第14章　Textural Properties of Pressure-Induced Gels of Food Proteins Obtained under Different Temperatures

M. Dumoulin：京都大学（農学部）
小澤省吾：京都大学（農学部）
林　力丸：京都大学（農学部）　農博

Abstract

Gelation process of ovalbumin, egg yolk and soy protein was carried out by pