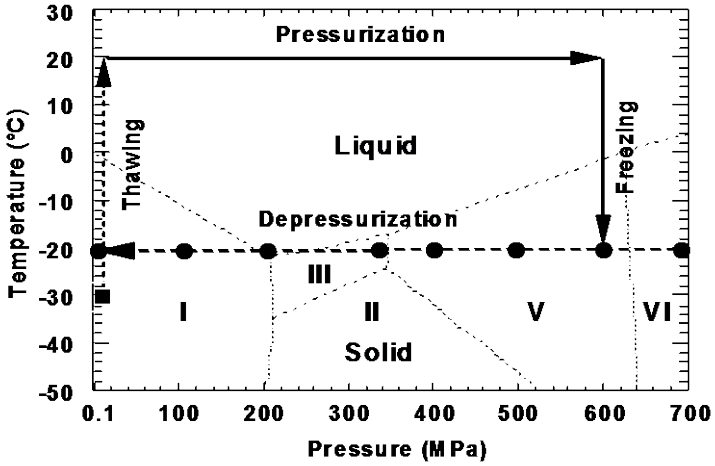


Fig. II-1. Phase diagram of freezing and thawing methods

The densities of ice I, ice II, ice III, ice V and ice VI are
0.92, 1.17, 1.14, 1.23 and 1.31 g/cm^3, respectively.
Ice II~V have crystals with bent structures (much denser than ice I);
the length and angle of hydrogen bonds grow, shrink, or bend.
Ice VI has a dual structure with two sets of crystals formed in one.
● points of pressurization, ■ storage.

3. Texture measurement

Egg custard gel was cut into 10 mm in length for texture measurement. The rupture stress and strain of the egg custard gel were measured using a creepmeter (Rheoner, RE-33005, Yamaden Co., Ltd., Tokyo) (Fuchigami and Teramoto, 1997c). The thickness of the samples was measured using a sample-height counter (HC-3305, Yamaden Co., Ltd., Tokyo), then punctured using a plunger (cylindrical shape: 3 mm diameter, 22 mm long) for 1 mm/s stopping at 99% of the thickness using a loadcell of 200g. Maximum force (breaking load) was used as the index of stress (rupture stress) (N / m^2), and (the distance of the breaking point ÷ the thickness of the sample) × 100 was used as the index for rupture strain (%).

4. Structure measurement

The structure of the central part of the samples was observed with a cryo-scanning electron microscope (S-4500, Hitachi Co., Ltd., Tokyo) (Fuchigami *et al.*, 1995). Gel samples were cut into 6 mm × 1 mm × 1 mm and dehydrated with 20%, 40% and 50% ethanol. A specimen was contained in a metal holder and quickly frozen by immerson in LN_2. The frozen specimen was transferred to the cold stage of a cryo-system for scanning electron microscopy and then cut with a knife (-150°C). After etching at -85°C, the surface was coated with gold, then observed at -120°C under low acceleration voltage (1kV). The magnifications used to observe ice crystals and gel networks were ×100 and ×20,000, respectively. The mean of the area of ice crystal traces, the dark-colored area in the micrograph, was caluculated using image analysis software (Mac-scope, Mitani Co., Ltd., Fukui).

RESULTS AND DISCUSSION

1. Changes in temperature of samples during freezing under high pressure

Changes in pressure and temperature during freezing in a pressure vessel were evaluated (Fig. II-2). The temperature of the pressure medium rose slightly at the beginning due to heat from pressurization and the samples, then decreased quickly and was maintained at -20 ~ -18°C.

Changes in the temperature of the samples show when the phase transition of liquid to ice occurred. The initial temperature of the samples was about 10 ~ 20°C. When the gels were frozen in a pressure vessel at atmospheric pressure and -20°C, a freezing plateau was detected at 0°C due to the release of latent heat. When pressurized at 100 MPa, the gel samples were cooled rapidly to about -8°C, and the temperature of the gels then decreased gradually to -20°C. When the pressure was released, the temperature decreased slightly. This indicated that all gels froze during pressurization.

When sugar-free gel was pressurized at 200 ~ 400 MPa and -20°C, no exothermic peak was detected in any gel for 90 min. However, when depressurized, the temperature rose quickly then decreased to -20°C (data for 400 MPa shown, but data for 200 and 340 MPa are not shown because the same behavior was observed at 200, 340 and 400 MPa.) Thus, the gel cooled at -20°C under these pressures showed no phase transition and froze quickly when the pressure was released through pressure-shift-freezing

(Kanda *et al.*, 1992). On the other hand, 5% sugar-gels were supercooled to -20°C at 200 ~ 500 MPa, then pressure-shift-frozen after depressurization. Therefore, depression of the freezing point for the 5% sugar-gel was greater than that for the sugar-free gel. Because the temperature of the pressure medium decreased greatly at a higher pressure due to decompression, the freezing time was shortened as the pressure rose.

When a sugar-free gel was pressurized at 500, 600 or 686 MPa at -20°C, an exothermic peak was detected during pressurization and then gradually decreased to -20°C. This indicated that the sugar-free gel froze during pressurization. However, in the 5% sugar-gel, an exothermic peak was detected during pressurization above 600 MPa. When the pressure was released, the temperature decreased quickly and an endothermic peak was then detected. Therefore, a phase transition of liquid to ice occurred under high pressure.

During pressurization at 100, 500, 600 and 686 MPa, the phase transition of liquid to ice occurred in a sugar-free gel, while at 100, 600 and 686 MPa, it occurred in the 5% sugar-gel. However, the sugar-free and 5% sugar-samples were cooled without a phase transition at -20°C at 200 ~ 400 MPa and 200 ~ 500 MPa, respectively, phase transition occurred when the pressure was released.

Changes in the temperature of the gel during freezing in freezers were also evaluated. When the egg custard gel was frozen at -20, -30 and -80°C in freezers, the temperature decreased from 0 to -5°C (freezing plateau), and the time duration of the freezing plateau for sugar-free, glucose-, trehalose-, and sucrose-added gel was as follows: at -20°C, 76, 66, 72 and 81 min; at -30°C, 57, 29, 46 and 52 min; at -80°C, 29, 18, 23 and 22 min, respectively. Although the time duration of the freezing plateau for the glucose-added gel was shorter than that of the others, the presence of sugar did not affect the duration of the freezing plateau greatly.

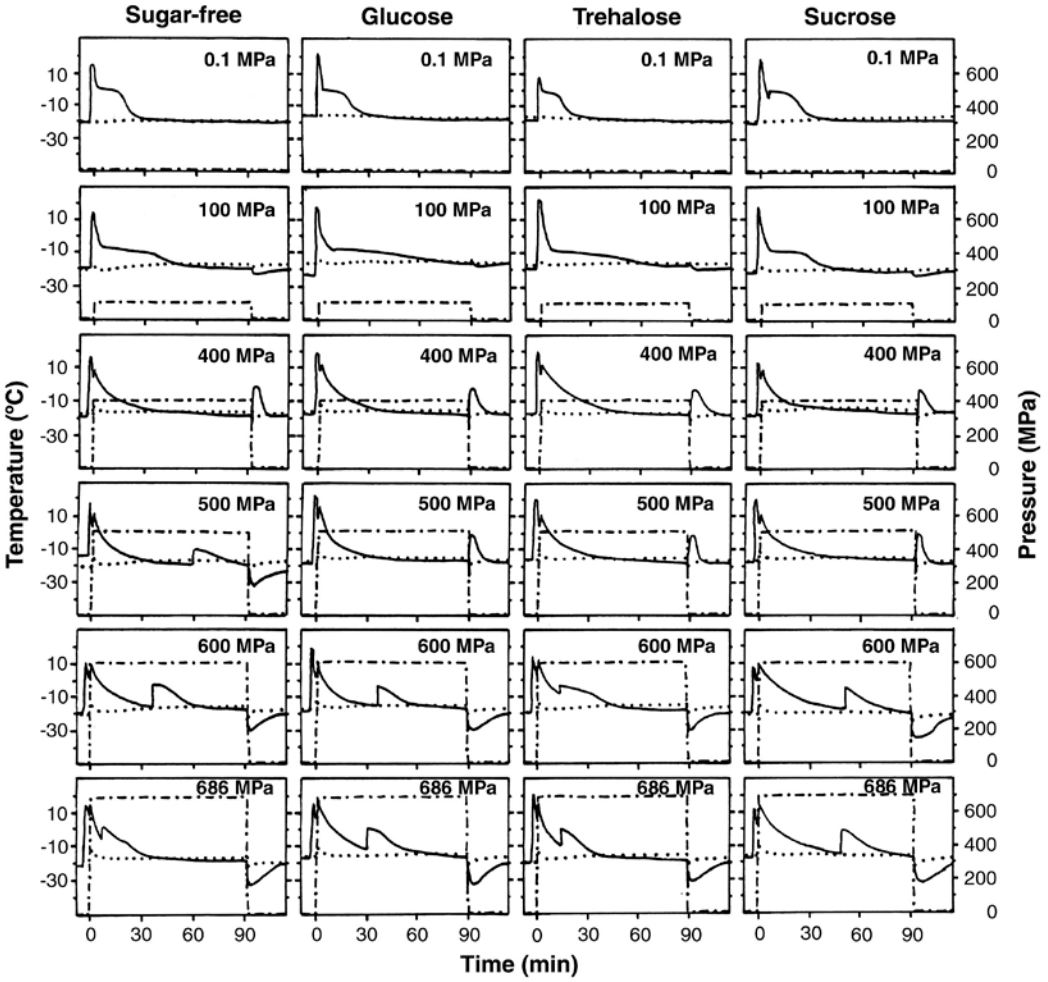


Fig. II-2. Changes in pressure and temperatures of egg custard gel and pressure medium during pressurization and depressurization at -20°C.

—— Temperature of egg custard gel, ---- temperature of pressure medium, and –·– pressure.

2. Syneresis from frozen-thawed egg custard gel

Syneresis from a frozen-thawed egg custard gel is shown in Table II-1. The amount of syneresis from a gel pressure-shift-frozen at 200 ~ 500 MPa was smaller than that frozen at 0.1, 100, 600, and 686 MPa. Therefore, pressure-shift-freezing was effective in reducing the syneresis. When a gel was frozen at 200 ~ 500 MPa, there was some significant difference between the sugar-free gel and the 5% sugar gel. However, the amount of syneresis was very small and relatively unimportant. On the other hand, when a gel was frozen at 0.1 MPa, there was no significant difference among sugar-free, sucrose-, glucose-, and trehalose-added gels. Thus, the addition of 5% sugar was not as effective in reducing syneresis.

Table II-1. The amount of syneresis from frozen egg custard gel

Freezing method	Pressure (MPa)	Temp. (℃)	Syneresis (%) 0% sugar	5% glucose	5% trehalose	5% sucrose
Frozen in pressure vessel	0.1	-20	18.9 ±11.5	10.4 ±4.3	9.8 ±4.9	10.5 ±6.5
	100	-20	28.3 ±9.2	21.2 ±16.0	29.6 ±6.2$^{\#\#\#}$	12.9 ±9.7
	200	-20	1.6 ±0.7$^{\#}$	3.4 ±1.3$^{*,\#\#}$	4.6 ±1.9*	3.4 ±1.1*
	340	-20	2.5 ±0.3$^{\#}$	3.7 ±0.6$^{*,\#\#}$	3.9 ±1.5	1.7 ±0.4$^{**,\#}$
	400	-20	1.8 ±0.7$^{\#}$	3.7 ±1.3$^{\#}$	3.2 ±1.0$^{*,\#}$	0.4 ±0.5$^{*,\#\#}$
	500	-20	2.7 ±1.9$^{\#}$	3.8 ±1.3$^{\#}$	2.9 ±0.4$^{\#}$	3.3 ±0.2
	600	-20	3.8 ±2.6$^{\#}$	10.3 ±7.9	12.8 ±3.3***	6.4 ±0.3
	686	-20	10.2 ±1.4	17.3 ±3.7$^{**,\#}$	15.1 ±5.4	20.2 ±6.5**
Frozen in freezers	0.1	-20	11.2 ±12.1	12.5 ±9.1	5.1 ±2.2	15.7 ±2.4
	0.1	-30	9.6 ±5.4	19.1 ±3.8$^{*,\#\#}$	13.7 ±6.6	14.4 ±6.5
	0.1	-80	19.0 ±5.9	13.3 ±5.6	11.7 ±0.4*	14.1 ±1.3

Means ± standard deviations (n=3)

There were significant differences by T-test.

*p=0.05, **p=0.01, ***p=0.001 (pair to 0% sugar gel)

$^{\#}$p=0.05, $^{\#\#}$p=0.01, $^{\#\#\#}$p=0.001 (pair to gel frozen in pressure vessel at 0.1MPa)

3. Texture of frozen-thawed egg custard gel

Both the rupture stress and strain of frozen-thawed egg custard gel are shown in Table II-2. The stress and strain of the non-frozen gel (control) decreased with the addition of glucose but did not change when either trehalose or sucrose was added. When gels were pressurized at 100 ~ 686 MPa and 20°C, the stress and strain of the gels did not change (Teramoto *et al*., 2001).

When sugar-free egg custard gel was frozen at 100, 500, 600, or 686 MPa, the rupture stress and strain increased 4~9 times and 2~3 times those of a non-frozen control, respectively. The stress and strain of a 5% sugar-added gel frozen at 100, 600, or 686 MPa increased 3~5 times and 2~3 times those of the non-frozen control, respectively. The rupture stress and strain of frozen-thawed gels increased greatly when the gels froze during pressurization. However, the stress and strain of pressure-shift-frozen gels (sugar-free gel frozen at 200 ~ 400 MPa, and 5% sugar-gel frozen at 200 ~ 500 MPa) did not change greatly (increase within 2 times). This indicates that pressure-shift-freezing protects against damage from freezing. Furthermore, the change in the stress of frozen-thawed gels was reduced with the addition of 5% sugar; however, the effects of sucrose, glucose, and trehalose were almost the same.

Changes in the stress and strain of sugar-free egg custard gels frozen at 0.1 MPa in freezers or a pressure vessel were greater than those of pressure-shift-frozen gels. However, there was no great difference in the stress, and only some differences in the strain of a 5% sugar-gel frozen at 0.1 MPa or a pressure-shift frozen sample. The texture of gels frozen at 0.1 MPa improved with the addition of 5% sugar. The addition of 5% sugar was effective in reducing the changes in rupture stress and strain; however, there was no significant difference among sucrose, glucose, and trehalose.

Table II-2. Effects of high pressure and sugar on rupture stress and strain of frozen-thawed egg custard gel

Freezing method	Pressure (MPa)	Temp. (℃)	Rupture stress ($\times 10^5 N/m^2$)				Rupture strain (%)			
			0% sugar	5% glucose	5% trehalose	5% sucrose	0% sugar	5% glucose	5% trehalose	5% sucrose
Unfrozen control			8.8 ±1.0	5.2 ±0.7	8.9 ±0.6	8.5 ±0.7	33.1 ±2.8	22.0 ±2.2	31.9 ±2.8	27.7 ±2.5
Frozen in pressure vessel	0.1	-20	48.5 ±8.7	8.5 ±2.1	14.2 ±2.1	13.6 ±2.2	72.1 ±4.9	43.2 ±11.2	51.2 ±7.1	34.5 ±3.1
	100	-20	79.7 ±5.9	16.3 ±2.7	45.5 ±3.5	39.2 ±6.4	72.9 ±3.5	47.6 ±4.6	65.1 ±2.0	70.4 ±3.6
	200	-20	24.0 ±1.5	8.6 ±0.7	15.3 ±0.8	15.3 ±5.1	40.3 ±1.8	29.2 ±2.2	36.2 ±1.6	37.4 ±1.8
	340	-20	25.4 ±8.3	9.1 ±0.9	15.3 ±1.4	14.9 ±6.7	39.0 ±2.7	29.6 ±1.7	36.0 ±2.0	37.0 ±3.5
	400	-20	26.1 ±3.0	9.1 ±1.1	16.7 ±1.8	15.2 ±1.4	43.8 ±3.4	31.7 ±2.8	39.1 ±2.8	36.4 ±2.7
	500	-20	41.0 ±2.2	7.8 ±0.7	14.5 ±1.1	15.8 ±1.0	54.3 ±2.8	28.3 ±2.7	34.1 ±2.2	37.9 ±1.8
	600	-20	40.9 ±8.6	16.3 ±2.2	34.1 ±3.5	27.7 ±3.9	69.7 ±5.3	50.7 ±2.8	70.0 ±4.8	60.5 ±3.4
	686	-20	77.4 ±14.9	25.8 ±5.2	34.7 ±6.3	33.1 ±6.4	78.1 ±5.5	68.5 ±6.9	70.4 ±4.4	65.1 ±3.3
Frozen in freezers	0.1	-20	50.0 ±5.8	14.1 ±3.2	13.2 ±1.5	18.7 ±4.7	69.5 ±3.4	52.5 ±6.8	38.8 ±3.6	53.8 ±5.6
	0.1	-30	44.5 ±5.2	9.3 ±8.9	17.6 ±4.8	15.6 ±3.5	66.8 ±4.0	41.0 ±4.7	43.7 ±7.8	42.3 ±3.6
	0.1	-80	41.5 ±6.7	10.1 ±2.8	11.1 ±2.8	13.8 ±2.2	61.6 ±3.4	43.5 ±7.6	41.6 ±12.8	41.2 ±5.0

Means ± standard deviations

(n=15; 5 samples/experiment, experiment was operated 3 times.)

4. Structure of egg custard gel frozen under high pressure

Cryo-scanning electron micrographs of frozen egg custard gels (Figs. II-3 and II-4) and the size of ice crystals (Table II-3) were compared. The micrographs in Fig. II-3 show ice crystal traces in a gel frozen under high pressure. Ice crystal traces appear dark in the micrographs. The size of the ice crystals in egg custard gel pressure-shift-frozen at 200 ~ 500 MPa was smaller than that in the gel frozen at 0.1, 100, 600 and 686 MPa. A large number of small round ice crystals formed homogenously throughout the pressure-shift-frozen gel. This indicated that ice formation was instantaneous.

However, when egg custard gel was frozen in freezers at 0.1 MPa, ice crystals were bigger in egg custard gels frozen at -20°C > -30°C > -80°C (Fig. II-4). Furthermore, when egg custard gel was frozen at -20°C and 0.1MPa, ice crystals in the egg custard gel frozen in a freezer were bigger than in those frozen in a pressure vessel (Figs. II-3 and II-4), because the freezing rate of air-cooling (in freezers) was slower than that of immersion in a pressure vessel. Thus, the freezing rate had an important influence on the growth of ice crystals. As the temperature of supercooling fell and the freezing time shortened, many small ice crystals were found.

Furthermore, the size of ice crystal traces in gels frozen in freezers decreased with the addition of 5% sugar. The structure of egg custard gel frozen at 0.1 ~ 686 MPa in a pressure vessel slightly improved with the addition of 5% sugar. This indicates that sugar appears to protect against the growth of ice crystals. However, there was no significant difference in the size of ice crystals when sucrose, glucose, or trehalose was added.

Table II-3. The size of ice crystal traces in frozen egg custard gel image-analyzed by a mac-scope

Freezing method	Pressure (MPa)	Temp. (℃)	Size of ice crystal traces (μm^2)			
			0% sugar	5% glucose	5% trehalose	5% sucrose
Frozen in pressure vessel	0.1	-20	2218	2415	2763	1644
	100	-20	2720	2139	2501	1627
	200	-20	852	644	745	757
	340	-20	775	734	484	508
	400	-20	1412	321	515	377
	500	-20	1216	337	157	241
	600	-20	1780	1897	1291	791
	686	-20	4409	1138	1380	1203
Frozen in freezers	0.1	-20	9698	3612	6668	4560
	0.1	-30	3930	1198	1793	1927
	0.1	-80	2166	1643	694	1191

n=1 (number of SEM micrograph)

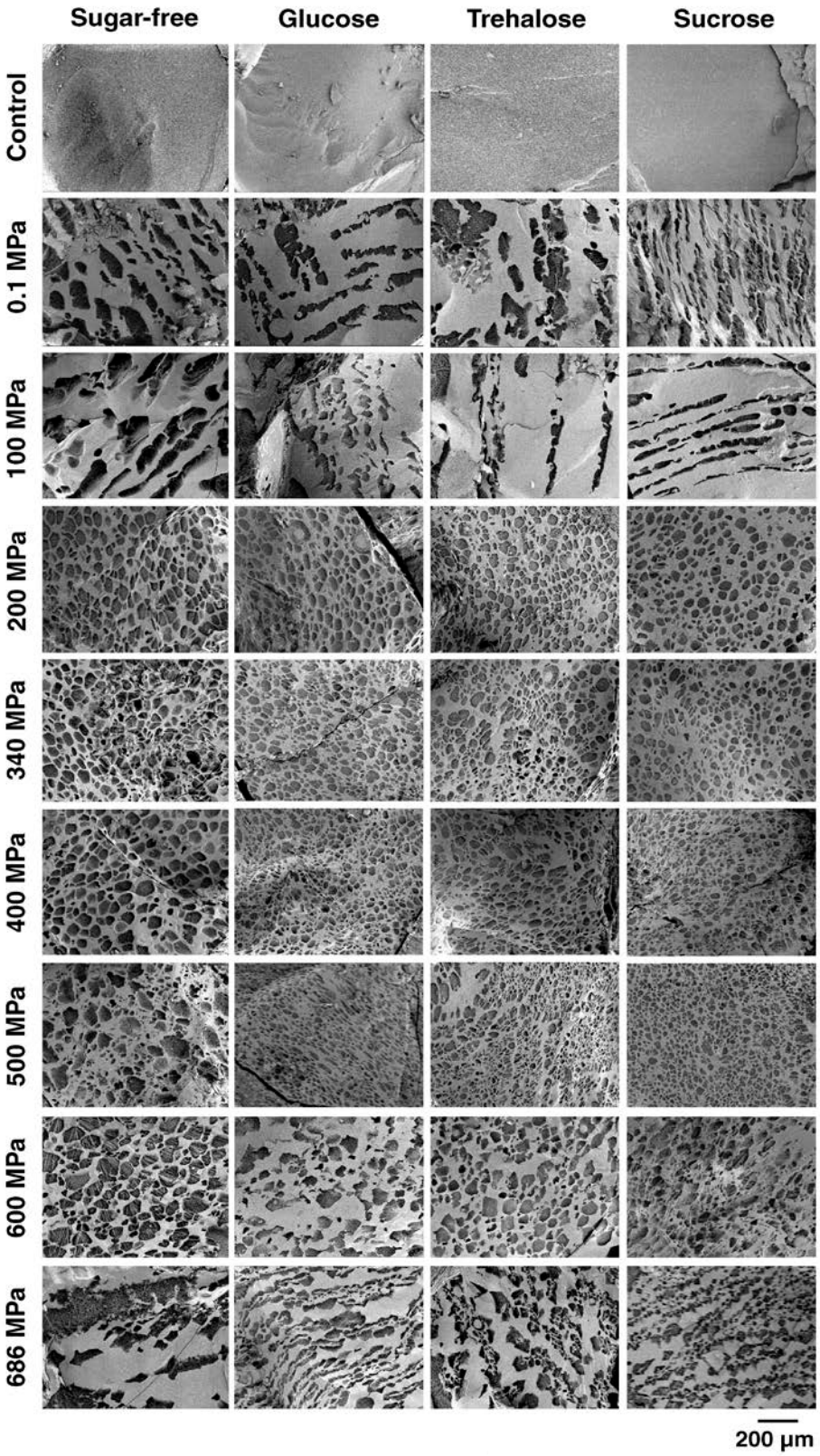


Fig. II-3. Cryo-scanning electron micrographs of high-pressure-frozen egg custard gel.

Control: non-frozen egg custard gel

Either glucose, trehalose or sucrose (5%) was included.

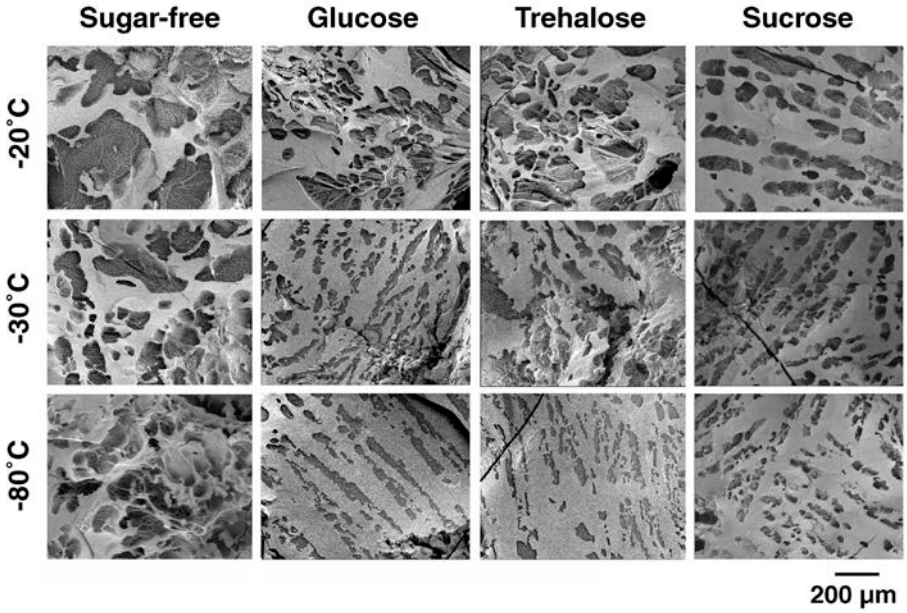


Fig. II-4. Cryo-scanning electron micrographs of egg custard gel frozen in freezers at atmospheric pressure

SUMMARY

The objective of this study is to determine the effects of high pressure and the addition of glucose, trehalose, or sucrose on the improvement in texture of frozen egg custard gel. Egg custard gel with 5% sugar (glucose, trehalose, or sucrose) was frozen at 0.1 ~ 686 MPa and -20°C. The gels with 0% and 5% sugar did not freeze at -20°C during pressurization at 200 ~ 400 MPa and 200 ~ 500 MPa, respectively. When the pressure was released, the supercooled gel froze quickly by pressure-shift-freezing, and small, granular-shaped ice crystals formed. Therefore, a change in texture (rupture stress and strain) of the pressure-shift-frozen gel was slightly prevented. The addition of 5% sugar to the gel was effective in improving the structural and textural quality of the frozen egg custard gel. However, there was no great difference in ice crystal size among the three kinds of sugar

Chapter III

Effects of High Pressure with the Addition of Sugar-alcohol on the Improvement in Texture and Structure of Frozen Egg Custard Gel

INTRODUCTION

Egg custard gel is formed by heat-induced aggregation of egg protein molecules. A smooth taste is considered to be highly important in egg custard gel used in typical Japanese steamed dishes (chawan-mushi and tamago-tofu), custard soup and custard pudding. However, the texture of egg custard gel when it is frozen-thawed at atmospheric pressure (0.1 MPa) is unsuitable for consumption because it is spongy.

A non-freezing region (liquid phase) below 0°C exists under high pressure (Fletcher, 1970; Hobbs, 1974). When water is pressurized at 200 MPa at -20°C, it does not freeze. However, when the pressure is released, water freezes quickly by pressure-shift-freezing (Kanda *et al*., 1992). If this is done to a frozen gel with high water content, damage to the gel may be reduced. Therefore, high-pressure-freezing effects on egg custard gel have been previously investigated (Chapter II; Teramoto *et al*., 2001; 2006; Teramoto & Fuchigami, 2002). In these studies, exothermic peaks were detected when frozen at -20°C at 0.1, 100, 500 ~ 686 MPa. This showed that a phase transition from liquid-to-ice occurred during pressurization (freezing). However, at 200 ~ 400 MPa, gel did not freeze but froze during release of pressure. Thus, the texture and structure of gel pressure-shift-frozen at 200 ~ 400 MPa then thawed was better than that of other samples due to quick freezing.

When 5% or 10% sucrose was added to egg custard gel, the damage to texture and structure of frozen-thawed gels was reduced with increases in the rate of freezing and the amount of sucrose (Teramoto *et al*., 2001), and also the addition of 5% glucose or 5% trehalose improved freezing tolerance of frozen-thawed egg custard gel (Chapter II). The addition of 1% NaCl or 1.28% KCl (Teramoto & Fuchigami, 2002) improved the quality of frozen-thawed gel. When sugars or salts were added, gels were pressure-shift-frozen at even 500 MPa.

Thus, the addition of 5% or 10% sucrose prevents damage of frozen egg custard gel. However, the taste of egg custard gel such as chawan-mushi and tamago-tofu with 5% or 10% sucrose (high-sweetness cryoprotectant) was too sweet. Therefore, a 5% low-sweetness cryoprotectant (i.e., glucose (Chapter II), trehalose (Chapter II), sorbitol or maltitol) was used. The sweetness of sucrose: glucose: trehalose: sorbitol: maltitol was 1: 0.6 ~ 0.7: 0.3 ~ 0.4: 0.6 ~ 0.7: 0.8, respectively. The objective of this chapter is to determine the effects of high pressure with the addition of sorbitol or maltitol on the improvement in texture and structure of frozen egg custard gel.

MATERIALS AND METHODS

1. Sample preparation

Egg whites and yolks were separated then remixed in a ratio of 2:1 (w/w), and strained. Fifty percent water and 5% sugar-alcohol (sorbitol or maltitol) of the final weight were added to the egg mixture and stirred. The mixture was preheated for 20 min at 60°C for degassing. The mixture (20 g) was vacuum packed in heat-sealed bags (25 mm in width and 150 mm in length), then heated for 15 min at 85°C in a water bath.

2. Method of freezing at high pressure and measurements of texture and structure

Three packs of egg custard gel with 0% (sugar-free) and 5% sorbitol or maltitol were put into pressure medium (propylene glycol) in a pressure vessel and immediately pressurized for 90 min at -20°C at 0.1 ~ 686 MPa using a Dr. Chef high pressure food processor (Kobe Steel Ltd., Kobe, Japan) (Teramoto *et al*., 2001; 2006; Teramoto & Fuchigami, 2002). After depressurization, gel was stored for 1 day at -30°C, then thawed at 20°C. Changes in the temperature of samples during freezing were determined. Also, texture and structure of high-pressure-frozen gel were compared with non-frozen and frozen (in freezers at -20°C, -30°C or -80°C at atmospheric pressure) gels using a creepmeter (Rheoner, RE-33005, Yamaden Ltd., Tokyo, Japan) and a cryo-scanning electron microscope (S-4500, Hitachi Ltd., Tokyo, Japan), respectively. The size of ice-crystal traces was measured using a Mac-scope (Mitani Ltd., Fukui, Japan).

RESULTS AND DISCUSSION

1. Changes in temperature during freezing

Changes in pressure and temperature of samples during freezing in a pressure vessel were evaluated (Fig. III-1). During pressurization at 100, 500, 600 and 686 MPa, an exothermic peak was detected, so the phase transition of liquid to ice occurred in sugar-free-gel under high pressure. However, the sugar-free and 5% sugar-samples were supercooled at about -20°C during pressurization at 200 ~ 400 MPa and 200 ~ 500 MPa, respectively. When the pressure was released, the temperature rose quickly then decreased to about -20°C. Thus, the supercooled gel froze quickly (data for 200 ~ 400 MPa are not shown, because the same changes in temperature as 500 MPa was observed).

When frozen in freezers, the time duration of the freezing plateau (0°C ~ -5°C) for sugar-free, sorbitol- and maltitol-gels was as follows: at -20°C, 47, 46 and 46 min; at -30°C, 44, 16 and 23 min; at -80°C, 23, 11 and 14 min, respectively. As the freezing temperature was lower and the sugar-alcohol was added, gel froze quickly. However, pressure-shift-frozen gels froze more rapidly than the gels frozen at the other pressures.

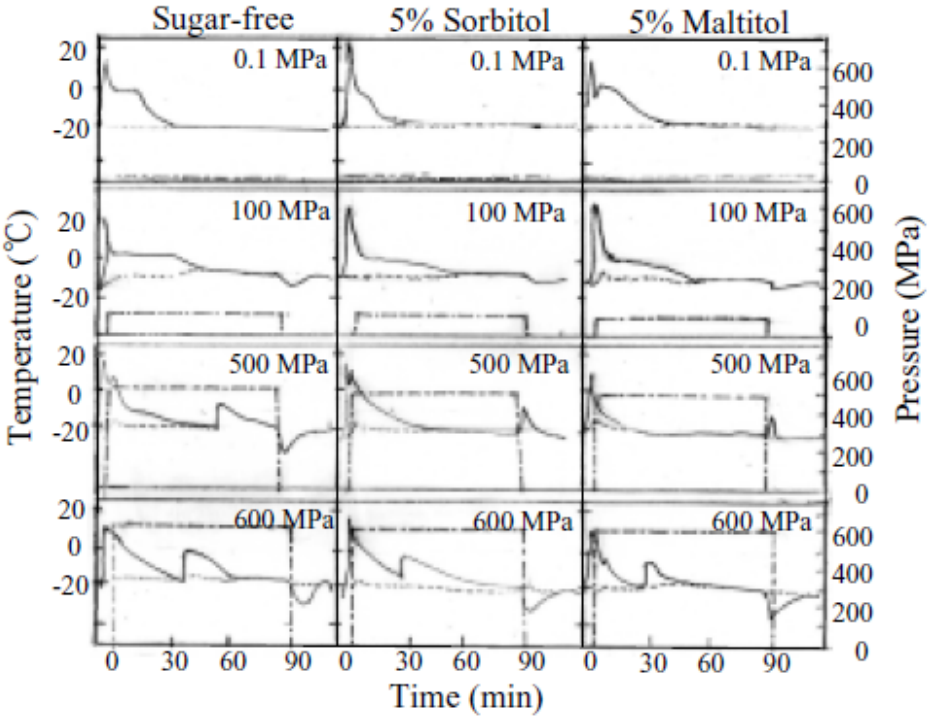


Fig. III-1. Changes in pressure and temperature of egg custard gel and pressure medium during pressurization and depressurization at -20°C

2. Syneresis from frozen-thawed egg custard gel

Syneresis from frozen-thawed egg custard gels was compared (Fig. III-2). The amount of syneresis from gel pressure-shift-frozen at 200 ~ 400 MPa was smaller than that frozen at the other pressures. Therefore, pressure-shift-freezing was effective in reducing the syneresis, but the addition of 5% sugar-alcohol to pressure-shift-frozen gel was not effective in reducing the syneresis. However, when a gel was frozen at 0.1 MPa, the addition of 5% sugar-alcohol appeared to reduce syneresis, although there was no significant difference.

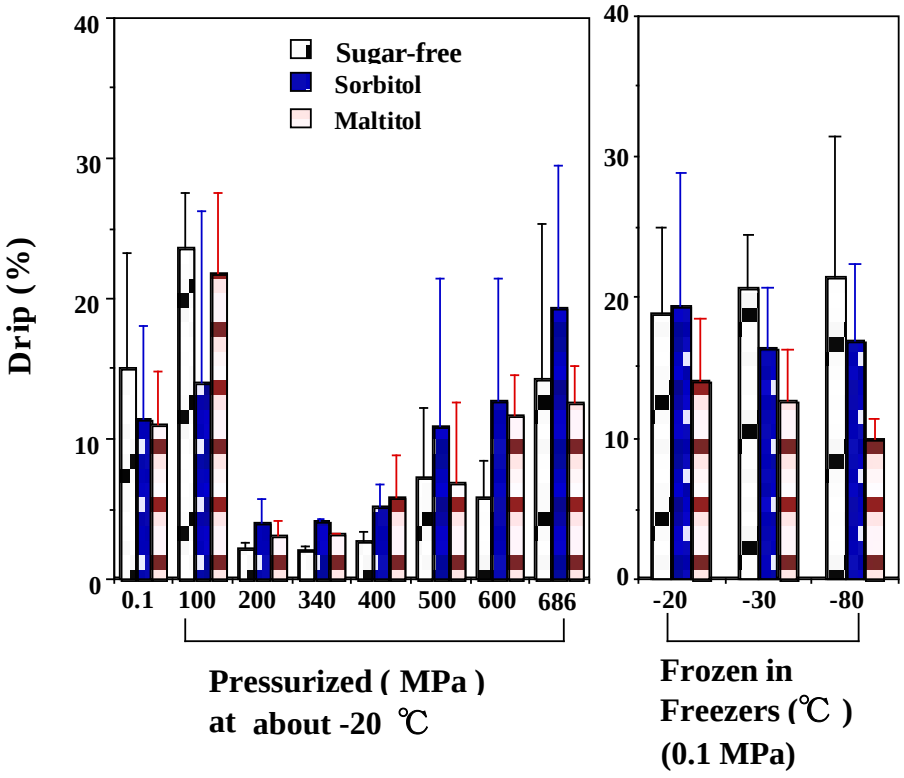


Fig. III-2. The amount of syneresis from frozen egg custard gel

3. Texture of frozen-thawed egg custard gel

The rupture stress and strain of frozen-thawed gels were compared (Fig. III-3). When the gels were frozen in freezers at 0.1 MPa or in a pressure vessel at 0.1, 100, 500, 600, or 686 MPa, the rupture stress and strain of these gels increased significantly. However, that of pressure-shift-frozen gels increased slightly. Furthermore, the changes in the rupture stress and strain of all gels were reduced with the addition of 5% sugar-alcohol; however, there was no significant difference between the two kinds of sugar-alcohol.

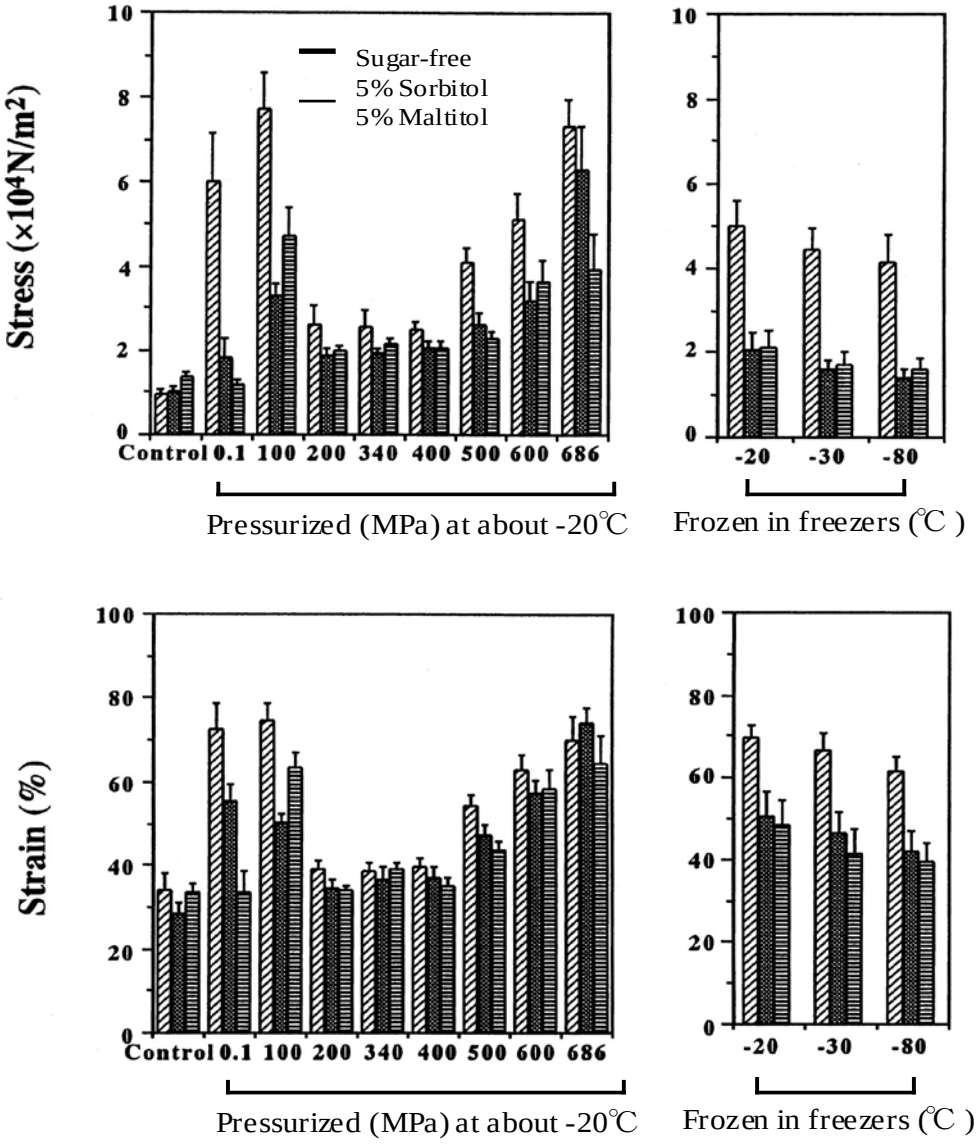


Fig. III-3. Effects of high pressure and sugar-alcohol on rupture stress and strain of frozen-thawed egg custard gel.

4. Structure of frozen-thawed egg custard gel

Cryo-scanning electron micrographs of frozen-thawed egg custard gels (Figs. III-4 ~ 6) and the size of ice crystals (Fig. III-7) were compared. The size of the ice crystals in gel pressure-shift-frozen at 200 ~ 500 MPa was smaller than that frozen at other pressures. A large number of small round ice crystals formed homogenously throughout the pressure-shift-frozen gel. This indicated that ice formation was instantaneous (Fig. III-4). However, when gel was frozen in freezers at 0.1 MPa, ice crystals were bigger in gels frozen at -20°C > -30°C > -80°C (Fig. III-5). Furthermore, these were bigger than in those frozen in a pressure vessel, because the freezing rate of air-cooling (in freezers) was slower than that of immersion in a pressure vessel. Thus, the freezing rate had an important influence on the size of ice crystal. As the temperature of supercooling fell and the freezing time shortened, many small ice crystals were found.

The size of ice crystal traces in gels frozen in freezers decreased with the addition of 5% sugar-alcohol. However, there was no significant difference in the size of ice crystals with the addition of soribitol or maltitol, and also glucose, sucrose and trehalose (Chapter II). The minute structure of the gel frozen in freezers was compared with non-frozen gel (Fig. III-6). The network of non-frozen control gel was coarse, but that of frozen gel was compressed. However, it became coarse with the addition of sugar-alcohol. This indicates that sugar-alcohol appears to protect against the growth of ice crystals and the compression of gel; consequently, changes in the texture of the gel were reduced.

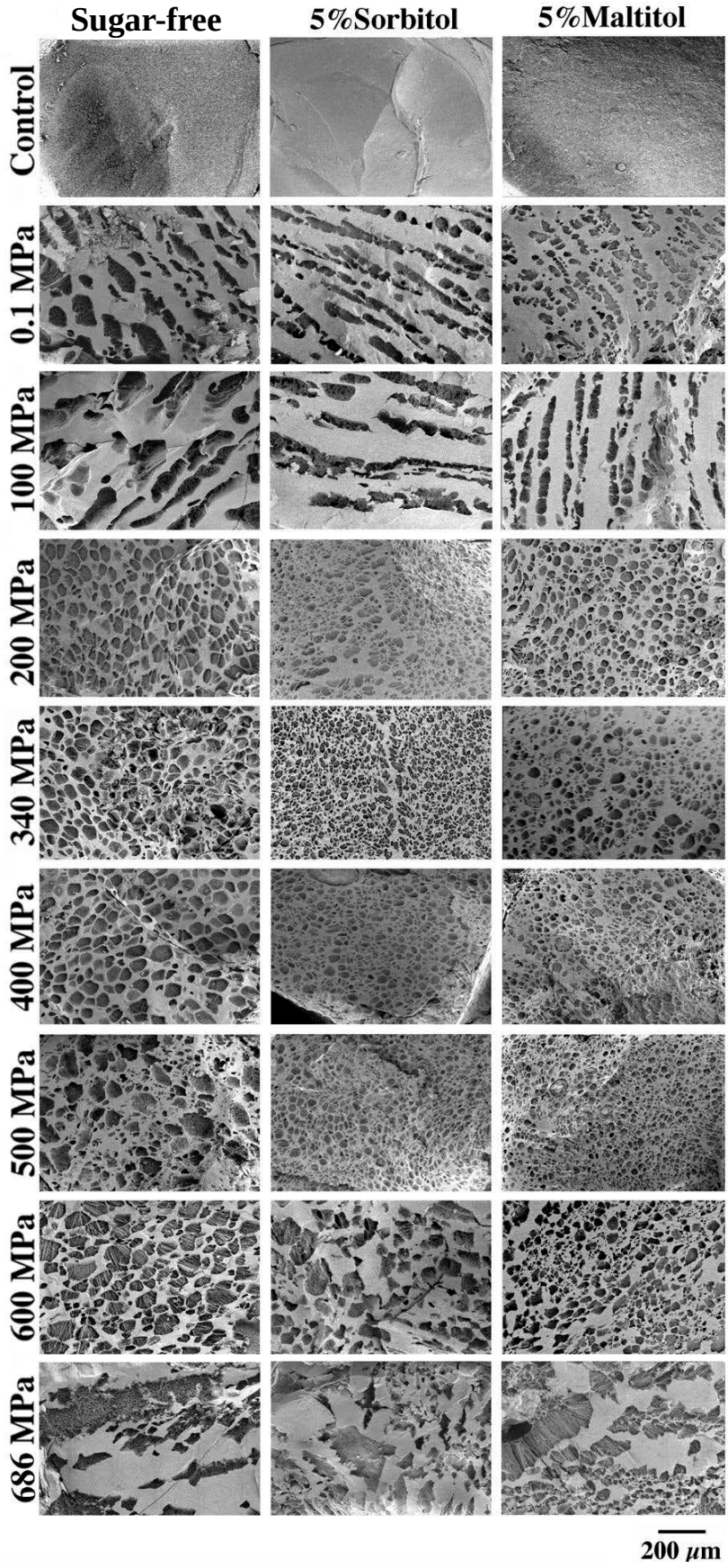


Fig. III-4. Cryo-scanning electron micrographs of high-pressure-frozen gel

Control: non-frozen egg custard gel. Either sorbitol or maltitol (5%) was included.

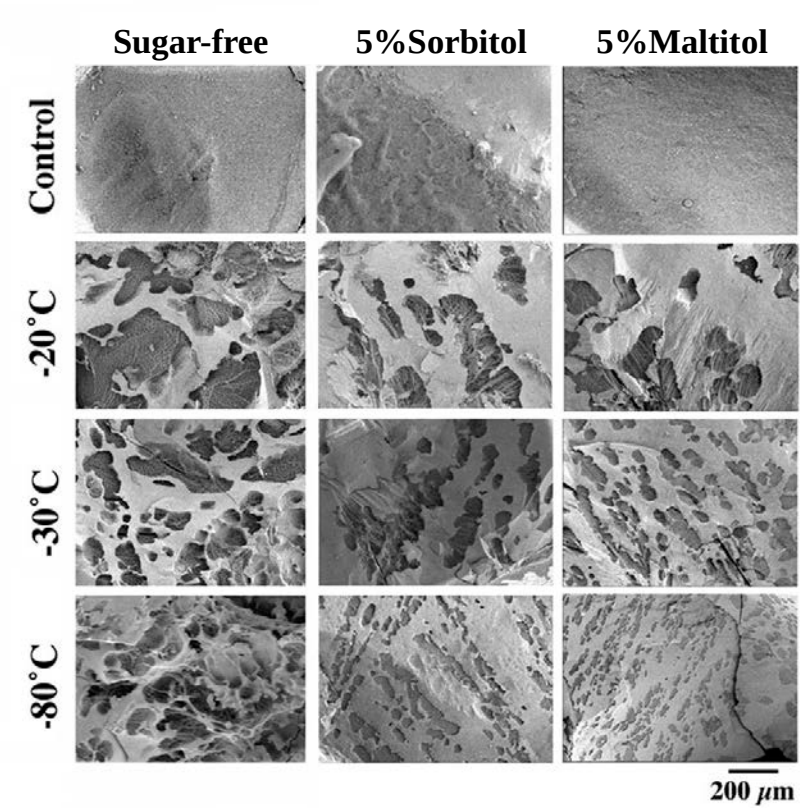


Fig. III-5. Cryo-scanning electron micrographs of egg custard gel frozen in freezers at atmospheric pressure.

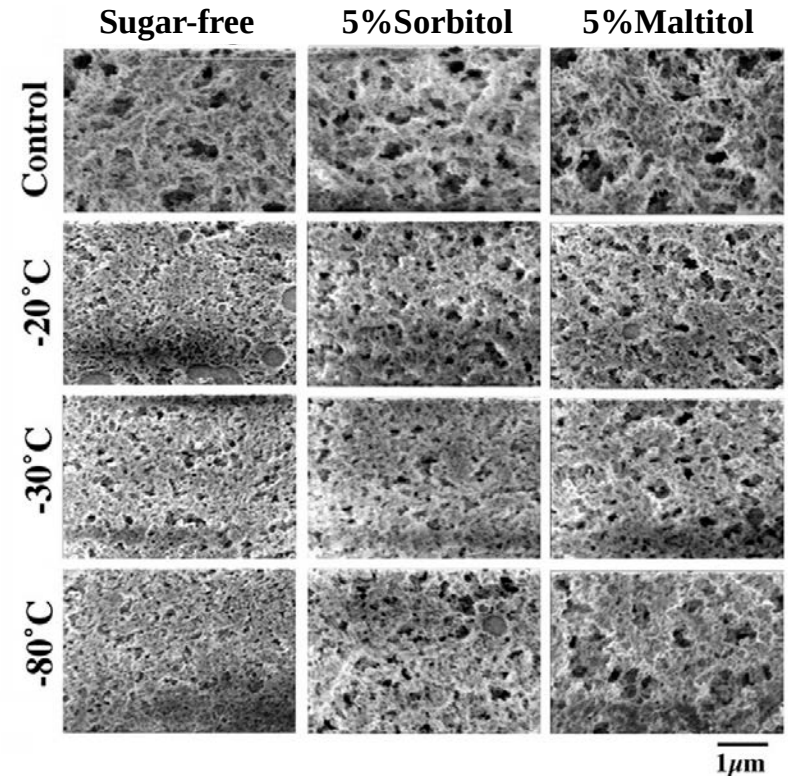


Fig. III-6. Network-structure of egg custard gel frozen in freezers at atmospheric pressure.

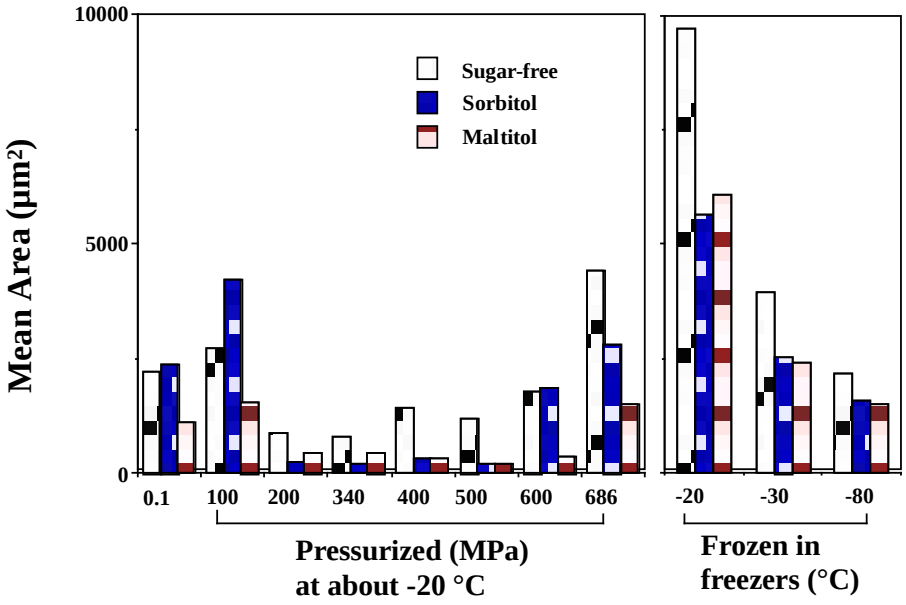


Fig. III-7. The size of ice crystal traces in frozen egg custard gel image-analyzed by a mac-scope

SUMMARY

Smooth taste is a very important quality of egg custard gel. However, egg custard gel frozen at atmospheric pressure (0.1 MPa) is unsuitable for consumption because it is spongy. A non-freezing region (liquid phase) below 0°C exists under high pressure. If high pressure applied to the gel freezing, damage to the gel may be prevented. The objective of this study is to determine the effects of high pressure and the addition of sugar-alcohol on the improvement in texture of frozen egg custard gel. Egg custard gel with 5% sugar-alcohol (sorbitol or maltitol) was frozen at 0.1 ~ 686 MPa and about -20°C. The gels with 0% and 5% sugar-alcohol did not freeze at -20°C during pressurization at 200 ~ 400 MPa and 200 ~ 500 MPa, respectively. When the pressure was released, the supercooled gel froze quickly by pressure-shift-freezing, and small, granular-shaped ice crystals formed. The amount of syneresis from gel pressure-shift-frozen at 200 ~ 400 MPa was smaller than that frozen at the other pressures. Thus, a change in texture (rupture stress and strain) of the pressure-shift-frozen gel was prevented. The addition of 5% sugar-alcohol to the gel was effective in improving the structural and textural quality of the frozen egg custard gel. However, there was no great difference in ice crystal size among the two kinds of sugar-alcohol.

Chapter IV

Rheology of Pressure-shift-frozen Hen Egg Yolk

INTRODUCTION

When raw egg is frozen-thawed, the quality of yolk is worse than albumen. Due to damage of emulsion, frozen yolk without sugar becomes unacceptable. Freezing reduces the concentration of water by forming ice crystals, which leads to partial dehydration of proteins, coupled with a rearrangement of lipoproteins. The dehydrated proteins aggregate and form a three-dimensional linked network with micro structural inhomogeneities (Chang *et al*., 1977; Miyawaki *et al*., 1992). As a consequence of the freeze induced gelation, frozen-thawed egg yolk forms a gel-like, gummy structure, which can be prevented by high dosages (up to 10%) of edible ingredients such as sugar and salt (Jaekel *et al*., 2008).

A non-freezing area (liquid phase) below 0°C exists under high pressure (Hobbs, 1974). When food is pressurized to liquid phase, it does not freeze. However, when pressure is released, it freezes quickly (Fuchigami & Teramoto, 1997c). The objective of this study is to research the effect of pressure-shift-freezing on improving the quality of frozen egg yolk.

MATERIALS AND METHODS

1. Sample preparation

One-day-old egg yolk was filtered using a strainer. 7g of egg yolk with 0%, 5% 10% or 20% sucrose (w/w) was poured into a polypropylene test tube (11mm inside diameter by 100mm high) and sealed with a silicon cap.

2. Method of freezing at high pressure

Three test tubes were pressurized using a Dr. Chef high pressure food processor (Kobe Steel Ltd., Kobe). Propylene glycol (the pressure medium) was first placed in a pressure vessel (6 cm inside diameter by 20 cm high) and kept at -20°C using a cooler, then removed. Next, the thermocouple (k-type) was inserted in the center of the sample in a tube. Then, the pressure medium (-20°C) was added to the pressure vessel. The

sample was immediately pressurized for 60 min at 100 ~ 686 MPa and -20°C. The sample was also pressure-shift-frozen at 100 MPa and -10°C or at 150 MPa and -15°C. After reduction of pressure, the samples were stored for 1 day at -30°C then thawed at 20°C in a low temperature incubator.

2. Texture measurement

Steady-flow viscosity, thixotropy and dynamic-viscoelasticity (G', G'' and dynamic-viscosity) of yolk were measured using a Rheosol-G3000 (UBM Ltd., Kyoto). They were compared with the non-frozen egg yolk and the yolk placed in a pressure vessel at 0.1 MPa and -20°C.

RESULTS AND DISCUSSION

When yolk was either pressurized at 100 MPa and -10°C, or at 150 MPa and -15°C, or pressurized at 200~600 MPa and -20°C, and then released from pressure, the temperature of the samples increased immediately. Therefore, they froze quickly by pressure-shift-freezing.

The steady-flow viscosity, G', G'' and dynamic-viscosity of frozen-thawed yolk increased according to the increase of pressure, especially above 200 MPa. The area of hysteresis-loop of thixotropy also increased above 200 MPa due to pressure-denaturation of protein. However, as the addition of sucrose increased, an increase of viscosity was prevented. Especially, with the addition of 20% sucrose, prevention was effective.

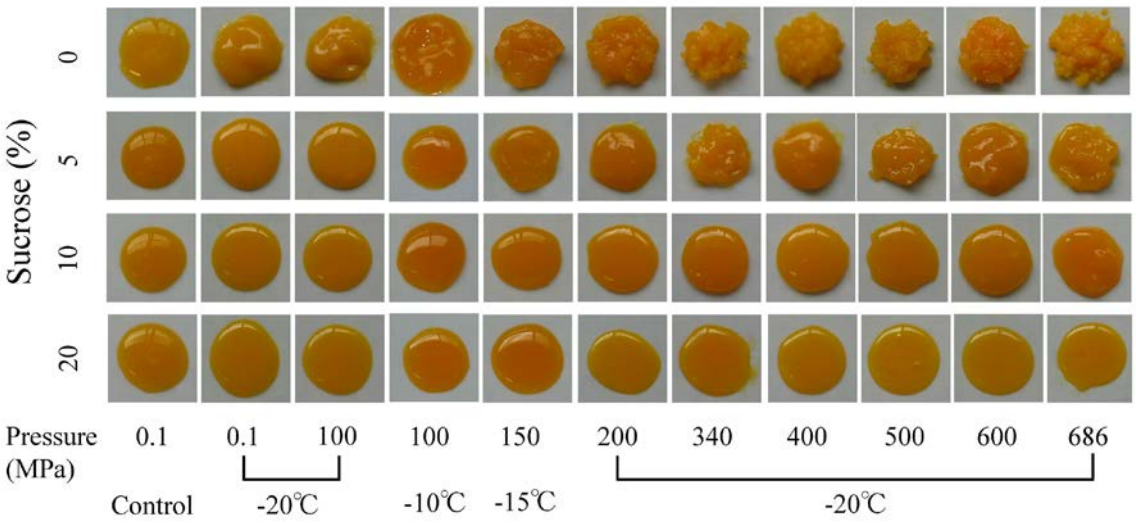


Fig. IV-1. Visual appearance of frozen-thawed egg yolk

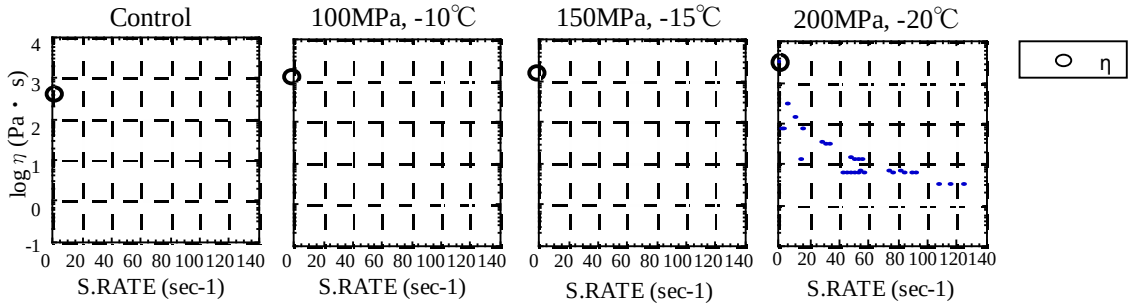


Fig. IV-2. Thixotropy of egg yolk frozen at various pressure and temperature

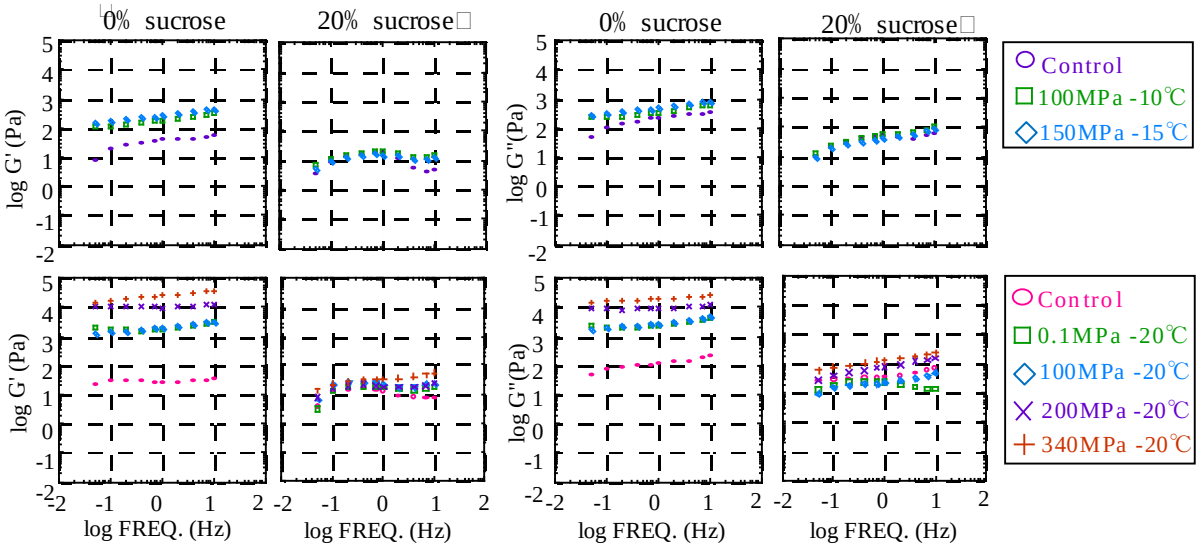


Fig. IV-3. Effect of sucrose on storage modulus and loss modulus of egg yolk frozen at various pressure ant temperature

SUMMARY

The effect of pressure-shift-freezing on improving the quality of frozen egg yolk was investigated. Egg yolk with 0%, 5% 10% or 20% sucrose was pressurized for 60 min at 100 ~ 686 MPa and -20°C. The sample was also pressure-shift-frozen at 100 MPa and -10°C or at 150 MPa and -15°C. The steady-flow viscosity, G', G'' and dynamic-viscosity of frozen-thawed yolk increased according to the increase of pressure, especially above 200 MPa. Pressure-shift-freezing at 100 MPa and -10°C, at 150 MPa and -15°C and the addition of sugar were effective in improving the quality of frozen yolk.

Chapter V

Texture and Structure of Pressure-shift-frozen Agar Gel with High Visco-elasticity

INTRODUCTION

Agar is a colloid extracted from seaweed (*Gelidiaceae* and *Gracilariaceae*), and currently used as a gelling, thicking and stabilizing additive (Armisen 1999). In Japan, many kinds of dessert gel with agar have been used in traditional cooking. Recently produced in Japan are many kinds of agar powder having various textural properties (from soft to firm gel). In this paper, the freezing tolerance of two kinds of agar gel were compared; A gel with high visco-elasticity and B gel (an ordinary dessert gel) discussed in the previous paper (Fuchigami & Teramoto, 2003b). The molecular weight of A is higher, about 2 times more than that of B. Also, the melting temperature of A is higher than that of B, the texture (strength of gel) of A is softer than B, the content of ester sulfate of A is higher than B, and the amount of syneresis of A is smaller than B.

The main structure of agar is chemically characterized by repeating units of D-galactose and 3,6-anhydro-L-galactose with a few variations, and it has a low ester sulphate content. Agar is also a strongly gelling hydrocolloid. Typically, 1% agar is enough to make a rigid gel suitable for most applications. Thus, because of a high water content, damage to structures of agar gel through freezing is extensive. Agar gel, frozen by air blast method at atmospheric pressure, collapses and does not recover its gel phase when thawed, so texture after thawing becomes unacceptable. However, if high pressure is applied to frozen agar gel, the damage of gel may be prevented.

A non-freezing area (liquid phase) below 0°C exists under high pressure (Fletcher, 1970; Hobbs, 1974). In previous studies (Kanda, Aoki, & Kosugi, 1992) when tofu was pressurized at 200 MPa and -18°C, it did not freeze. However, when pressure was released, it froze quickly. This method was designated as “pressure shift-freezing”. Also, in previous studies (Fletcher 1970; Hobbs 1974) it was found that a specific volume of water increases during water-ice I transition, whereas the opposite is observed for “high pressure ices” (II ~ IX). Ice I is less dense than liquid water and floats. The density of high pressure ices is higher.

In fact, the effect of high pressure on the improvement in quality (texture and structure) of frozen food has been discussed previously: tofu (Kanda *et al*., 1992; Fuchigami & Teramoto, 1997c; Fuchigami *et al*., 1998b; 2002; Teramoto & Fuchigami, 1999); carrots (Fuchigami *et al*., 1997a; 1997b); Chinese cabbage (Fuchigami *et al*., 1998a); eggplant (Otero *et al*., 1998); potato (Knorr *et al*., 1998); emulsion (Levy, *et al*., 1999); lobster (Chevalier *et al*., 2000); konnyaku (Teramoto and Fuchigami, 2000); gellan gum gel (Fuchigami & Teramoto, 2003a); and agar gel (Fuchigami & Teramoto, 2003b). The results of these studies were as follows: pressure-shift-freezing at 200 ~ 400 MPa and -18°C ~ -20°C appeared to be effective in improving both the texture and/or histological structure of frozen food except for konnyaku (Teramoto and Fuchigami, 2000). When pressure-shift-frozen, nuclei formed during pressure release, and phase transition from liquid-to-ice I occurred quickly. Therefore, small ice crystals formed. Leading to a beneficial effect on texture. However, the effect of high pressure on improving the quality of frozen food appeared to be related to the type of food.

In a previous paper (Fuchigami & Teramoto, 2003b), changes in temperature and structure of agar gel, as affected by sucrose during high-pressure freezing, was investigated. Agar gel with 0, 5, 10 or 20% sucrose were pressurized at 0.1 ~ 686 MPa and -20°C. Exothermic peaks were detected at 0.1, 100, 500 ~ 686 MPa (freezing). However, at 200 ~ 400 MPa, gel did not freeze but froze during pressure release. Thus, structure of gel frozen at 200 ~ 400 MPa was better than other samples due to quick freezing. The phase transition from high-pressure-ices to ice I at -20°C might have promoted the growth of ice crystals. With the addition of sucrose, the initial freezing temperature decreased and structural quality improved. However, changes in texture and syneresis during freezing-thawing were not discussed.

Therefore, the first objective was to determine the difference of texture between A gel and B gel during high pressure-freezing and conventional freezing at atmospheric pressure. The second objective was to determine the amount of syneresis from frozen-thawed gels. Finally, the third objective was to determine what affect addition of sucrose might have on the structure of A.

In this report, the freezing method of 100 ~ 686 MPa at -20°C is referred to as high-pressure-freezing, although gel was not frozen at all these pressure levels. Also, the freezing method is designated as pressure-shift-freezing when samples are cooled under pressure to –20˚C without ice formation then frozen when pressure is released.

MATERIALS AND METHODS

1. Sample preparation

Two kinds of agar powder, Yamato and Kanten Cook, (Ina Food Co., Ina, Nagano, Japan) were used. Yamato-gel has a high visco-elasticity, while Kanten Cook is ordinary agar used for dessert gel. In this paper, Yamato and Kanten Cook are designated A and B, respectively. Samples were prepared as described previously (Fuchigami & Teramoto, 2003b). Agar powder was mixed with distilled and deionized water (1.5% w/w) for 10 min and heated to melting then boiled for 2 min to ensure the complete hydration of agar. Then, 0%, 5%, 10% or 20% (w/w) sucrose (saccharose, extra-pure reagent, Ishizu Seiyaku Ltd., Osaka) was added, and the mixture was heated for 2 min. After deairing for about 2 min using a vacuum pump, weight was adjusted using hot water, then the mixture was poured into plastic trays (gel thickness: 10 mm) and stored in a 5°C refrigerator over night (about 20 h) to achieve complete stabilization. The gel samples were then cut into cylinders (15 mm in diameter and 10 mm height). Seven pieces of agar gel were vacuum packed in heat-sealed polyethylene bags. Four packs of samples with various concentrations of sucrose were frozen at the same time. These agar gel samples were designated as 0%, 5%, 10% or 20% sucrose-gel.

2. Method of freezing at high pressure

High hydrostatic pressure treatments were carried out using a high pressure food processor (Dr. Chef, Kobe Steel Ltd., Kobe) as described previously (Kato *et al*., 1997; Fuchigami & Teramoto, 2003b). Propylene glycol, the pressure medium, was first placed in a pressure vessel (6 cm inside diameter and 20 cm high, surrounded with a jacket) and kept at -20°C by a cooler (-35°C ~ 10°C) then removed. Next, samples were placed in a pressure vessel, and the thermocouple (k-type) was inserted in the center of the gel sample. The pressure medium (-20°C) was then added to the pressure vessel. Samples were immediately pressurized at 100 ~ 686 MPa. With the addition of the pressure medium, it took within 2 min to reach the defined pressure. The operation was fully automated and both pressure and temperature of the sample (upper section of the pressure vessel) and pressure medium (lower section) were recorded in intervals of 5 sec using Thermodac E/Ef (Eto-denki Ltd., Tokyo).

After pressurization at 100, 200, 600 or 686 MPa and -20°C for 63 min, pressure was released and gel samples were left for about 20 min in a pressure vessel to ensure complete freezing. They were immediately stored for 1 day in a freezer (-30°C) at

atmospheric pressure then thawed at 20°C in a low temperature incubator. This experiment was repeated 5 times.

3. Method of freezing at atmospheric pressure

The samples were also frozen at atmospheric pressure (0.1 MPa) in a pressure vessel at -20°C or in freezers (-20°C, -30°C or -80°C). The temperature of the sample was measured at intervals of 30 sec using a data collector (AM-7002 K, Anritsu-keiki Ltd., Osaka). This experiment was repeated 5 times.

4. Texture measurement

After the amount of syneresis was measured, texture of gel samples was measured by a creepmeter (Rheoner, RE 33005, Yamaden Ltd., Tokyo). Thickness of samples was measured using a sample-height counter (HC-3305, Yamaden Ltd., Tokyo) then punctured by using a plunger (cylindrical shape: 3 mm dia, 22 mm long) at 1 mm/sec, stopping at 99% of the thickness using a loadcell of 200 g. Rupture stress, rupture strain and rupture energy were indicated.

5. Structure measurement

Structure of the gel samples was observed with a cryo-scanning electron microscope (S-4500, Hitachi Ltd., Tokyo) (Fuchigami, Hyakumoto & Miyazaki, 1995). Gel samples were cut into 6 mm × 1 mm × 1 mm, and dehydrated with 20%, 40% and 50% ethanol. The specimen was contained in a metal holder and quickly frozen by immersing in LN_2, transferred to the cold stage of a cryo-system for scanning electron microscopy and cut with a knife (-150°C). After etching at -85°C, the surface was coated with gold then observed at -120°C under low acceleration voltage (1kV). The magnifications used to observe ice crystals and gel networks were ×100 and ×10,000, respectively.

RESULTS AND DISCUSSION

1. Changes in temperature of samples during freezing

Change in pressure and temperature for A gels during pressurization and depressurization at -20°C was shown in Fig. V-1. Because change in the temperature of A and B gels was almost the same, only temperature of A gels is shown. Within 1 min, the defined pressure was reached and maintained for 63 min, then immediately (about 20 sec) reduced to atmospheric pressure.

When the gel was pressurized at 200 MPa and -20°C, all gel samples were cooled to about -20°C; an exothermic peak was not detected during pressurization. However, when depressurized, the temperature of the gel decreased due to heat absorbed by decompression, then rose quickly and decreased to -20°C. Thus, the gel froze partially when pressure was released. This indicates that the gel must have frozen through pressure-shift-freezing (Kanda *et al*., 1992).

For gels with 0%, 5%, and 10% sucrose frozen at 100 or 600 MPa and -20°C, and gels with 0 ~ 20% sucrose frozen at 686 MPa and -20°C, exothermic peaks were detected during pressurization and endothermic peaks were detected during depressurization. Therefore, these gel samples froze during pressurization, and ice I, ice V or ice VI formed, respectively. While the gels with 20% sucrose were pressurized at 100 or 600 MPa and -20°C were pressure-shift-frozen.

When 0% sucrose-gel was frozen at atmospheric pressure in freezers, freezing time (freezing plateau at 0°C) was 9.5 min at -80°C, 32 min at -30°C and 37 min at -20°C. Freezing time for the immersion method in a pressure vessel (-20°C) was 11.6 min and was shorter than for the air blast method in a freezer (-20°C). As the concentration of sucrose in gel increased, the initial freezing temperature/temperature of supercooling decreased and freezing time became shorter.

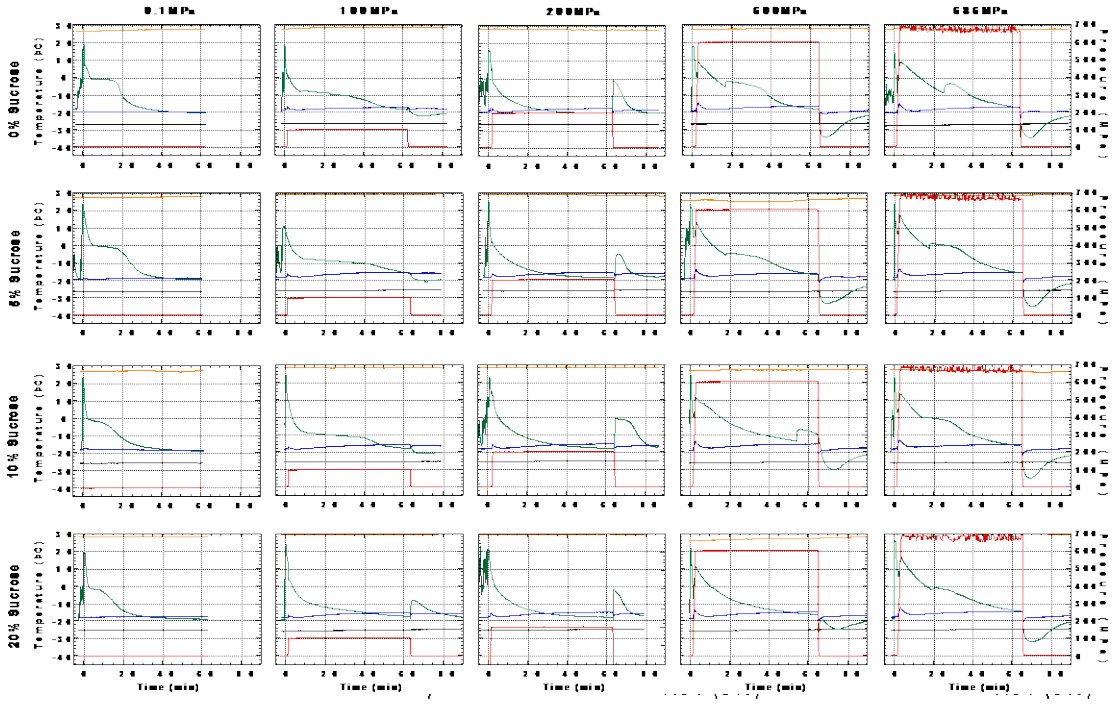


Fig. V-1. Changes in pressure and temperatures of A gels and pressure medium during pressurization and depressurization at -20°C

2. Visual appearance of frozen-thawed agar gels

Visual appearance of frozen-thawed A (Fig. V-2) was compared. The appearance of 0% sucrose-gel pressure-shift-frozen at 200 MPa was same as control. However, that of frozen at 0.1, 100, 600 and 686 MPa differed greatly from A control due to syneresis and a volumetric shrinkage of the gel. As the concentration of sucrose in the gel increased, the appearances of gels improved. In fact, all 20% sucrose-gels frozen at 0.1 ~ 686 MPa, or the 20% sucrose-gels frozen in freezers were the same as the original gels with 20% sucrose. Visual appearance of frozen-thawed B (data not shown) was the same as A.

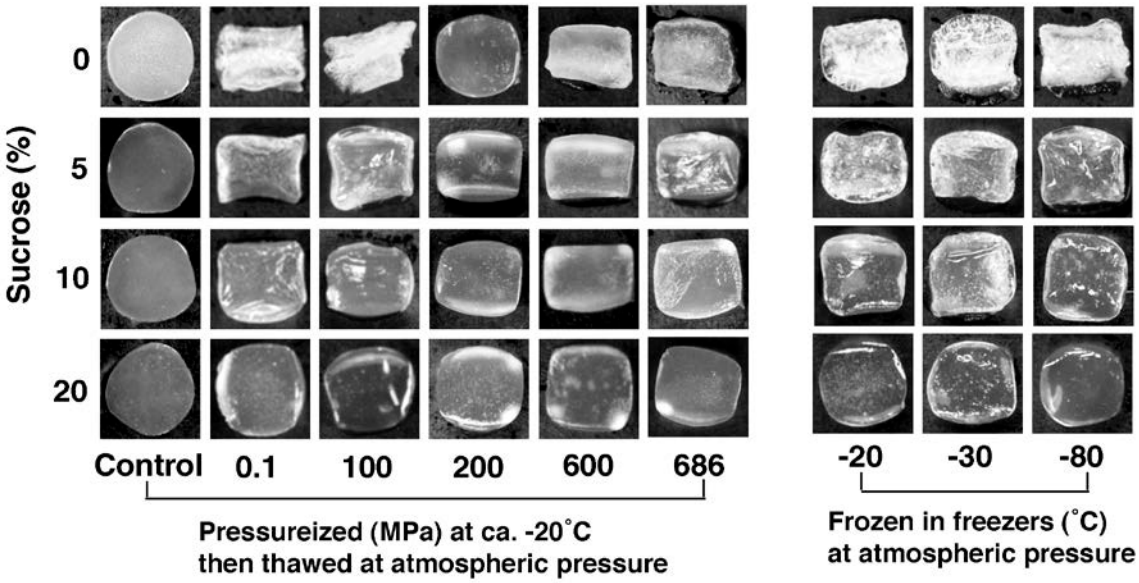


Fig. V-2. Visual appearance of frozen-thawed agar gels with high visco-elasticity (A)

3. Syneresis from frozen-thawed agar gels

The amount of syneresis from frozen-thawed A and B gels is shown in Fig. V-3. The amount of syneresis from 0%, 5% and 10% sucrose-gel pressure-shift-frozen at 200 MPa was smaller than that from gel frozen at other pressures significantly. The amount of syneresis from pressure-shift-frozen (at 200 MPa) A and B gels without sucrose was about 20% and 40%, respectively. Also, syneresis from 0% sucrose A gels frozen at other pressures was smaller than that from B gels although there is no significant difference. As the concentration of sucrose in the gel increased, the amount of syneresis extremely decreased. Thus, the addition of sucrose to gel appeared to be effective in preventing syneresis.

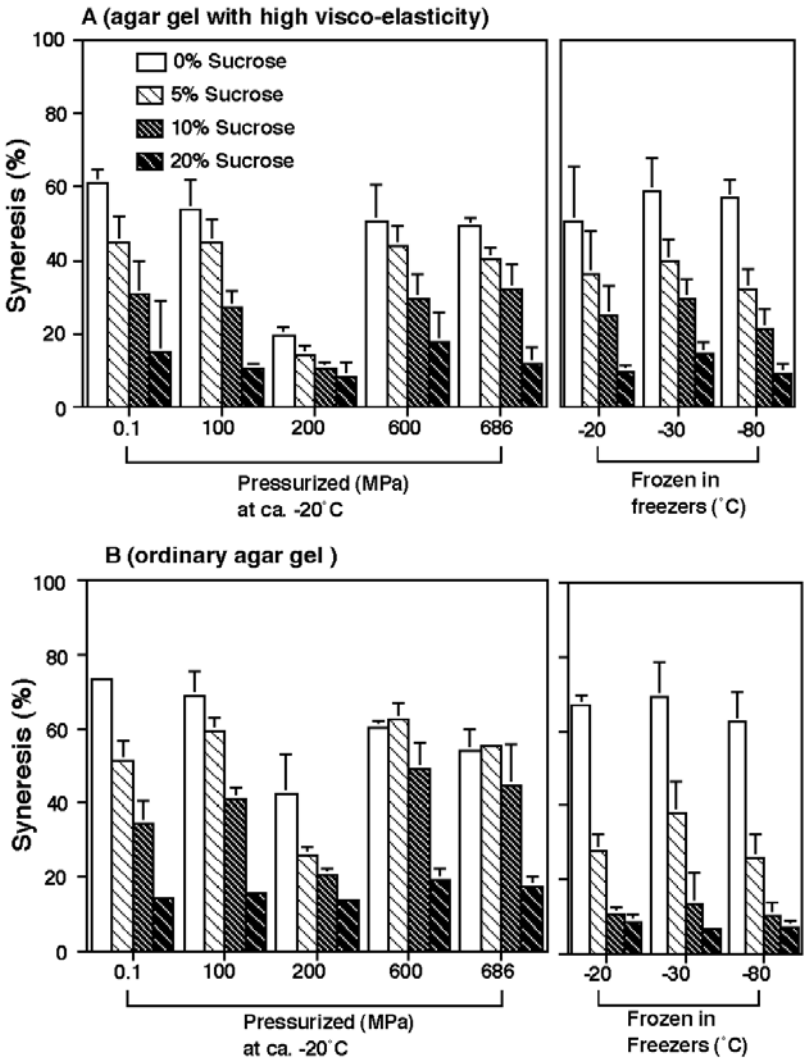


Fig. V-3. Amount of syneresis from frozen-thawed agar gels

4. Texture of frozen-thawed agar gels

Typical stress-strain curves of frozen-thawed agar gel (A and B) are compared (Fig. V-4). Only 3 curves (control gel and gel frozen at 0.1 and 200 MPa) were shown because Fig. V-4 would have became too complex, and the curves of gel frozen at 100, 600 and 686 MPa were similar to gel frozen at 0.1 MPa. The average values of rupture stress, rupture strain and rupture energy of all gels (A and B) were compared in Tables V-1 ~ 3, respectively.

The rupture stress of A control was smaller (about $90\times10^3 N/m^2$) than that of B control (about $200\times10^3 N/m^2$). Therefore, rupture energy of A control was smaller (about $114\times10^2 J/m^3$) than that of B control (about $237\times10^2 J/m^3$). The control gel A and B, when punctured to about 30% and 28% of its thickness, respectively, became broken.

Rupture strain of A control was a little greater than that of B control. This indicated that the control gel B was slightly brittle. However, there was no notable difference in rupture strain between A and B gels due to size of sample used in this experiment. In data using larger samples (60 mm in diameter and 65 mm high) the difference in rupture strain (A>B) became clear. Stress and energy increased slightly as the amount of sucrose increased, but the concentration of sucrose did not affect the texture of the unfrozen control greatly.

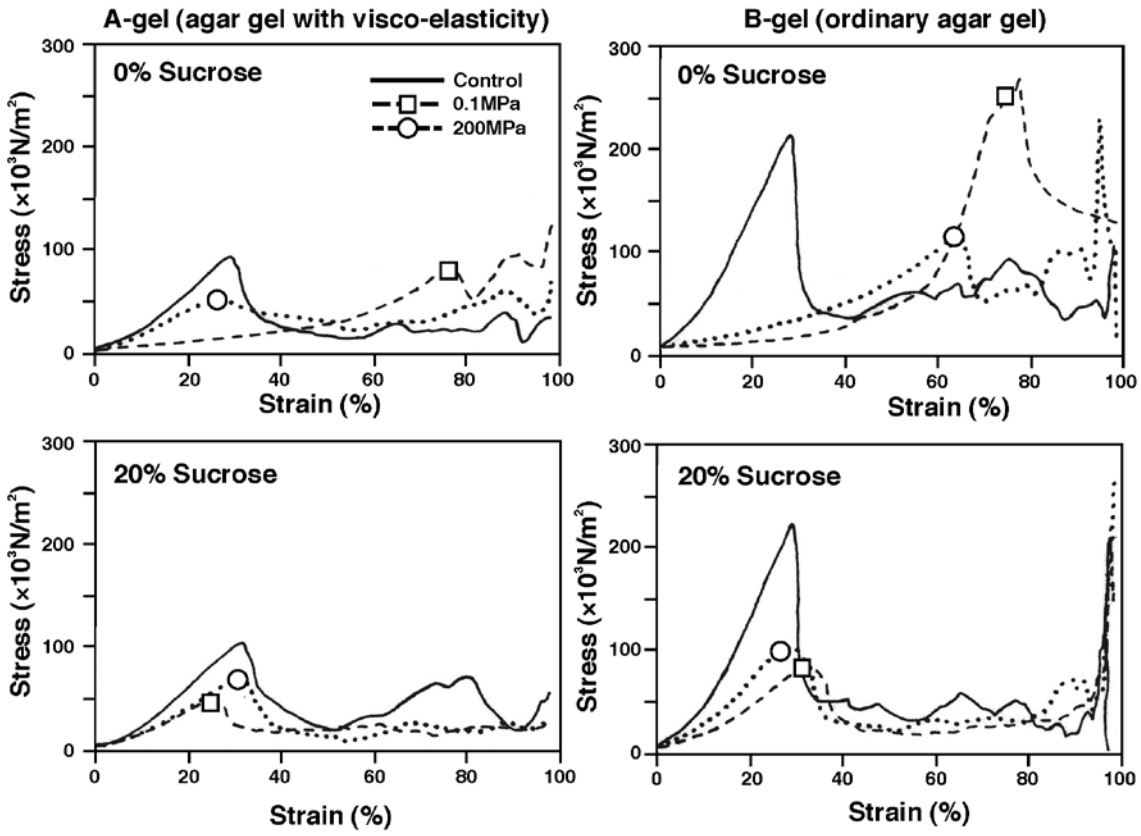


Fig. V-4. Typical stress-strain curves of agar gels
Control (unfrozen),
(□) frozen in a pressure vessel at 0.1 MPa and -20°C, and
(○) pressure-shift-frozen at 200 MPa and -20°C.

Pressurization at 20°C did not affect the texture of gels (data not shown). On the other hand, the texture of all frozen-thawed gels differed greatly from control gels. The rupture stress of frozen-thawed gels decreased, while the rupture strain increased. When high-pressure-frozen agar gels A and B were thawed, stress-strain curves differed greatly from the control gel (Fig. V-4). The initial curve angles of 0% sucrose-gel

decreased sharply when frozen. When compressed to about 30% of thickness, a great difference in stress among these groups occurred. The control gel was firmest, followed by gel pressure-shift-frozen at 200 MPa > 0.1MPa, respectively. However, the final rupture stress of gel frozen at 0.1MPa was closer the control gel than to the gel which was frozen at 200 MPa. It was also interesting to note that the pattern of stress-strain curves differed greatly; the curve of gel frozen at 200 MPa was of an intermediate pattern, between the control gel and gels frozen at 0.1, 100, 600 and 686 MPa. After thawing, rupture strain of 0% sucrose A and B gel increased from 30% (control) to 60 ~ 80% (frozen samples), except pressure-shift frozen A gel. The rupture strain of 0% sucrose A gel pressure-shift frozen at 200 MPa was the same (about 30%) as that of control after thawing. Thus, freezing tolerance of A was better than B. As the concentration of sucrose increased, the strain of both gels improved gradually. The strain of all 20% sucrose gels became the same as that of the control gel. However, the stress of 20% sucrose-gel was less than the control significantly. Thus, it appeared rupture strain was more suitable in the evaluation of quality of frozen-thawed agar gel than rupture stress. Also, texture quality of gel samples frozen at 200 MPa was greater than that of those frozen at 0.1, 100, 600 and 686 MPa.

The texture of A and B gels, pressure-shift-frozen at 200 MPa, was also better than gels frozen in freezers of -20, -30 or -80°C at atmospheric pressure due to quick freezing. As the concentration of sucrose in gels frozen in freezers increased, the rupture strain approached the value of the control gels, but only a small improvement in rupture stress was observed.

Table V-1. Average of rupture stress ($\times 10^3 N/m^2$) of frozen-thawed agar gels (A and B)

	Temperature (℃)	Pressure (MPa)	Agar gel with visco-elasticity (A) Sucrose 0%	5%	10%	20%	Ordinary agar gel (B) Sucrose 0%	5%	10%	20%
	Control		90 ± 10	90 ± 8	91 ± 12	99 ± 14	195 ± 9	206 ± 7	210 ± 10	217 ± 11
Under pressure	-20℃	0.1	81 ± 21	23 ± 9	24 ± 6	42 ± 9	254 ± 11	135 ± 26	62 ± 7	87 ± 4
	-20℃	100	54 ± 20	30 ± 9	31 ± 5	54 ± 6	183 ± 27	70 ± 17	52 ± 7	73 ± 4
	-20℃	200	51 ± 18	37 ± 6	43 ± 6	66 ± 10	118 ± 16	57 ± 16	60 ± 5	103 ± 8
	-20℃	600	84 ± 31	45 ± 10	36 ± 10	51 ± 14	253 ± 12	149 ± 24	125 ± 8	84 ± 6
	-20℃	686	41 ± 11	33 ± 8	31 ± 6	43 ± 4	182 ± 31	130 ± 27	132 ± 9	82 ± 14
In freezers	-20℃	0.1	79 ± 46	33 ± 11	25 ± 6	36 ± 6	113 ± 69	59 ± 22	52 ± 28	69 ± 7
	-30℃	0.1	86 ± 27	28 ± 9	32 ± 8	43 ± 5	76 ± 29	47 ± 12	49 ± 7	53 ± 25
	-80℃	0.1	94 ± 20	23 ± 8	26 ± 7	40 ± 10	93 ± 27	34 ± 16	35 ± 10	76 ± 4

Table V-2. Average of rupture strain (%) of frozen-thawed agar gels (A and B)

	Temperature (℃)	Pressure (MPa)	Aar gel with visco-elasticity (A) Sucrose 0%	5%	10%	20%	Ordinary agar gel (B) Sucrose 0%	5%	10%	20%
	Control		30 ± 2	30 ± 2	31 ± 2	32 ± 3	28 ± 0	28 ± 0	27 ± 1	27 ± 1
Under pressure	-20℃	0.1	78 ± 5	54 ± 13	43 ± 9	29 ± 7	78 ± 4	74 ± 15	58 ± 8	31 ± 6
	-20℃	100	60 ± 13	52 ± 10	34 ± 5	30 ± 2	56 ± 17	58 ± 11	36 ± 3	27 ± 1
	-20℃	200	30 ± 7	23 ± 2	27 ± 2	32 ± 2	57 ± 7	31 ± 3	28 ± 2	28 ± 2
	-20℃	600	70 ± 7	51 ± 6	39 ± 5	33 ± 4	69 ± 16	66 ± 4	56 ± 3	36 ± 1
	-20℃	686	53 ± 9	44 ± 12	34 ± 8	29 ± 2	76 ± 12	71 ± 5	54 ± 8	31 ± 2
In freezers	-20℃	0.1	62 ± 15	49 ± 13	36 ± 6	28 ± 3	61 ± 15	58 ± 15	31 ± 4	34 ± 3
	-30℃	0.1	72 ± 8	51 ± 9	42 ± 7	29 ± 3	60 ± 19	51 ± 12	34 ± 2	26 ± 10
	-80℃	0.1	75 ± 7	44 ± 8	33 ± 4	26 ± 2	65 ± 17	47 ± 18	33 ± 10	34 ± 2

Table V-3. Average of rupture energy ($\times 10^2 J/m^3$) of frozen-thawed agar gels (A and B)

	Temperature (℃)	Pressure (MPa)	Aar gel with visco-elasticity (A) Sucrose 0%	5%	10%	20%	Ordinary agar gel (B) Sucrose 0%	5%	10%	20%
	Control		114 ± 19	117 ± 16	118 ± 23	133 ± 28	237 ± 6	243 ± 7	253 ± 9	254 ± 25
Under pressure	-20℃	0.1	207 ± 48	64 ± 32	54 ± 20	57 ± 11	220 ± 54	136 ± 53	100 ± 24	69 ± 27
	-20℃	100	135 ± 54	81 ± 33	55 ± 13	72 ± 8	155 ± 90	99 ± 30	65 ± 14	61 ± 7
	-20℃	200	74 ± 33	45 ± 8	53 ± 8	91 ± 19	156 ± 26	59 ± 13	56 ± 8	85 ± 12
	-20℃	600	224 ± 70	97 ± 24	70 ± 24	74 ± 20	172 ± 70	182 ± 35	132 ± 15	91 ± 8
	-20℃	686	106 ± 24	72 ± 32	55 ± 21	58 ± 9	191 ± 44	203 ± 39	143 ± 29	85 ± 53
In freezers	-20℃	0.1	179 ± 107	76 ± 32	47 ± 15	50 ± 9	220 ± 138	169 ± 67	85 ± 22	122 ± 20
	-30℃	0.1	197 ± 55	74 ± 28	69 ± 23	60 ± 8	179 ± 83	111 ± 45	90 ± 15	87 ± 42
	-80℃	0.1	220 ± 38	54 ± 20	45 ± 13	51 ± 15	281 ± 113	100 ± 54	76 ± 14	119 ± 7

5. Structure of frozen-thawed agar gels

Cryo-scanning electron micrographs of high-pressure-frozen A are compared (Fig. V-5). When 0% sucrose-gel was thawed, a small amount of large ice crystal traces in gels frozen at 0.1 and 100 MPa was observed. Conversely, large amounts of small ice crystals formed in the gel which was pressure-shift-frozen at 200 MPa, and size of ice crystals began to increase above 600 MPa. The ice crystals in 0% sucrose-gel pressure-shift-frozen at 200 MPa were smaller than in 0% sucrose gels frozen at 0.1, 100, 600 and 686 MPa. With an increase of sucrose, the size of ice crystals in these gel samples decreased. These results indicated that as the initial freezing temperature became lower, the ice-nuclei numbers increased and growth of ice crystals was

prevented; consequently, ice crystals became smaller. Also, the textural quality (rupture strain) of the gel was good. It appeared that the size of ice crystals affected the amount of syneresis and rupture strain of the thawed gel. Because ice crystals were larger, syneresis promoted shrinkage of the gel and rupture strain increased. As the concentration of sucrose increased, the amount of water in the gel decreased and also freezing temperatures decreased. As a consequence of this, freezing time was shorted. Thus, size of ice crystals became smaller and texture improved. The same results were observed in B (Fig. V-6); however, the size of ice crystals in B was somewhat bigger than that of A. Therefore, textural quality of A gel was somewhat better than that of B.

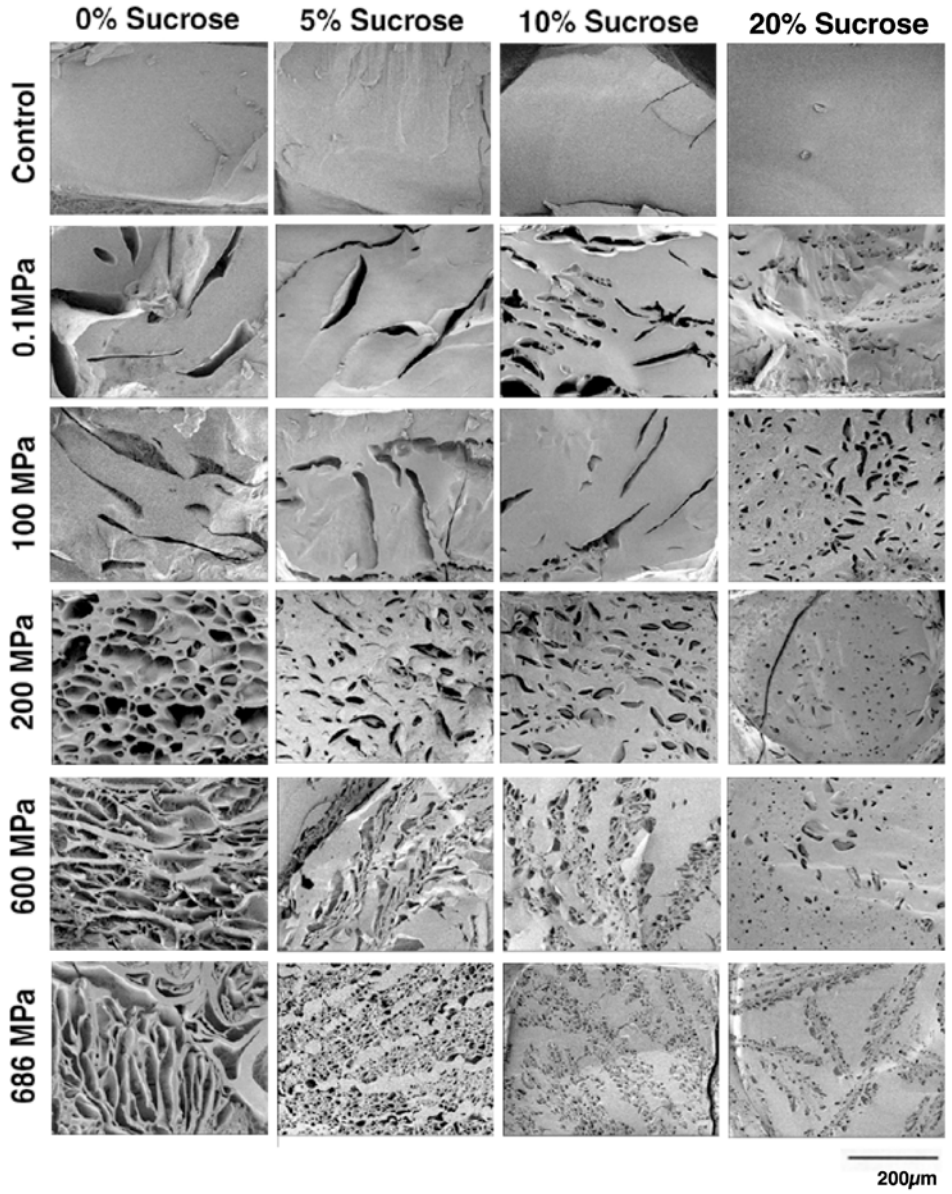


Fig. V-5. Cryo-scanning electron micrographs of high-pressure-frozen agar gels with high visco-elasticity (A)

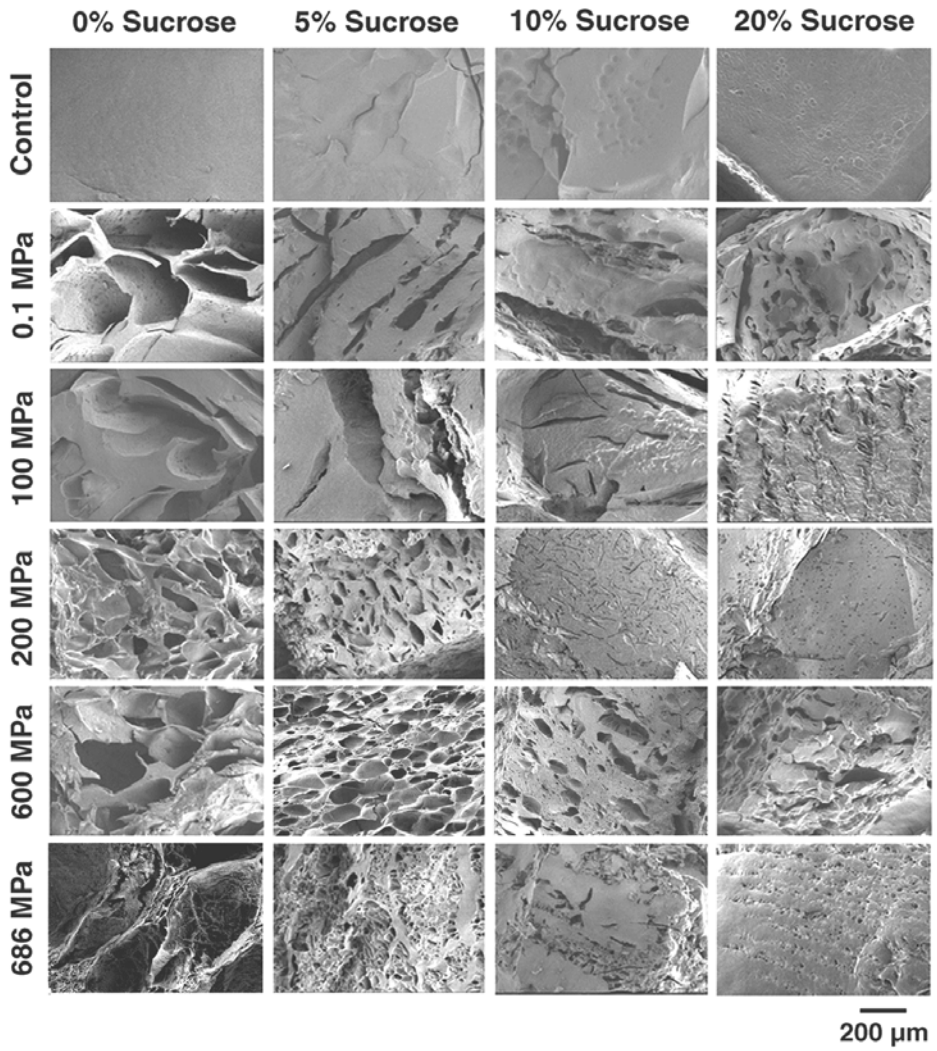


Fig. V-6. Cryo-scanning electron micrographs of high-pressure-frozen agar gels (B)

The network of high-pressure frozen A gels is compared (Fig. V-7). The gel network of A control was coarse. The amount of sucrose did not affect the mesh of the gel network (coarse or fine). When 0% sucrose-gel samples were frozen then thawed, the gel network contracted. However, with an increase in sucrose above 5%, the structure of A improved, but there was no notable change in the gel network above 5% sucrose; the same comparatively coarse network as the control gel was maintained. The network of high-pressure frozen 0% sucrose B gel (Fig. V-8) was more contracted and size of ice crystals was larger than that of A gel. These results indicate that the growth of ice crystals in 0% sucrose-gel may adhere closely to the gel network. With the addition of sucrose, the gel network in frozen-thawed A and B became coarse and thus maintained conformation of the network.

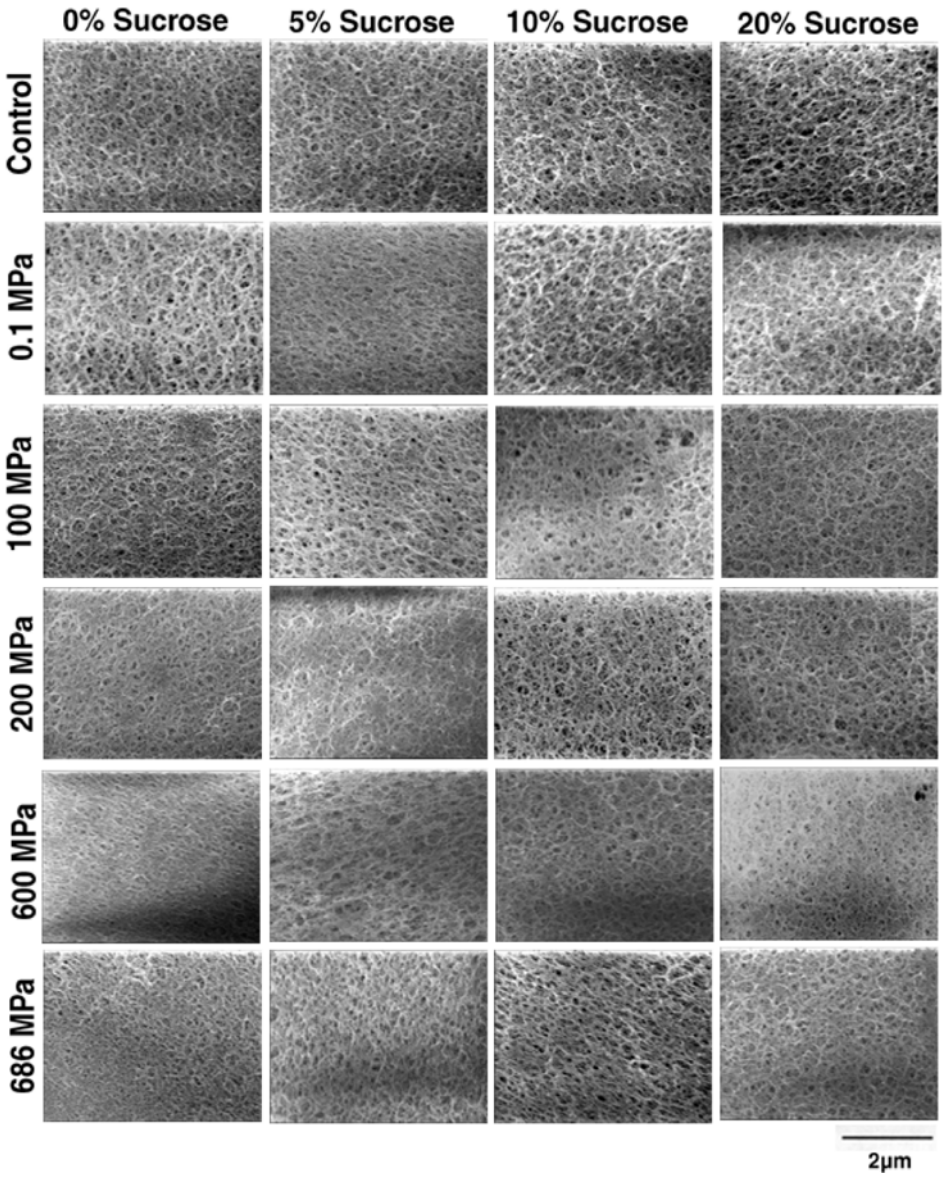


Fig. V-7. Network-structure of high-pressure-frozen agar gels with high visco-elasticity (A)

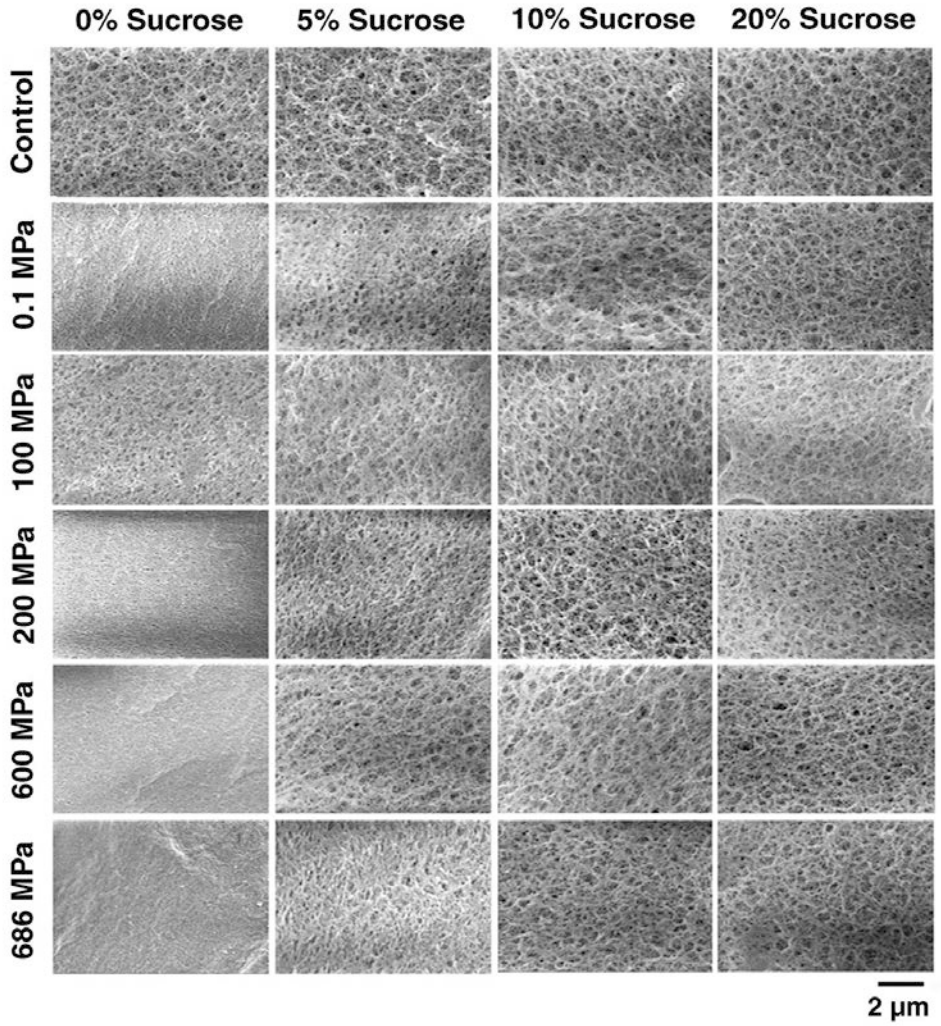


Fig. V-8. Network-structure of high-pressure-frozen agar gels (B)

Cryo-scanning electron micrographs taken of A and B frozen in freezers at atmospheric pressure are compared (Fig. V-9). The size of ice crystals of B was larger than A. As the concentration of sucrose increased, size of ice crystals became smaller. Thus, the addition of 5% sucrose notably improved the structure of both A and B gel samples.

When agar gel was frozen at 0.1 MPa (in a pressure vessel or freezers) or 100 MPa and -20°C or pressure-shift-frozen at 200 MPa, ice I formed in all gels. However, size of ice crystals differed greatly. The initial freezing temperatures and also freezing time appeared to affect the size of ice I; consequently, pressure-shift-freezing appeared to be more effective.

Finally, the addition of sucrose decreased supercooling and freezing temperatures; therefore, texture and structure of frozen agar gel with sucrose became comparatively good. Previously, when tofu was frozen, rupture stress increased due to physical

compression caused by the growth of ice crystals and the increase of chemical interactions caused by concentration during freezing of protein and salts (Fuchigami and Teramoto, 1997c). Conversely, rupture stress of agar gel decreased due to the structural damage caused by ice crystals.

Probably, because agar A has more sulfate than agar B, the amount of syneresis from A gels was less than B gels and freeze-thaw tolerance of A gels was better than B gels (Thomas, 1999). Therefore, the A gels were of better quality (texture and structure) than B.

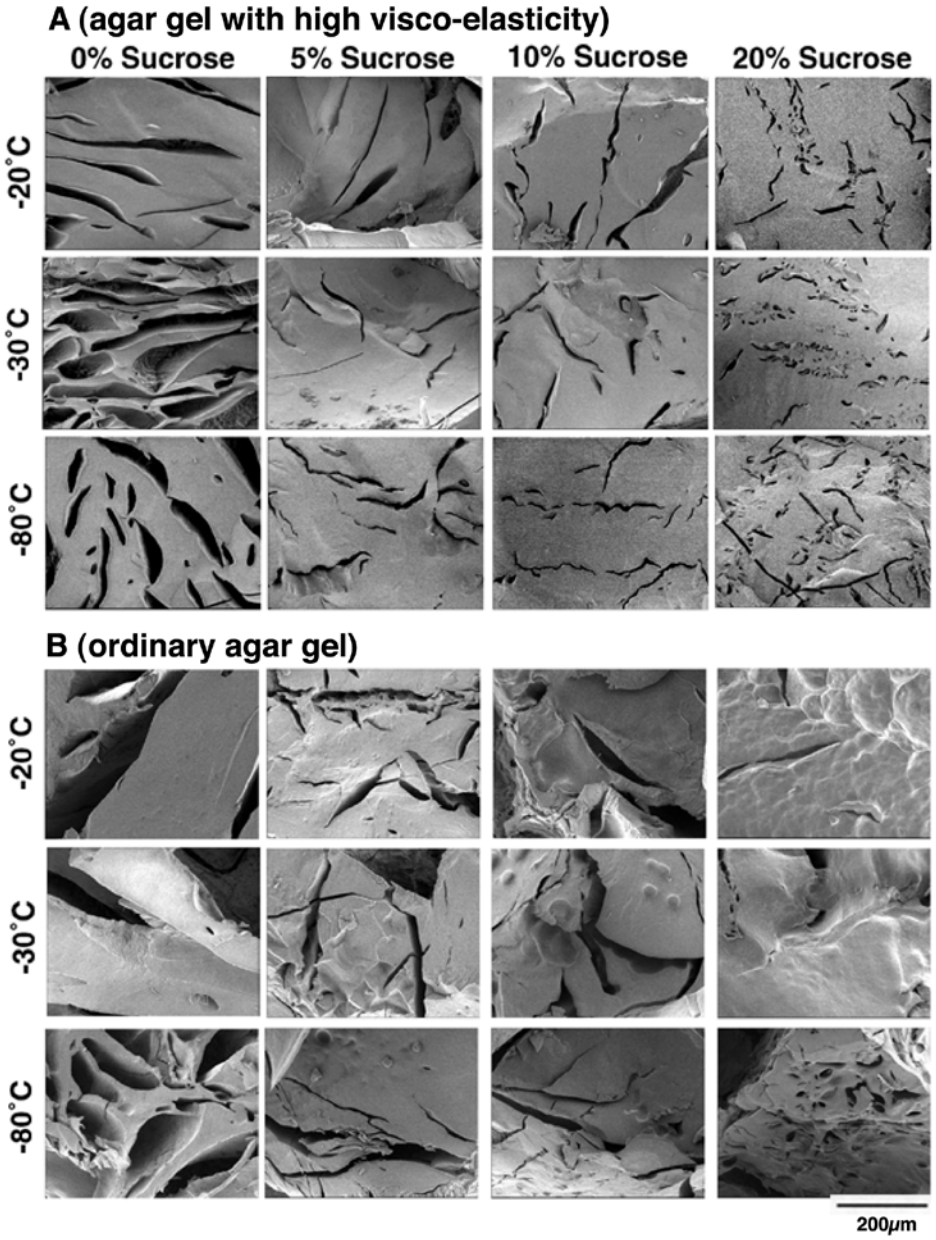


Fig. V-9. Cryo-scanning electron micrographs of agar gels frozen in freezers at atmospheric pressure

SUMMARY

To determine the effects of sucrose and high-pressure-freezing on the improvement in quality (texture and structure) of frozen gel, two kinds of agar gel were compared; A gel with high visco-elasticity and B gel, an ordinary dessert gel. Both agar gels with 0, 5, 10 or 20% sucrose were frozen at 0.1 ~ 686 MPa and -20°C. They were frozen during pressurization, and exothermic peaks were detected at 0.1, 100, 600 and 686 MPa and -20°C (freezing). However, at 200 MPa, they did not freeze but froze with released pressure (pressure-shift-freezing). Thus, the amount of syneresis from gel pressure-shift-frozen at 200 MPa was smaller than that from gel frozen at other pressures. Also, amount of syneresis from A was smaller than B. In addition, compared to control gels, the appearance of 0% sucrose-agar gels frozen at 0.1, 100, 600 and 686 MPa differed greatly due to syneresis and a volumetric shrinkage of the gel. It was apparent that the rupture stress of the gels decreased, strain and size of ice crystals increased and quality declined. Conversely, due to quick freezing, the texture and structure of both A and B pressure-shift-frozen at 200 MPa were better than the other pressure-treated gels and gels frozen in freezers (-20, -30 or -80˚C) at atmospheric pressure. Consequently, pressure-shift-freezing was more effective. However, texture, structure and syneresis of A were somewhat better than that of B. It was found that the addition of sucrose to the gel was effective in improving the quality of frozen agar gels.

Chapter VI

Effects of High Pressure and Addition of Sucrose on the Quality Improvement of Frozen-thawed Carrageenan Gels

Part 1. Comparison of Kappa and Iota Carrageenan Gels

INTRODUCTION

Carrageenan is a naturally occurring polysaccharide material which fills a void in cellulosic plant structure. As a result of its water-gelling and milk-protein interaction, this extracted hydrocolloid material is extensively used by the food industry as a gel for thickening and stabilizing food systems (Thomas, 1992).

Red seaweeds produce extracts which compose a family of hydrocolloids including agar, furcellaran and three types of carrageenan (kappa: κ, iota: ι, lambda: λ). All of these hydrocolloids have a galactose backbone joined together by alternating the glycosidic linkage. However, the number and position of ester sulfate groups, and the amount of 3, 6-anhydro-D-galactose (3,6-AG) which they contain, differ. The results in a wide range of gelling properties, from the very brittle agar gels to carrageenan gels and the non-gelling λ-carrageenans (Thomas, 1999).

Carrageenan is made up of repeating galactose units and 3,6-AG, both sulfated and non-sulfated. The units are joined by alternating α-1-3 and β-1-4 glycosidic linkages, and κ-carrageenan contains approximately 25% ester sulfate and 34% 3,6-AG (Thomas, 1999). The addition of potassium ions causes helices to aggregate and the gel to contract and become brittle. Potassium salts are essential in order to form this firm gel structure.

On the other hand, ι-carrageenan contains approximately 32% ester sulfate and 30% 3,6-AG (Thomas, 1999). The structure is about 10 percent of 3,6-AG 2-sulfate residues replaced by D-galactose 2,6-disulfate (Rees, 1969). The 2-sulfate group on the outside of ι-carrageenan molecule does not allow the helices to aggregate to the same extent as κ-carrageenan. Thus, the texture (gel strength) of ι-carrageenan gel is softer than κ-carrageenan gel. The clarity and freeze-thaw tolerance of gels increase, though strength decreases, as the content of ester sulfate groups increases.

With food gels that have a high water content, damage to structures through freezing is extensive and texture after thawing becomes unacceptable. Such food gels frozen at atmospheric pressure (0.1 MPa) do not recover their gel phase when thawed. It has been already established that a non-freezing area (liquid phase) below 0°C exists under high pressure (Fletcher, 1970; Hobbs, 1974). In previous studies (Kanda et al., 1992), when tofu was pressurized at 200 MPa and -18°C, it did not freeze. However, when pressure was released, it froze quickly. This method was designated as "pressure-shift-freezing". Also, in previous studies (Fletcher, 1970; Hobbs, 1974) it was found that a specific volume of water increases during water-ice I transition, whereas the opposite is observed for "high pressure ice" (II-IX). When pressure was raised to 2400 MPa at -200°C ~ 80°C, several kinds of high pressure ices (ice I ~ ice IX) with different structures and properties were formed (Fletcher, 1970; Hobbs, 1974; Maeno, 1881). Ice at atmospheric pressure is denoted as ice I (density, 0.92). Ice I is less dense than liquid water and floats. Thus, the density of high pressure ices is higher, and their crystal structure is very complex. The density of ice II, ice III, ice V, ice VI, ice VII and ice VIII are 1.19, 1.14, 1.23, 1.31, 1.50 and 1.50g/cm^3, respectively (Fletcher, 1970; Hobbs, 1974; Maeno, 1881).

The effect of high pressure on the improvement in quality (texture and/or structure) of frozen food has been discussed previously: tofu (Kanda *et al.*, 1992; Fuchigami & Teramoto, 1997; Fuchigami *et al.*, 1998; 2002; Teramoto & Fuchigami, 1999); gellan gum gel (Fuchigami & Teramoto, 2003a); agar gel (Fuchigami & Teramoto, 2003b; Fuchigami *et al.*, 2006); gelatin (Zhu *et al.*, 2005); egg custard gel (Teramoto *et al.*, 2006) and boiled egg (Jibu *et al.*, 2009). Also, pressure-shift-freezing effects on several kinds of food have been reviewed (Fuchigami, 1996, 2001, 2006; Fuchigami *et al.*, 2008).

The results of these studies were as follows: pressure-shift-freezing at 200-400 MPa and -18 to -20°C appeared to be effective in improving both the texture and/or histological structure of frozen food, because, when pressure-shift-frozen, nuclei formed during pressure release, and phase transition from liquid-to-ice I occurred quickly. Therefore, small ice crystals formed. Thus, this led to a beneficial effect on texture. However, the effect of high pressure on improving the quality of frozen food appeared to be related to the type of food.

Therefore, the first objective of this research is to compare the freeze-thaw tolerance of κ- and ι-carrageenan gels with different amount of ester sulfate. K-carrageenan gel, frozen by air blast method at atmospheric pressure, collapses and does not recover its gel phase when thawed, so texture after thawing becomes unacceptable. However, if high pressure is applied to frozen carrageenan gel, the damage of gel may be prevented. The second objective is to research the effect of high pressure on improving the quality of frozen carrageenan gel. The third objective is to determine the effect of the addition of sucrose on the quality of frozen-thawed carrageenan gels.

MATERIALS AND METHODS

1. Sample preparation

K-carrageenan gel: 2% κ-carrageenan (San-eigen FFI Ltd., Osaka) and 0.2% potassium dihydrogenphosphate (extra-pure reagent, Wako Pure Chemical Industries, Ltd., Osaka) became swollen in distilled and deionized water over night, heated to melting and boiled for 5 min. Then, 0%, 5%, 10% or 20% (w/w) sucrose (saccharose, extra-pure reagent, Ishizu Seiyaku Ltd., Osaka) was added then heated for 5~8 min.

I-carrageenan gel: 5% genutine 9404 (Sansho Ltd., Osaka) and 0%, 5%, 10% or 20% (w/w) sucrose became swollen in distilled and deionized water. Then they were heated to 85°C, stirring constantly.

These two samples were deaired for about 5 min using vacuum pump, weight was adjusted using hot water, then poured into plastic trays (gel thickness: 10 mm), respectively. They were stored in a 5°C refrigerator over night (about 20 hr) to achieve complete stabilization of the gels. The gels were then cut into cylinders (15 mm in diameter and 10 mm height). Seven pieces of gel were vacuum packed in heat-sealed polyethylene bags. Four packs of samples with various concentrations of sucrose were frozen at the same time.

2. Method of freezing at high pressure

Samples were frozen at 100~686 MPa using a Dr. Chef high pressure food processor (Kobe Steel Ltd., Kobe) (Kato *et al.*, 1997). Weston brine PS (66% propylene glycol, CCI Ltd., Gifu) was used for the pressure medium. The pressure medium was first placed in a pressure vessel (6 cm inside diameter and 20 cm high) and kept at -20°C by a cooler, then removed. Next, samples were placed in a pressure vessel, and the thermocouple (k-type) was inserted in the center of the sample. Then, the pressure medium (-20°C) was added to the pressure vessel. The samples were frozen at atmospheric pressure (0.1 MPa) in a pressure vessel or immediately pressurized at 100~686 MPa for 60 min. After reduction of pressure, the samples were stored for 1 day at -30°C then thawed at 20°C.

The operation was fully automated and both pressure and temperature of the samples (upper section of the pressure vessel) and pressure medium (lower section) were recorded in intervals of 5 sec using Thermodac E/Ef (Eto-denki Ltd., Tokyo).

3. Texture measurement

After the amount of syneresis was measured, a photograph of the gel samples was taken. Then, texture of the gel samples was measured by a creepmeter (Rheoner, RE-33005, Yamaden Ltd., Tokyo). Thickness of samples was measured using a sample-height counter (HC-3305, Yamaden Ltd., Tokyo) then punctured by using a plunger (cylindrical shape: 3mm dia., 22 mm long) at 1 mm/sec stopping at 99% of the thickness using a loadcell of 200 g. Rupture stress and rupture strain were indicated.

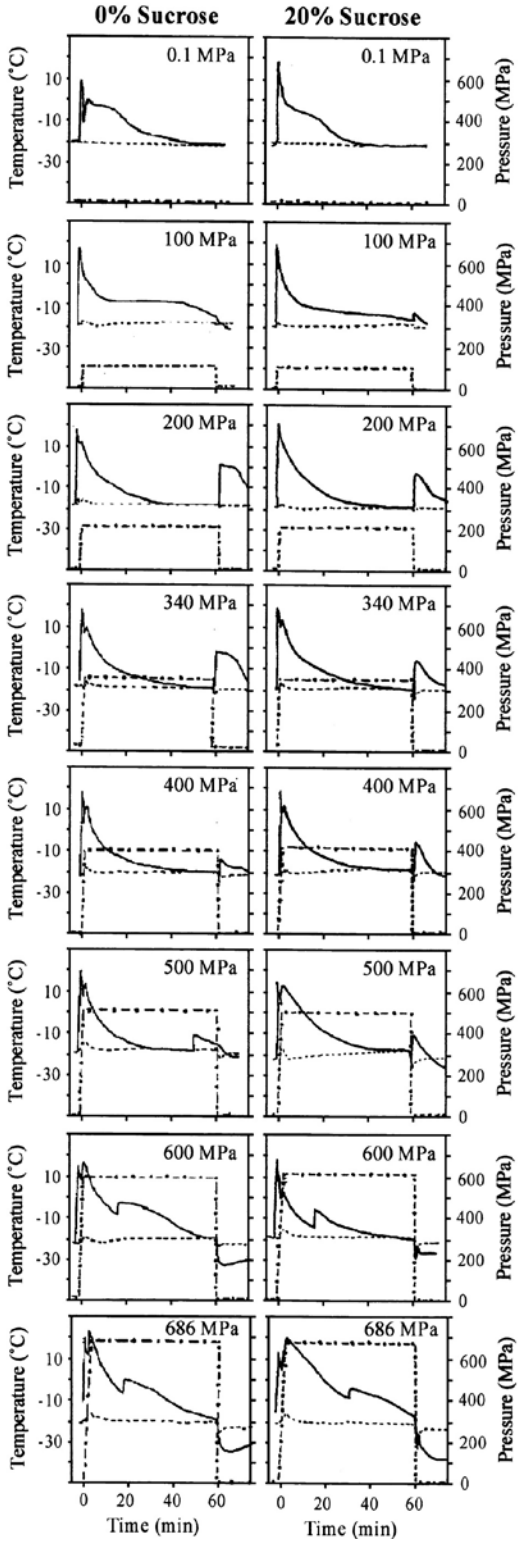
4. Structure measurement

Structure of the gel samples was observed with a cryo-scanning electron microscope (S-4500, Hitachi Ltd., Tokyo) (Fuchigami *et al.*, 1995). Gel samples were cut into 6 mm × 1 mm × 1 mm, and dehydrated with 20, 40 and 50% ethanol. The specimen was contained in a metal holder and quickly frozen by immersing in LN_2, transferred to the cold stage of a cryo-system for scanning electron microscopy and then cut with a knife (-150˚C). After etching at -85˚C, the surface was coated with gold then observed at -120˚C under low acceleration voltage (1kV). The magnifications used to observe the traces of ice crystals and gel networks were ×100 and ×10,000, respectively.

The pore size of frozen-thawed gels was measured using software for image analysis (Mac-scope, Mitani Co. Ltd., Fukui, Japan). Area of pores and means in the micrographs (magnification: ×100) were calculated.

RESULTS AND DISCUSSION

1. Changes in temperature of samples during freezing



Changes in pressure and temperatures of κ-carrageenan gels with 0% and 20% sucrose during pressurization are shown in Fig. VI-1. Within 1 min, the defined pressure was reached and was maintained for 60 min then reduced to atmospheric pressure within about 20 sec.

Changes in the temperatures of κ- and ι-carrageenan gels during pressurization were almost the same. Therefore, only temperatures of κ-carrageenan gels are shown. The initial temperature of samples was about 10~20°C. When the gels were frozen in a pressure vessel at atmospheric pressure (0.1 MPa) and -20°C, the temperature decreased to 0°C and the duration of the freezing plateau (from 0 to -5°C) for gels with 0% and 20% sucrose was about 15 min and 12 min, respectively. Then the temperature of the gels decreased gradually to about -20°C.

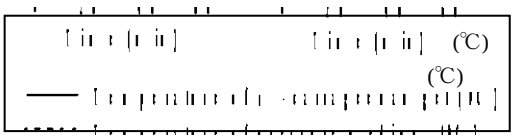


Fig. VI-1. Changes in pressure and temperatures of κ-carrageenan gels and pressure medium during pressurization and depressurization at –20°C

When pressurized at 100 MPa and -20°C, the gel with 0% sucrose were cooled rapidly to about -8°C and maintained -8°C for 40 min. When the pressure was released, the temperature decreased slightly. This indicated that the gels froze during pressurization, and ice I formed. The gel with 20% sucrose was cooled rapidly to -10°C, then the temperature decreased gradually. The ice I, which is formed at 0.1 MPa or 100 MPa, is with a large increase in volume when water transformed into ice I (Fletcher, 1970; Hobbs, 1974; Maeno, 1881). This is a cause of structure damage during slow freezing at 0.1 MPa or 100 MPa.

When pressurized at 200~400 MPa and -20°C, all gel samples cooled to about -20°C, and an exothermic peak was not detected during pressurization. However, when pressure was released, the temperature of the samples rose quickly then decreased to about -20°C. Therefore, they partially froze during depressurization. This indicates that the gels must have frozen quickly through pressure-shift-freezing (Kanda *et al.*, 1992).

When the gels with 0% and 5% sucrose were pressurized at 500 MPa and -20°C, an exothermic peak was detected during pressurization, while the gels with 10% and 20% sucrose were pressure-shift-frozen.

For the gels pressurized at 600 and 686 MPa and -20°C, an exothermic peak was detected during pressurization and then gradually decreased to about -15 to -20°C. Therefore, when these gel samples froze during pressurization, ice V or ice VI formed, respectively.

The concentration of sucrose became higher and the initial freezing temperature of gels frozen at 0.1, 100, 500, 600 and 686 MPa decreased.

2. Visual appearance of frozen-thawed carrageenan gels

Visual appearances of frozen-thawed κ- and ι-carrageenan gels were compared (Fig. VI-2). The appearances of κ-carrageenan gels with 0% and 5% sucrose frozen at 0.1, 100 and 500~686 MPa differed from the original gel (non-frozen control), due to syneresis and a volumetric shrinkage of gel. As the concentration of sucrose in the gels increased, the appearance of gels improved. The appearances of κ-carrageenan gels (with 20% sucrose) pressure-shift-frozen at 200~400 MPa were similar to the original gel.

On the other hand, the appearances of frozen-thawed ι-carrageenan gels were almost the same as the original gel (Fig. VI-2), but the appearances of ι-carrageenan gels without sucrose, frozen at 0.1 and 100 MPa, differed from the original gel. However, as the concentration of sucrose in the gel increased, the appearance of gels improved.

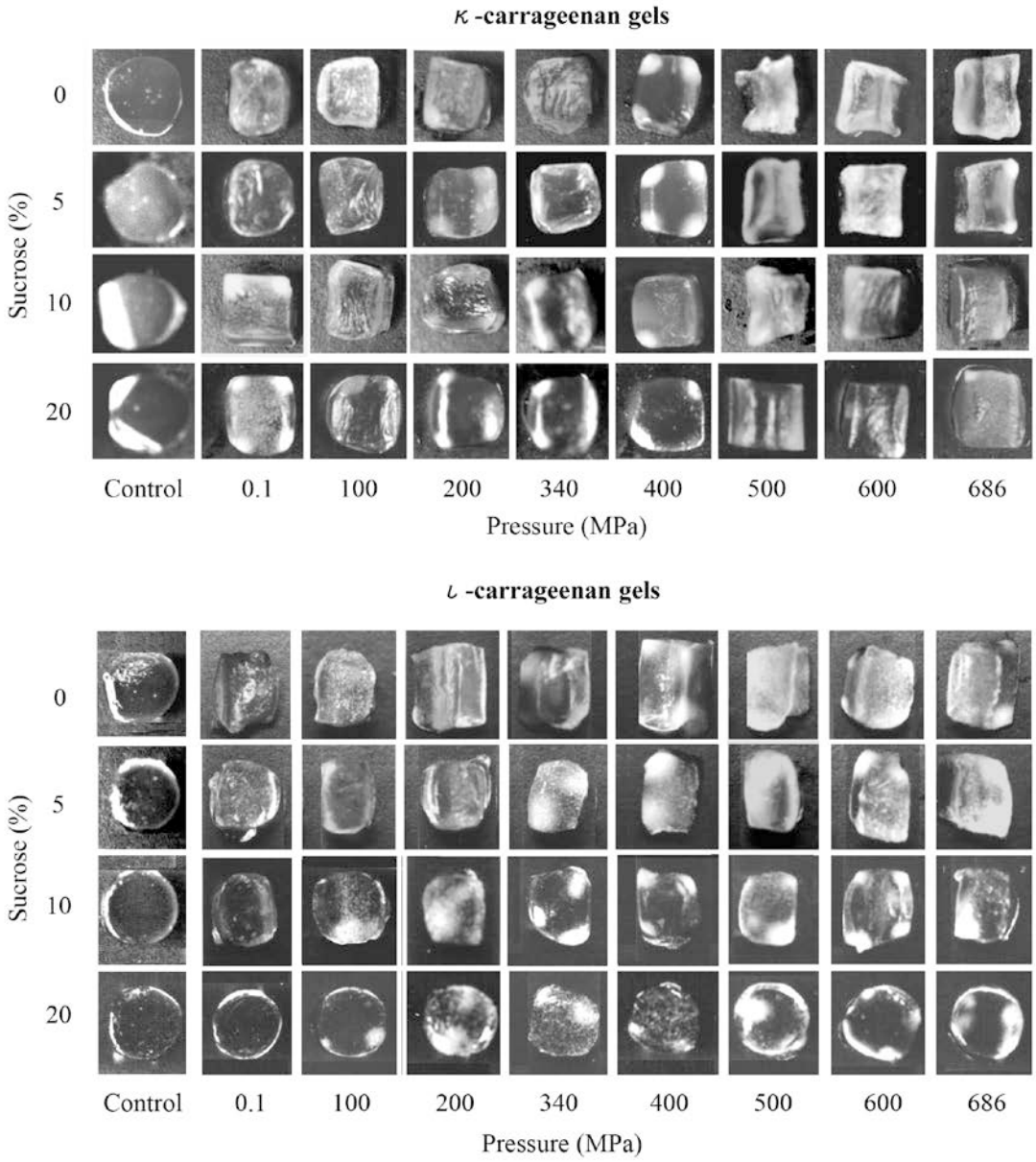


Fig. VI-2. Visual appearance of non-frozen and frozen-thawed κ- and ι-carrageenan gels
Control: non-frozen gel

3. The amounts of syneresis from frozen-thawed carrageenan gels

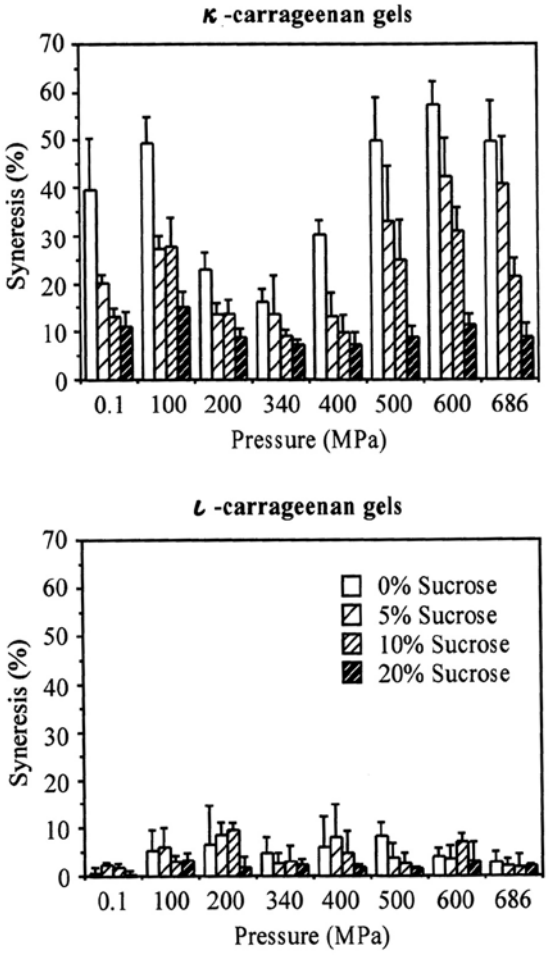


Fig. VI-3. The amounts(%) of syneresis from frozen-thawed κ- and ι-carrageenan gels

The amounts (%) of syneresis are shown in Fig. VI-3. The syneresis (%) from κ-carrageenan gels (with 0%, 5% and 10% sucrose) pressure-shift- frozen at 200, 340 and 400 MPa were significantly smaller than that from gels frozen at the other pressures.

As the concentration of sucrose in gel increased, syneresis decreased. There was no significant difference in syneresis among frozen-thawed gels with 20% sucrose. Thus, the addition of sucrose to gel appeared to be effective in preventing syneresis.

The syneresis from ι-carrageenan gels was smaller than that from κ-carrageenan gels. The syneresis (%) from ι-carrageenan gels pressurized at -20°C was almost the same as that from ι-carrageenan gels pressurized at 10°C (not shown). Therefore, the cause of syneresis might be by pressurization and not by freezing-thawing. Thus, the increase of syneresis by freezing-thawing appeared very small. Also, when ι-carrageenan gels were frozen-thawed, there was not a great difference in syneresis (%) from the freezing temperature and/or pressure. The syneresis from all frozen-thawed ι-carrageenan gels was smaller than that from κ-carrageenan gels.

4. Texture of frozen-thawed carrageenan gels

Typical stress-strain curves of κ- and ι-carrageenan gels are compared in Fig. VI-4 and the average values of rupture stress and rupture strain are compared in Fig. VI-5. Rupture stress of the non-frozen κ-carrageenan gel was greater than that of ι-carrageenan gels. When κ- and ι-carrageenan gels were punctured to about 25% and 68% of their thickness, they broke, respectively. Rupture strain of κ-carrageenan gel was smaller than that of ι-carrageenan gel. This indicated that κ-carrageenan gel was firm and brittle, and ι-carrageenan gel was soft and elastic.

The stress and strain of ι-carrageenan gel increased as the amount of sucrose increased. On the other hand, the concentration of sucrose did not affect the rupture stress of κ-carrageenan gel.

The texture of all frozen-thawed κ-carrageenan gels differed significantly from the non-frozen original gel. The rupture stress of frozen-thawed gels decreased greatly, while the rupture strain increased except for gels pressure-shift-frozen at 200 and 340 MPa. Thus, textural quality of κ-carrageenan gel pressure-shift-frozen at 200 and 340 MPa was better than that of gels frozen by other pressures.

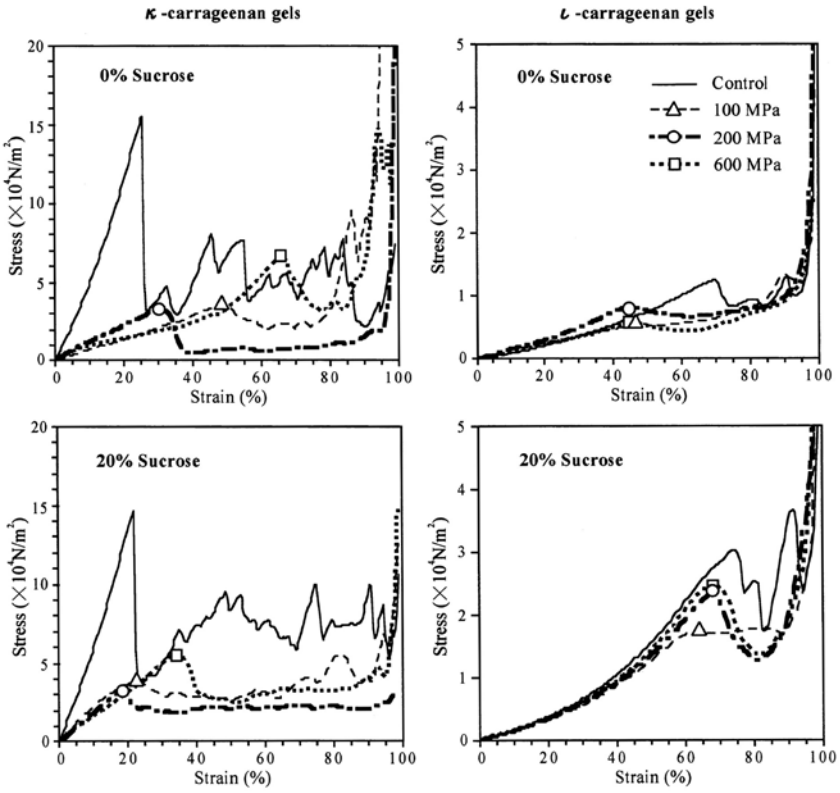


Fig. VI-4. Typical stress-strain curves of non-frozen and frozen-thawed κ- and ι-carrageenan gels
Control: non-frozen gel

As the concentration of sucrose in κ-carrageenan gel increased, the strain of frozen-thawed gels improved gradually. The strain of gels with 20% sucrose became the same as that of the original gel, except the gels frozen at 500~686 MPa. However, the rupture stress of all gels with 20% sucrose was smaller than the original gel.

On the other hand, when ι-carrageenan gels were frozen-thawed, the rupture stress and strain slightly decreased. As the concentration of sucrose increased, the strain of gels improved. There was no significant difference in the texture (rupture stress and strain) from pressure. The texture of all frozen-thawed ι-carrageenan gels was similar to original gel. Thus, freezing tolerance of ι-carrageenan gel was better than κ-carrageenan gel.

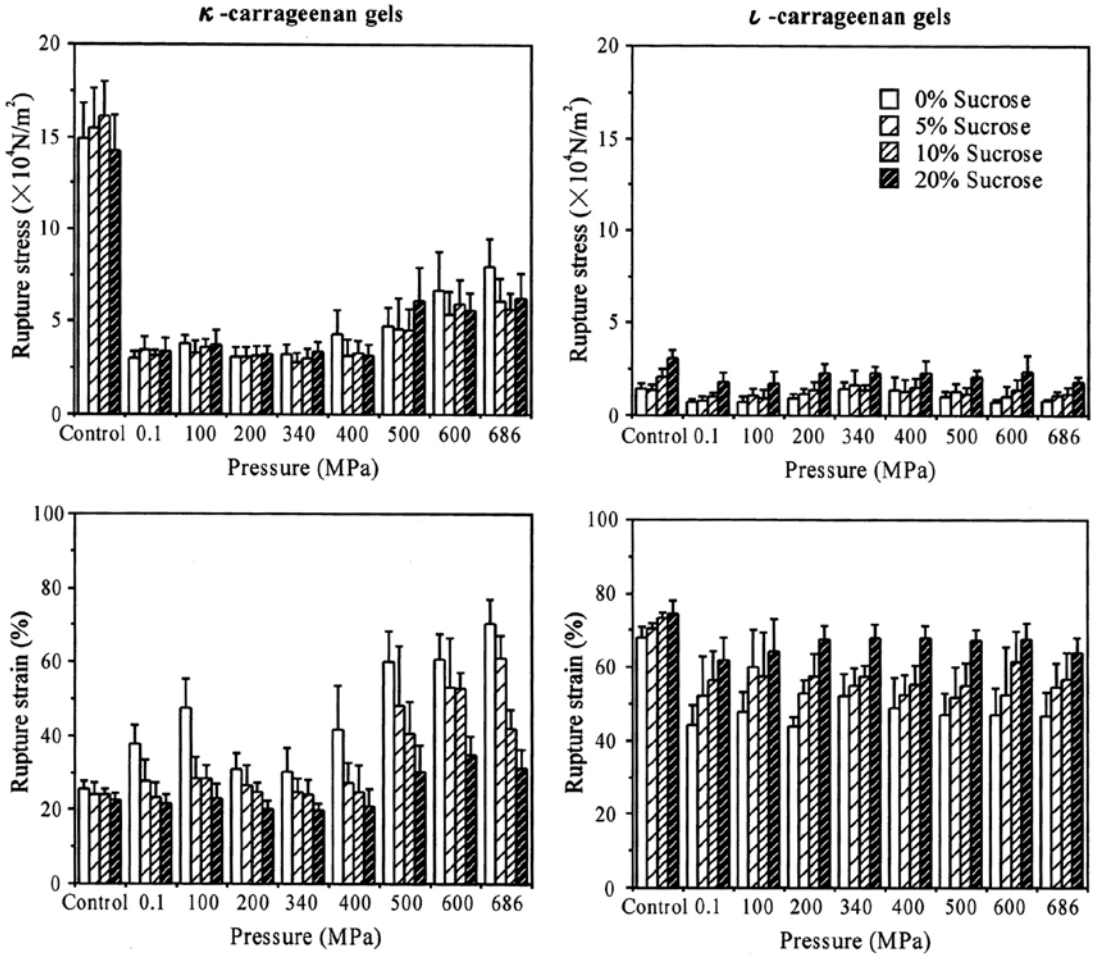


Fig. VI-5. Ruputure stress and ruputure strain of non-frozen and frozen-thawed κ- and ι- carrageenan gels
Control: non-frozen gel

5. Structure of frozen-thawed carrageenan gels

Cryo-scanning electron micrographs of frozen-thawed κ- and ι-carrageenan gels are compared in Fig. VI-6 and Fig. VI-7. Ice crystal traces appear dark in the micrographs. The pore size of ice crystal traces of κ-carrageenan gels is shown in Table VI-1.

When κ-carrageenan gels without sucrose were frozen at 0.1, 100, 500, 600 and 686 MPa then thawed, a small amount of large ice crystal traces was observed. Conversely, large amounts of small, long and thin ice crystals formed in the gels pressure-shift-frozen at 200~400 MPa. This indicated that ice formation was instantaneous. The size of ice traces began to increase above 500 MPa.

With an increase of sucrose, the size of ice crystal traces in these gel samples decreased slightly. When the gels with 10% and 20% sucrose were pressurized at 500 MPa and -20°C, they were pressure-shift-frozen. Consequently, the size of ice traces decreased. These results indicated that the initial freezing temperatures (super cooling temperature) became lower, and also freezing time (freezing rate) became shorter, the number of ice-nuclei increased and growth of ice crystals was prevented (Watanabe, 1995); consequently, ice crystals became smaller.

It appeared that the size of ice crystals affected the amount of syneresis and texture of the thawed gel. Because ice crystals in the gels were larger, the amount of syneresis increased, and syneresis promoted shrinkage of the gels; therefore, rupture stress decreased and rupture strain increased. As the concentration of sucrose increased, the amount of water in the gel and freezing temperatures decreased, shortening freezing time. Thus, size of ice crystals became smaller and texture improved.

The shape of ice crystals in pressure-shift-frozen gels differed. Those of tofu (Fuchigami & Teramoto, 1997; Fuchigami *et al.*, 1998; 2002; Teramoto & Fuchigami, 1999), egg custard gel (Teramoto, *et al*., 2006) and agar gels (Fuchigami & Teramoto, 2003b; Fuchigami *et al*., 2006) were round, while those of κ-carrageenan gels and un-substituted form-gellan gum gels (Fuchigami & Teramoto, 2003a) were thin and long. These results indicated that the shape of ice crystals might be affected by the kind, concentration, brittleness, elasticity and strength of the gels.

On the other hand, ice crystal traces were not observed in all frozen-thawed ι-carrageenan gels. Therefore, only non-frozen control and gels frozen at 600 MPa then thawed are shown in Fig. VI-7.

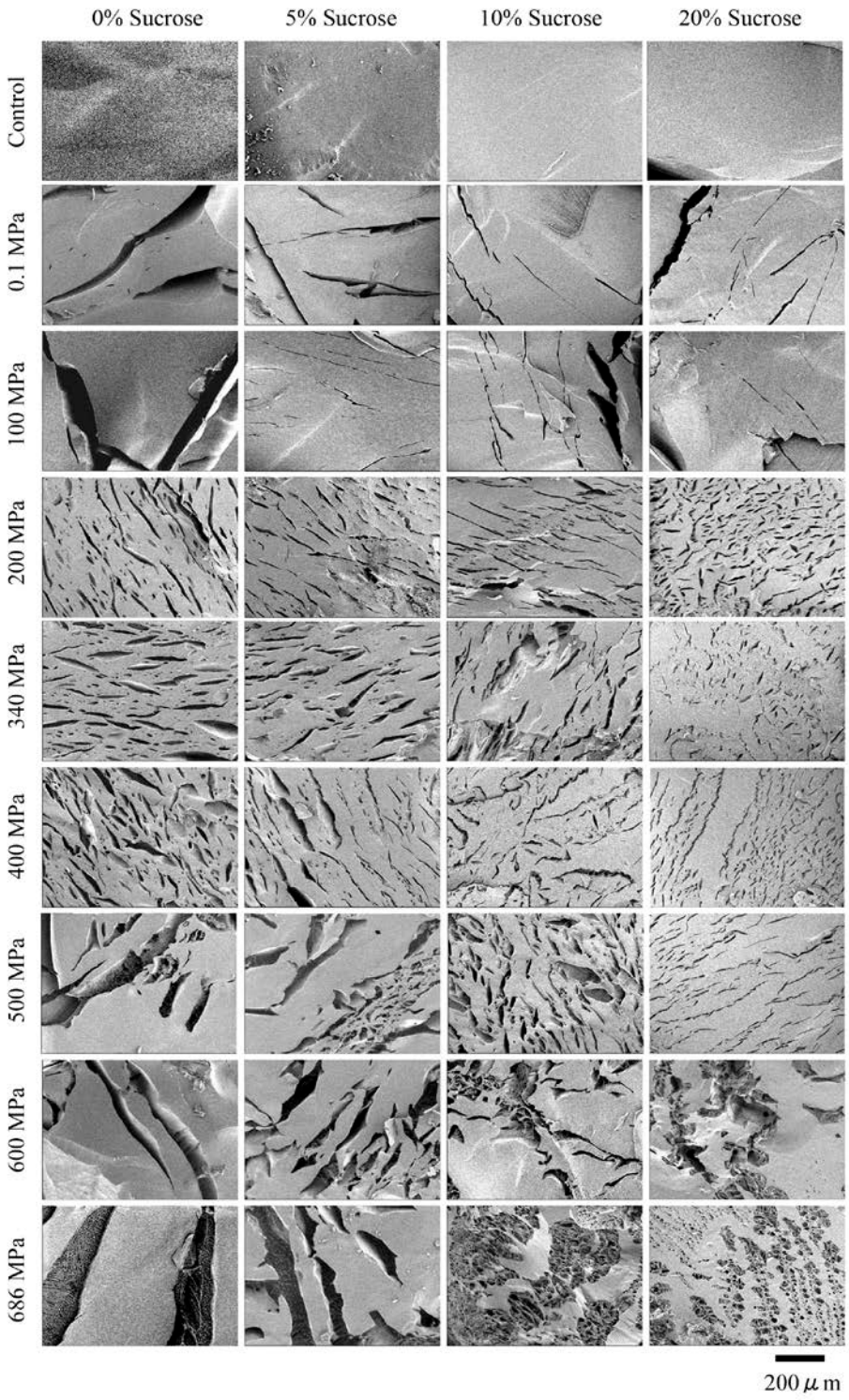


Fig. VI-6. Cryo-scanning electron micrographs of non-frozen and frozen-thawed κ- carrageenan gels
Control: non-frozen gel

Tabel 1. The size of ice crystal traces in frozen-thawed κ-carrageenan gels image-analyzed by a mac-scope

Pressure	Size of ice crystal trace ($\times 10^3 \mu m^2$)			
(MPa)	0% Sucrose	5% Sucrose	10% Sucrose	20% Sucrose
0.1	5.71 ± 1.49	4.03 ± 7.61	2.00 ± 2.07	1.08 ± 4.43
100	28.23 ± 3.08	0.36 ± 0.38	3.16 ± 6.66	3.00 ± 7.78
200	0.50 ± 1.06	0.40 ± 0.53	0.61 ± 0.96	0.33 ± 0.57
340	0.78 ± 1.64	0.76 ± 1.25	1.70 ± 4.64	0.26 ± 0.34
400	0.93 ± 1.64	0.71 ± 2.12	0.79 ± 1.10	0.21 ± 0.47
500	8.59 ± 23.43	1.32 ± 4.19	0.76 ± 1.34	0.30 ± 0.50
600	14.93 ± 20.45	2.96 ± 8.43	1.00 ± 3.37	1.64 ± 8.50
686	69.28 ± 56.82	14.65 ± 3.42	4.31 ± 2.39	4.03 ± 9.32

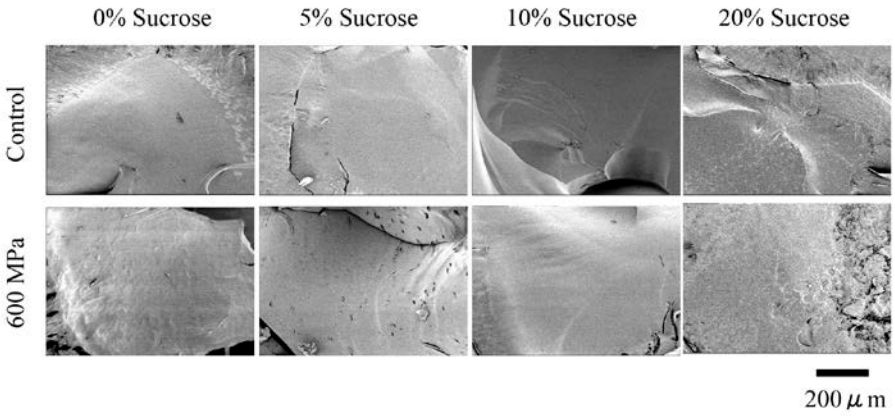


Fig. VI-7. Cryo-scanning electron micrographs of non-frozen and frozen-thawed ι- carrageenan gels
Control: non-frozen gel

The network of frozen-thawed κ- and ι-carrageenan gels are compared in Figs. VI-8 and VI-9. When κ-carrageenan gels without sucrose was frozen at 0.1, 100 and 500~686 MPa, the gel network contracted. These results indicate that the growth of ice crystals in gels may adhere closely to the gel network. However, with the addition of 20% sucrose-to-gel, the structure of the gels improved. The network of κ-carrageenan gels pressure-shift-frozen at 200~400 MPa was comparatively similar to non-frozen gel.

The gel network of ι-carrageenan gels was not observed in non-frozen gel nor in frozen-thawed gels (Fig. VI-9). These results were similar to native gellan gum gels (Fuchigami & Teramoto, 2003a). When ι-carrageenan gels were frozen-thawed, the same structure as the non-frozen gel was maintained.

When freeze-thaw tolerance of two kinds of agar gel was compared (Fuchigami *et al.*, 2006), the quality of agar gel with more ester sulfate and high visco-elasticity was better than that of ordinary agar gel with a low ester sulfate content (below 4.5%, typically 1.5~2.5%). These results are summarized below. The amount of ester sulfate was least to greatest in ordinary agar gel < agar gel with high visco-elasticity < κ-carrageenan < ι-carrageenan, respectively. Conversely, the syneresis from gels was least to greatest from ι-carrageenan < κ-carrageenan < agar gel with high visco-elasticity < ordinary agar gel, respectively. Because ι-carrageenan has more sulfate (approximately 32%) than κ-carrageenan (about 25%), the amount of syneresis from ι-carrageenan gels less than κ-carrageenan gels. Thus, carrageenan gel with more ester sulfate may have better freeze-thaw tolerance comparing to agar gel. Schematic forms of the proposed mechanism for gelation by ι- and κ-carrageenan are as follows: the chains are cross-linked by isolated double helices in ι-carrageenan, whereas, double helices are aggregated to an unknown extent in κ-carrageenan (Rees, 1969). It is considered that the aggregated gels increase firmness, but decrease freeze-thaw tolerance. Therefore, the quality of frozen-thawed ι-carrageenan gels was better than that of κ-carrageenan gels.

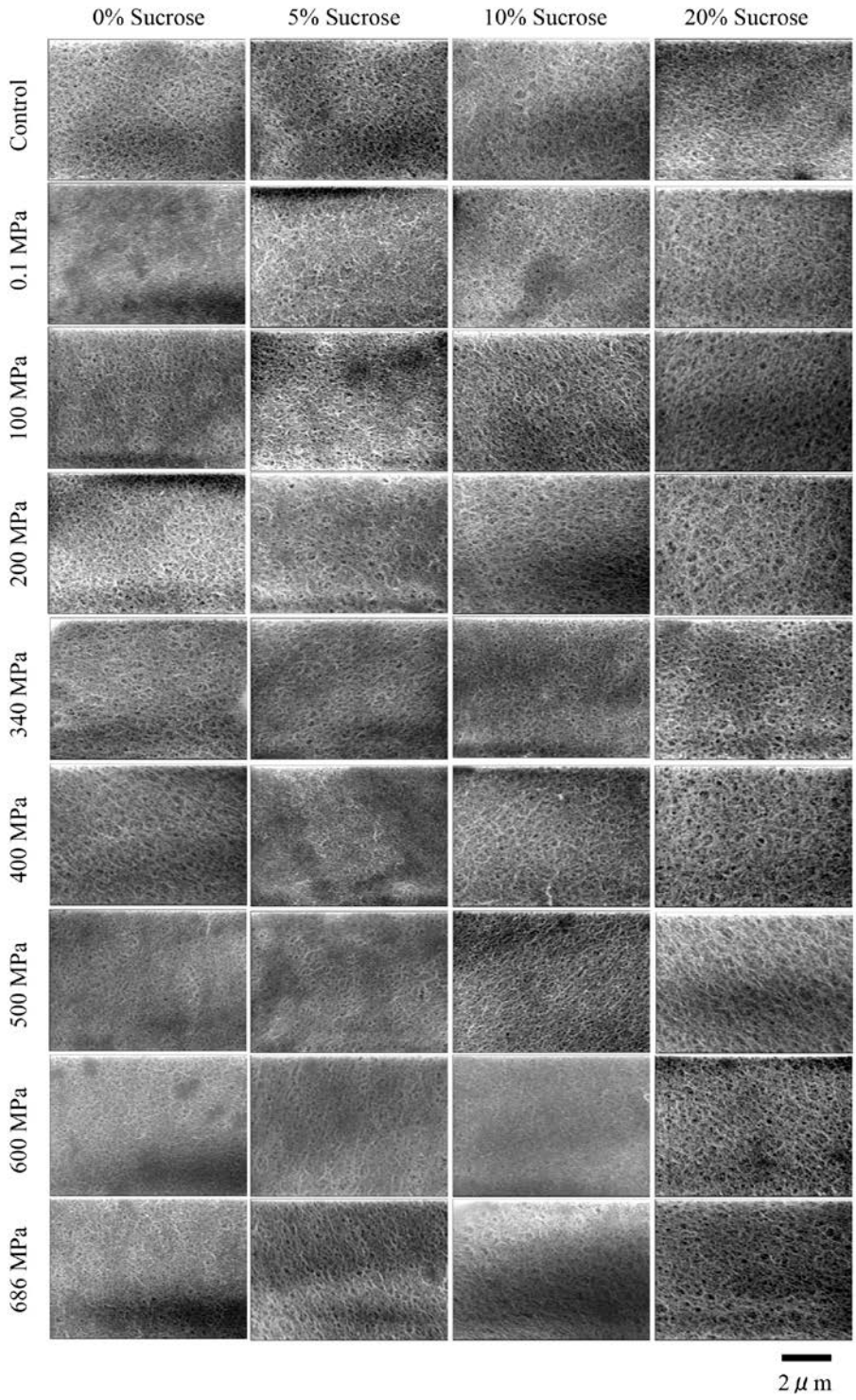


Fig. VI-8. Network-structure of non-frozen and frozen-thawed κ-carrageenan gels
Control: non-frozen gel

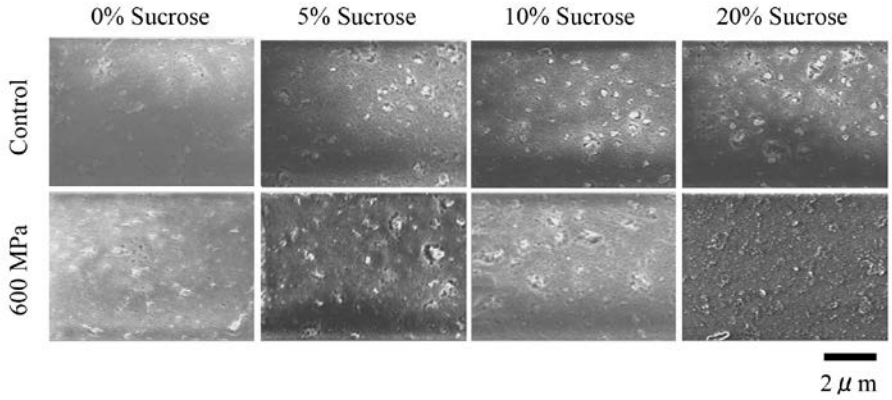


Fig. VI-9. Network-structure of non-frozen and frozen-thawed ι-carrageenan gels
Control: non-frozen gel

SUMMARY

To determine the effects of high pressure and sucrose on improving the quality of frozen-thawed gel, κ- and ι-carrageenan gels were compared. Rupture stress of the non-frozen κ-carrageenan gels was greater and rupture strain was smaller than that of ι-carrageenan gels, respectively. Thus, κ-carrageenan gel made a firm brittle gel whereas ι-carrageenan formed very soft and elastic gel.

When κ-carrageenan gels were frozen at 0.1, 100, 500, 600 and 686 MPa and -20°C then thawed, rupture stress of the gels decreased greatly while the rupture strain increased, except the gels pressure-shift-frozen at 200, 340 and 400 MPa. On the other hand, the texture of all frozen-thawed ι-carrageenan gels was similar to the original gel.

When κ-carrageenan gels were pressure-shift-frozen then thawed, the size of ice crystal traces and the amounts of syneresis were smaller than gels frozen at other pressures. Thus, pressure-shift-freezing was effective in improving the quality of frozen-thawed κ-carrageenan gels.

Conversely, the trace of ice crystals was not observed in all frozen-thawed ι-carrageenan gels, syneresis was slight, and the quality of gels was the same as non-frozen gel. Thus, freezing tolerance of ι-carrageenan gels with more sulfate was better than κ-carrageenan gels.

The addition of sucrose decreased the initial freezing temperature and prevented the growth of ice crystals; therefore, texture and structure of frozen-thawed carrageenan gels with sucrose were good. With the addition of 20% sucrose, the quality of frozen-thawed gels was improved.

Chapter VII

Effects of High Pressure and Addition of Sucrose on the Quality Improvement of Frozen-thawed Carrageenan Gels

Part 2. Effects of the Addition of Locust Bean Gum to Carrageenan Gels

INTRODUCTION

Carrageenan is a colloid extracted from red seaweed extensively used by the food industry as a gel for thickening and stabilizing food (Thomas, 1992). Carrageenans have a wide range of applications. Within the sub families of carrageenan hydrocolloids, a very broad range of properties can be developed from interactions between various types of carrageenans (kappa: κ, iota: ι, lambda: λ), milk proteins, and other hydrocolloids (konjac flour and locust bean gum) (Thomas, 1992).

To determine the effects of high pressure and sucrose on improving the quality of frozen-thawed gel, two kinds of carrageenan gel (2% kappa and 5% iota) were compared in Chapter VI. Both carrageenan gels with 0, 5, 10 or 20% sucrose were frozen at 0.1 MPa and pressurized at 100 ~ 686 MPa and -20°C. When pressurized at 200 ~ 400 MPa and -20˚C, both gels did not freeze. However, when pressure was released, they froze quickly by pressure-shift-freezing (Kanda *et al*., 1992). Consequently, the quality of pressure-shift-frozen-then-thawed gels was better than gels frozen at other pressures then thawed.

It was found that the addition of sucrose to both gels was effective in improving the quality of frozen-thawed carrageenan gels. However, freezing tolerance of ι-carrageenan gel was greater than κ-carrageenan gel. Traces of ice crystals were not observed in all frozen-thawed ι-carrageenan gels, the amount of syneresis was slight, and the quality of gel was the same as non-frozen gel.

Conversely, the size of ice crystals and the amount of syneresis from pressure-shift-frozen κ-carrageenan gel were smaller than those frozen at the other pressures, while rupture strain also improved. However, rupture stress of all frozen-thawed κ-carrageenan gels decreased. To prevent the decrease of rupture stress by freezing-thawing, locust bean gum was added to κ-carrageenan gels in this paper. Locust bean gum is a galactomannan (D-galactose : D-mannose = 1 : 4, molecular

weight: 200,000-300,000) in beans.

K-carrageenan is able to interact synergistically with other gums, such as locust bean gum and konjac mannan, to modify further the gel texture. Hot solutions of κ-carrageenan-locust bean gum form strong elastic gels with low syneresis when cooled below 50 ~ 60˚C.

Galactomannan, that is most effective in forming gels in interaction mixtures with other polysaccharides, has lower proportions of galactose residues. Therefore, it is suspected that “smooth” rather than “hairy” regions are predominantly involved in the interactions (Rees, 1972). Galactomannan can serve as spacing agents: smooth regions of the galactomannan with few or no galactose side-groups associate with a herix (Pilnik and Rombouts, 1985). Namely, the mannose regions of the locust bean gum are able to associate with the repeating helical structure of carrageenan dimers to form gels. The maximum interaction, and hence peak rupture gel strength, occurs at a ratios between 60:40 and 40:60 κ-carrageenan to locust bean gum (Imeson, 2000). While as desirable gelling agent for dessert jellies should be consist of κ-carrageenan and locust bean gum in a mixed ratio of 7:3 ~ 6:4, and KCl at 0.25%, based on the rheological measurements (Murayama, 1990).

κ-carrageenan is used with locust bean gum for clear water dessert gels to provide a more gelatin-like texture and to decrease syneresis (Thomas, 1992). Therefore, the carrageenan manufacturer already adds an appropriate amount of galactomannan to his products, which is sold for uses in pie fillings, dessert gels, gelled milk products (Pilnik and Rombouts, 1985). In Japan, some gelling agents (the mixed gel of κ-carrageenan and locust bean gum) such as “PEARLAGAR-8” (Fuji Shoji Ltd.) were marketed. A “PEARLAGAR-8” is a gelling agent which contains 7% κ-carrageenan, 8% locust bean gum, 2% potassium di-hydrogen-phosphate and 83% glucose.

The objective of this study is to research the effects of an addition of 0.8% locust bean gum on improving the quality of frozen-thawed 0.7% κ-carrageenan gel. Furthermore, the freezing tolerance of [A-gel]: the mixed gel of κ-carrageenan and locust bean gum (glucose-additive-free), [B-gel]: “PEARLAGAR-8” gel (with 8.3% glucose), and [C-gel]: 2% κ-carrageenan gel without locust bean gum gel (Chapter VI) is compared. The concentration of C-gel was 2%. However, the rupture stress of gels increased by the addition of locust bean gum. Therefore, the mixed gels (A and B) of 0.7% κ-carrageenan and 0.8% locust bean gum were used in this study.

MATERIALS AND METHODS

1. Sample preparation

Two kinds of gel were prepared. In this paper, a mixed gel of κ-carrageenan and locust bean gum and a “PEARLAGAR-8” gel were designated as A-gel and B-gel, respectively. A-gel was made using the same combination ratio as a “PEARLAGAR-8” except for glucose. For comparative study, 2% κ-carrageenan gel without locust bean gum (Chapter VI) was designated as C-gel.

A-gel consisting of 0.7% κ-carrageenan (San-eigen FFI Ltd., Osaka), 0.8% locust bean gum (San-eigen FFI Ltd.) and 0.2% potassium di-hydrogen-phosphate (extra-pure reagent, Wako Pure Chemical Industries, Ltd., Osaka) became swollen in distilled and deionized water over night, then heated until melting and boiled for 5 min.

B-gel consisting of 10% “PEARLAGAR-8” (Fuji shoji Ltd., Tokyo, with 7% κ-carrageenan, 8% locust bean gum, 2% potassium di-hydrogen-phosphate and 83% glucose) became swollen in water over night then heated until melting and boiled for 5 min.

After boiling, 0%, 5%, 10% or 20% (w/w) sucrose (saccharose, extra-pure reagent, Ishizu Seiyaku Ltd., Osaka) was added to both A and B and heated for 5 ~ 8 min. After deairing for about 5 min using a vacuum pump, the weight was adjusted using hot water. They were then poured into plastic trays (gel thickness: 10 mm). They were stored in a 5°C refrigerator over night (about 20 hrs) to achieve the complete stabilization of the gels. The gels were then cut into disks (15 mm in diameter and 10 mm height). Finally, seven pieces of gel were vacuum packed in heat-sealed polyethylene bags. Four packs of samples with various concentrations of sucrose were frozen at the same time.

2. Freezing method

Samples were frozen at 100 ~ 686 MPa and -20°C using a high pressure food processor by the method described in Chapter VI.

3. Texture measurement

After the amount of syneresis was measured, a photograph of the gel samples was taken. Then, texture of the gel samples were measured by a creepmeter (Rheoner, RE-33005, Yamaden Ltd., Tokyo). Texture was measured using the method described in Chapter VI.

4. Structure measurement

The structure of gel samples was observed with a cryo-scanning electron microscope (S-4500, Hitachi Ltd., Tokyo) (Chapter VI). The magnifications used to observe ice crystals and gel networks were ×100 and ×10,000, respectively.

RESULTS AND DISCUSSION

1. Changes in temperature of samples during freezing

Changes in temperature of A- and B-gels and the pressure medium (propylene glycol) during pressurization and depressurization at -20°C were compared. The changes in temperature of A- and B-gels were almost the same as C-gels (Chapter VI), therefore, data is not shown.

When the gels were pressurized at 200 ~ 400 MPa and -20°C, all gel samples cooled to about -20°C, and an exothermic peak was not detected. However, when pressure was released, the temperature of the samples rose quickly then decreased to about -20°C. Therefore, it froze through pressure-shift-freezing (Kanda *et al*., 1992).

When gels were frozen at 100, 600 or 686 MPa and -20°C, an exothermic peak was detected during pressurization then gradually decreased to about -15°C to -20°C. Therefore, the gel samples froze during pressurization, and ice I, ice V or ice VI formed, respectively.

2. Visual appearance of frozen-thawed carrageenan gels

The visual appearance of frozen-thawed A- and B-gels was compared (Fig. VII-1). The appearance of A-gels frozen at 0.1 and 100 MPa differed from the original gel (non-frozen control), due to syneresis and a volumetric shrinkage of gel. As the concentration of sucrose in gels increased, the appearance of the gels improved. The appearance of A-gels frozen with 0 ~ 20% sucrose at 200 ~ 686 MPa was similar to the original gel.

On the other hand, the appearance of all frozen-thawed B-gels was almost the same as the original gel, except for gels with 0% and 5% sucrose frozen at 0.1 and 100 MPa. Also, the visual appearance of frozen-thawed A- and B-gels was better than C-gels (Chapter VI).

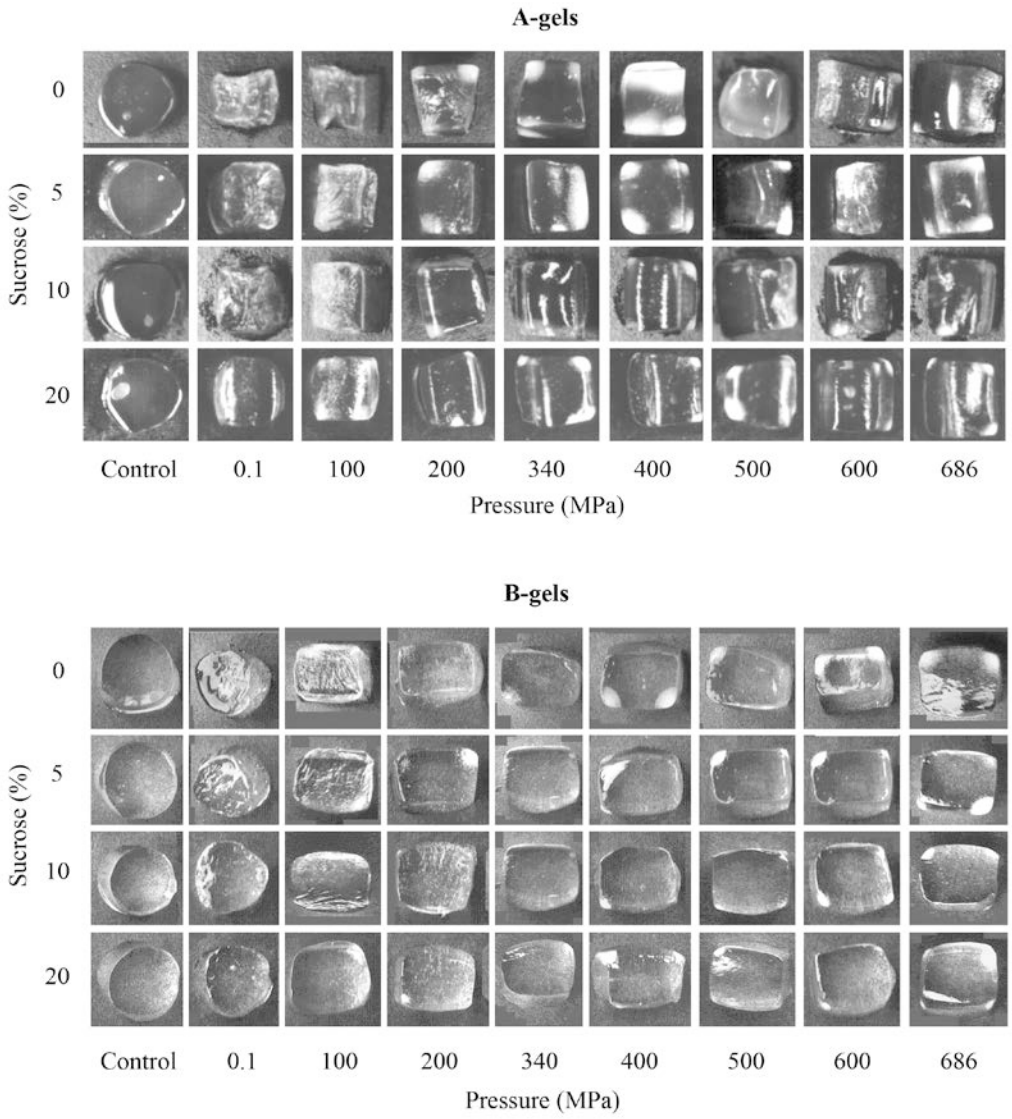


Fig. VII-1. Visual apperance of non-frozen and frozen-thawed carrageenan gels

Control: non-frozen gel

A-gels: the mixed gel of κ -carrageenan and locust bean gum

B-gels: PEARLAGAR-8 gels

3. Amount of syneresis from frozen-thawed carrageenan gels

The amount (%) of syneresis is shown in Fig. VII-2. The syneresis from A-gels pressure-shift-frozen at 200 ~ 400 MPa was smaller than that frozen at 0.1, 100 and 500 ~ 686 MPa. As the concentration of sucrose in gels increased, the syneresis decreased. The amount (%) of syneresis from B-gels was smaller than A-gels. When B-gels were frozen at 0.1 and 100 MPa, the syneresis was greater than that frozen at 200 ~ 686 MPa.

The syneresis from 0% sucrose-gels (A, B and C gels) frozen at 100 MPa was about 45%, 30% and 50%, and that frozen at 500 ~ 686 MPa was about 35~45%, 10%, 50~60%, respectively. The syneresis was greater from C-gels > A-gels > B-gels, respectively. Thus, the addition of sucrose/glucose and locust bean gum to gel appeared to be effective in preventing syneresis.

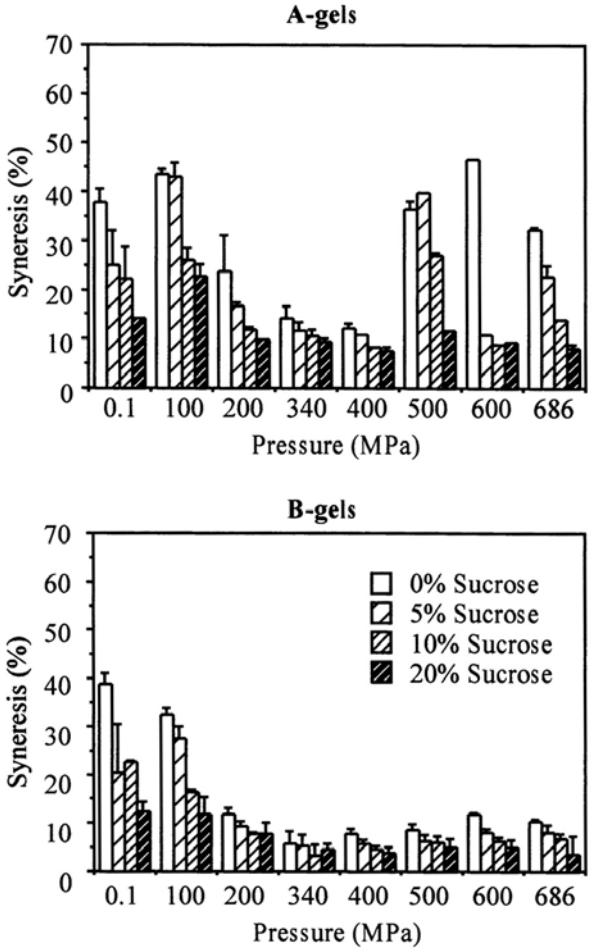


Fig. VII-2. The amounts (%) of syneresis from frozen-thawed carrageenan gels
A-gels and B-gels: see Fig. VII-1.

4. Texture of frozen-thawed carrageenan gels

Typical stress-strain curves of control and frozen-thawed A-gels with 0% and 20% sucrose are shown in Fig. VII-3. The stress-strain curve of frozen-thawed 0% sucrose-gel differed from the non-frozen control gel. However, when 20% sucrose was added, that of pressure-shift-frozen gel became the same as control gel.

The average values of rupture stress and strain of A- and B-gels are shown in Fig. VII-4, and compared with C-gels (Chapter VI). Rupture stress of control-A-gels (8 ~ 10×10^4 N/m^2) was smaller than control-B-gels (13 ~ 16×10^4 N/m^2) and control-C-gels (14 ~ 16×10^4 N/m^2). The concentration of C-gels was 2%, while that of A- and B-gels was 0.7%, therefore, the former was firmer than the latter. However, rupture stress of the former (C-gels) was smallest after freezing-thawing (Chapter VI).

The control-A-, B- and C-gels became broken when punctured to about 30%, 60% and 22% of thickness, respectively. Thus, rupture strain of control-B was greatest among all gels. These indicated that B-gel was more elastic than A- and C-gels. The rupture stress and strain of both control gels (A and B) increased with the addition of 20% sucrose.

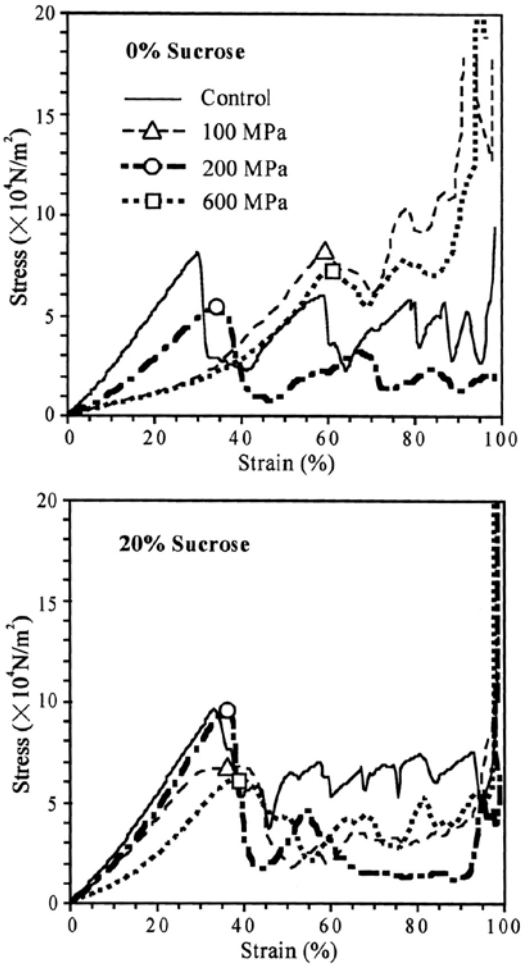


Fig. VII-3. Typical stress-strain curves of non-frozen and frozen-thawed A-gels

Control: non-frozen gel

A-gels: see Fig. VII-1.

When A-gels were frozen-thawed, the rupture stress decreased, while the rupture strain increased. When frozen at 0.1, 100 and 500 ~ 686 MPa then thawed, rupture strain increased, but it was similar to non-frozen gel when pressure-shift-frozen at 200 ~ 400 MPa then thawed. Thus, pressure-shift-freezing was effective in improving the quality of frozen-thawed gel.

On the other hand, when B-gels were frozen-thawed, both stress and strain were almost the same as non-frozen gel except for gels frozen at 0.1 MPa and 686 MPa. Thus, freezing tolerance of B-gel was greater than A-gel and C-gel. Also, when frozen-thawed, changes in texture was smallest to greatest in B-gel < A-gel < C-gel, respectively. As the concentration of sucrose increased, the stress and strain of all frozen-thawed gels improved.

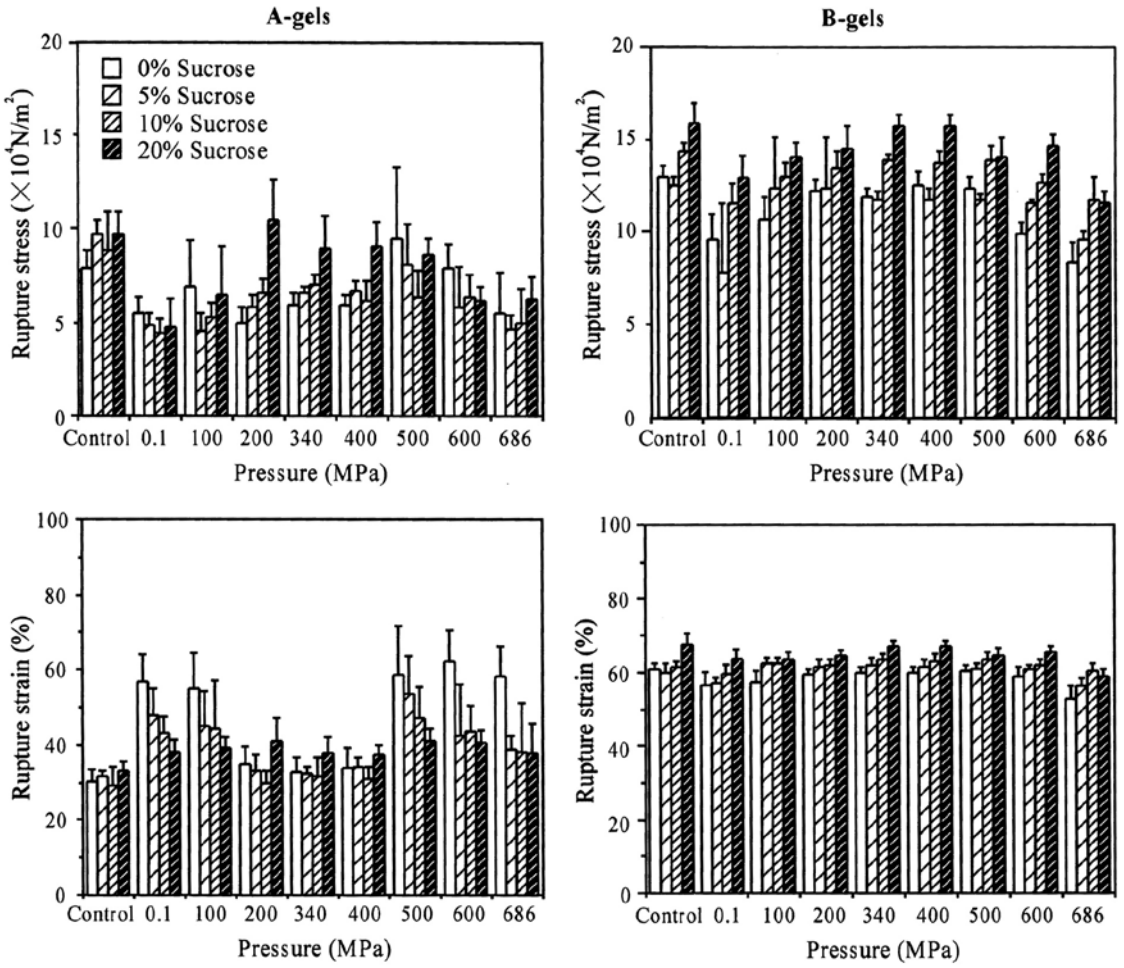


Fig. VII-4. Rupture stress and rupture strain of non-frozen and frozen-thawed carrageenan gels

Control: non-frozen gel

A-gels and B-gels: see Fig. VII-1.

5. Structure of frozen-thawed carrageenan gels

Cryo-scanning electron micrographs of ice crystal traces in frozen-thawed A- and B-gels are compared in Figs. VII-5 and VII-6, respectively. Ice crystal traces appear dark in the micrographs. When 0% sucrose-A-gel was frozen at 0.1 and 100 MPa, then thawed, a small amount of large ice crystal traces in gels was observed. Conversely, large amounts of small round ice traces formed when pressure-shift-frozen at 200 ~ 400 MPa. Thus, the size of ice traces began to increase above 500 MPa. With an increase of sucrose, the size of ice traces in these gels decreased.

On the other hand, small round ice traces were observed in all frozen-thawed B-gels (Fig. VII-6). With an increase of sucrose, ice traces were not observed in these gels. The size of ice traces was smallest to greatest in B-gel < A-gel < C-gel (Chapter VI), respectively. Ice traces in A- and B-gels were round, although those in C-gels were long and thin. The addition of locust bean gum prevented the growth of ice crystals, therefore, ice traces might be small and round.

The networks of frozen-thawed A- and B-gels are compared in Figs. VII-7 and VII-8, respectively. The network of both control gels was coarse, although that of C-gels was fine (Chapter VI), because the concentration of κ-carrageenan of C-gels (2%) was greater than A- and B-gels (0.7%). Also, the addition of locust bean gum led to a coarse network.

When A-gels were frozen, it was found that the network of only 0% sucrose-gels frozen 500 ~ 600 MPa contracted slightly. However, with the addition of sucrose, the structure of the gel improved. On the other hand, all frozen-thawed B-gel had the same coarse network as the non-frozen gel.

It appeared that the size of ice crystals affected the amount of syneresis and texture of the thawed gel, because, when ice crystals in the gel were large, the amount of syneresis increased and syneresis promoted shrinkage of the gel. As a result, texture became worse.

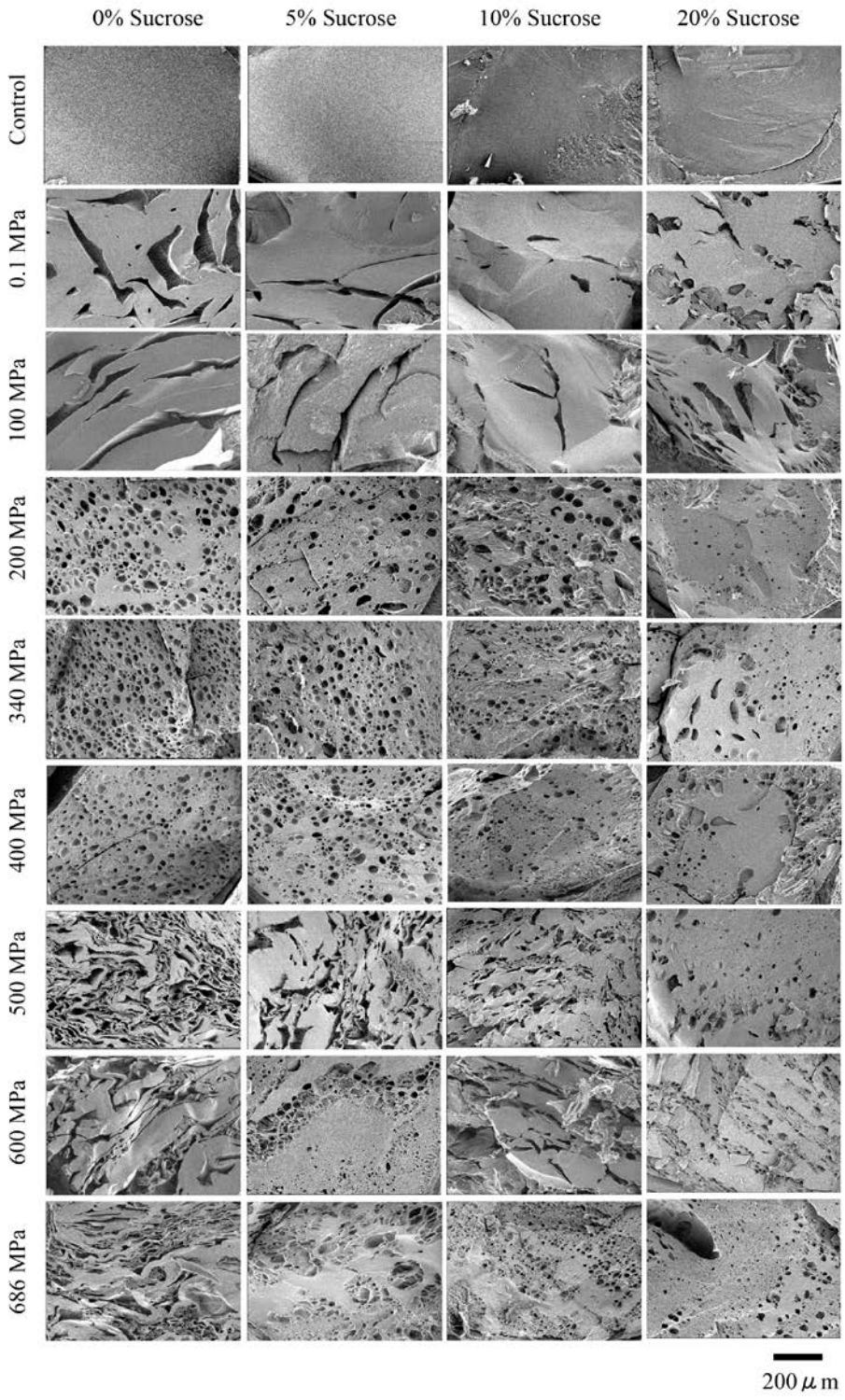


Fig. VII-5. Cryo-scanning electron micrographs of non-frozen and frozen-thawed A-gels

Control: non-frozen gel

A-gels: see Fig. VII-1.

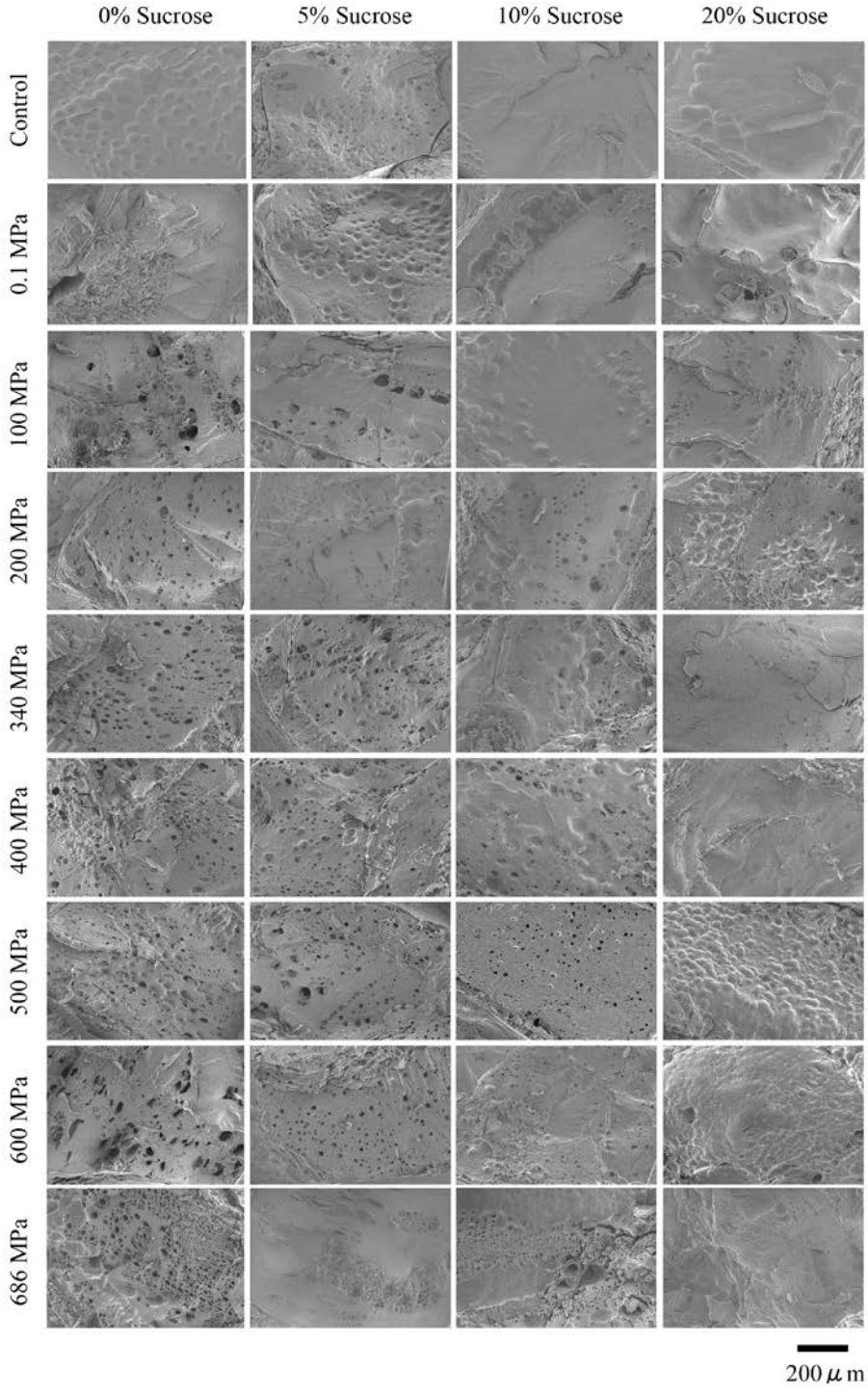


Fig. VII-6. Cryo-scanning electron micrographs of non-frozen and frozen-thawed B-gels

Control: non-frozen gel

B-gels: see Fig. VII-1.

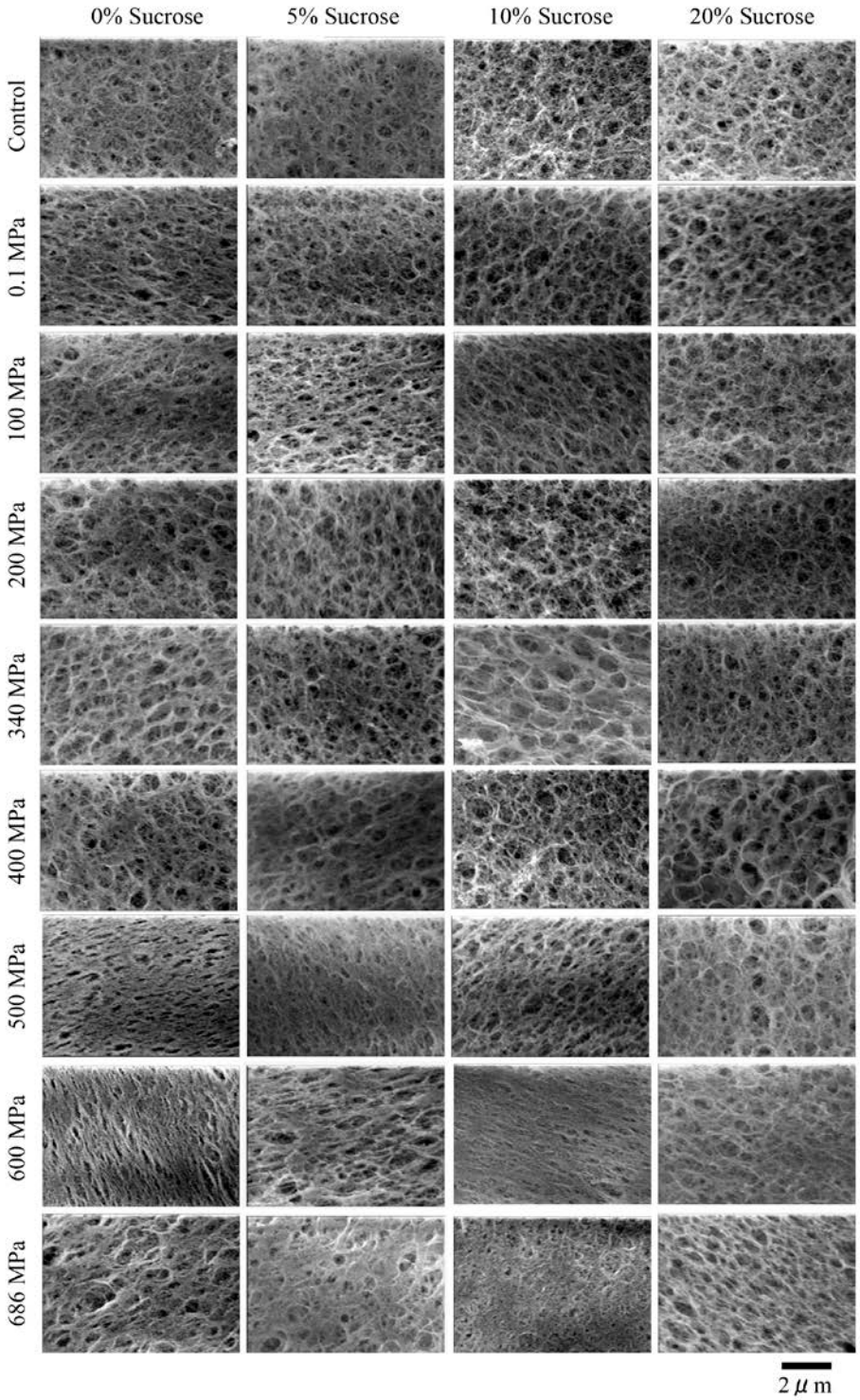


Fig. VII-7. Network-structure of non-frozen and frozen-thawed A-gels

Control: non-frozen gel

A-gels: see Fig. VII-1.

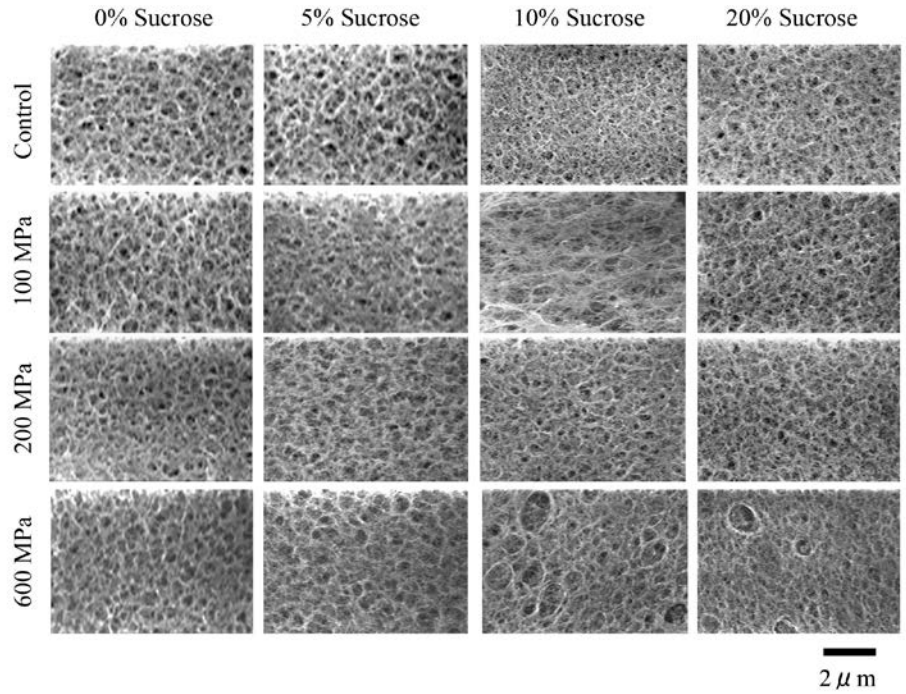


Fig. VII-8. Network-structure of non-frozen and frozen-thawed B-gels

Control: non-frozen gel

B-gels: see Fig. VII-1.

The development of ice crystals during cooling is integrated result of two processes: nucleation and crystal growth. The latter process is strongly dependent on the possibilities of heat and mass transfers through the system (Blond, 1985). Pressure-shift-freezing induces significant supercooling and enhances uniform ice nucleation throughout the sample (Cheftel *et al*, 2000). Supercooling enhances homogeneous nucleation. Also, pressure lowers the freezing point and reduces the rate of ice crystal growth. As the initial freezing temperature/supercooling temperature and freezing temperature were lower, ice crystal growth was prevented. Thus, as the size of ice crystals became smaller, texture was improved.

As the concentration of sucrose/glucose increases, the water-content in gel and initial freezing temperatures decrease. In consequence, freezing time was shortened. B-gel has glucose (8.3%) compared with A-gel (0%); therefore, the size of ice traces in B-gels was smaller than A-gels, and the amount of syneresis from B-gels was less than A-gels. Thus, freeze-thaw tolerance of B-gels was better than A-gels.

The depression of freezing point of solution is proportional to molarity. Molecular weight of sucrose and glucose is 342 and 180, respectively. Therefore, molarity of 8.3% glucose solution = 0.46 M. That of 5%, 10% and 20% sucrose solutions = 0.146 M, 0.29 M, 0.58 M, respectively. The addition of glucose was more effective on the depression of freezing point than that of sucrose. Therefore, when frozen-thawed, B-gels with 8.3% glucose maintained a better quality than A-gels without glucose.

The rheological properties of the polymeric matrix appear to be most important in the process of ice formation. In a truly elastic gel, nucleation at a given temperature is impeded, but the crystal growth is also strongly slowed down. The experimental study shows that the second of these two opposing processes exerts a predominant influence on the propagation rate of the ice front (Blond, 1985). As a result, water will freeze in an elastic gel as more numerous, smaller ice crystals than in a viscoelastic system or a solution (Blond, 1985). In this study, B-gel was most elastic, secondly A-gel. C-gel was brittle and not elastic. Therefore, freezing torelance was greatest to least in B-gels > A-gels > C-gels, respectively.

SUMMARY

The texture and structure of A-gels (the mixed gel of κ-carrageenan and locust bean gum) pressure-shift-frozen at 200 ~ 400 MPa and -20°C appeared better than that frozen at the other pressures. The addition of sucrose decreased supercooling and freezing temperatures, and prevented the growth of ice crystals; therefore, texture and structure of A- and B (PEARLAGAR-8)-gels with sucrose became good. Because B-gel has more glucose than A-gel, the quality of B-gel was better than A-gel when frozen-thawed. Also, texture and structure of frozen-thawed A- and B-gels were better than that of C-gels (κ-carrageenan gels without locust bean gum gels: Chapter VI). Thus, the additions of sucrose/glucose and locust bean gum to gel were effective in improving the quality of frozen-thawed κ-carrageenan gel.

SUMMARY AND CONCLUSION

With foods of high water content (i.e. egg, agar gel, carrageenan gel), damage to structures through freezing is extensive and the texture after thawing becomes unacceptable. When water is frozen at atmospheric pressure (0.1 MPa), volume increases. This volume increase by phase-transition (water to ice I) and the growth of ice crystal cause tissue damage during freezing at 0.1 MPa. However, under high pressure, several kinds of ices with different chemical structures and physical properties are formed. Thus, high pressure ices do not expand in volume during phase transition from water to ices. Therefore, the hypotheses that high pressure may be effective in improving the quality of frozen food was examined.

To determine the effect of high-pressure-freezing on quality (texture and structure), foods were frozen at 100 MPa (ice I), 200 MPa (liquid phase), 340 MPa (ice III), 400, 500, 600 MPa (ice V) or 686 MPa (ice VI) and ca. -20°C or 150 MPa and -15°C (liquid phase) or 100 MPa and -10°C (liquid phase). After reducing to atmospheric pressure, foods were stored at -30°C then thawed at 20°C. Texture and structure (cryo-SEM observation) were then compared with foods frozen (-20°C, -30°C or -80°C)-then-thawed at atmospheric pressure (0.1 MPa). Structural and textural changes due to high-pressure-freezing were also investigated in foods of various types; boiled egg (Chapter I), egg custard gel (Chapter II and Chapter III), egg yolk (Chapter IV), agar gel (Chapter V), carrageenan gel (Chapter VI and Chapter VII).

The structure, texture and amount of drip of boiled egg pressure-shift-frozen at 200 MPa and -18°C were compared to that frozen at atmospheric pressure in a pressure vessel at -18°C or to boiled egg frozen in freezers (-18, -30 or -80°C). Freezing time was shortest to longest, amount of drip was least to greatest and ice crystals were smallest to largest, when boiled egg was pressure-shift-frozen at 200 MPa and -18°C < frozen in a -80°C freezer < placed in a pressure vessel at 0.1 MPa and -18°C < -30°C freezer < -18°C freezer, respectively. Ice crystals were smaller in the pressure-shift-frozen samples than in the other treated samples. Thus, pressure-shift-freezing was effective in improving the quality of frozen boiled egg.

On the other hand, in non-sugar gels (egg custard gel, agar gel and carrageenan gel) frozen at 0.1, 100, 500, 600 and 686 MPa, texture and structure were seriously damaged. However, when all gels were frozen at 200 ~ 400 MPa, quality of frozen gels improved compared to gels frozen at 0.1 and 100 MPa (ice I). Also, the addition of sucrose to gels was effective in improving texture and structure of frozen gels. The

effects of high-pressure-freezing on the quality of frozen gels depended on the type of gels. The trace of ice crystals was not observed in all frozen-thawed ι-carrageenan gel, syneresis was slight, and the quality of gel was the same as non-frozen gel. Thus, freezing tolerance of ι-carrageenan gel was greater than κ-carrageenan gel. The difference in freezing tolerance seems to be related to the chemical structure of gels and the complicated manner in which water in gel networks behave. Change in texture after freezing depended on the type of gels; agar gel and carrageenan gel became soft, while egg custard gel became firmer. The softening of gels may have been due to irreversible damage to the structure by the growth of ice crystals, while the hardening of gels were caused by a concentration of protein through freezing.

When egg yolk was pressurized at 200 ~ 686 MPa and -20°C, the steady-flow viscosity, G', G'' and dynamic-viscosity of frozen-thawed egg yolk increased according to the increase of pressure, especially above 200 MPa due to pressure-denaturation of protein. However, pressure-shift-freezing at 100 MPa and -10°C, at 150 MPa and -15°C and the addition of sugar were effective in improving the quality of frozen egg yolk.

When foods were pressurized at 200 ~ 400 MPa and ca. -20°C or at 100MPa and -10°C, at 150MPa and -15°C, the foods did not freeze (supercooled), while after depressurization, foods froze quickly. Furthermore as the concentration of sucrose in the foods increased, the amount of water in the foods and freezing temperatures decreased, shortening freezing time. The initial freezing temperatures (super cooling temperature) became lower, and also freezing time (freezing rate) became shorter, ice crystals became smaller and texture improved. Thus, pressure-shift-freezing and the addition of sucrose to foods were effective in improving the quality of all frozen foods due to quick freezing.

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Jibu, Y., Teramoto, A., Kuwada, H. and Fuchigami, M. (2014), Effects of High Pressure and Addition of Sucrose on the Quality Improvement of Frozen-thawed Carrageenan gels, Part 1. Comparison of Kappa and Iota Carrageenan Gels, *Nihon Cyorikagaku Kaishi*, **47**, 143-154.

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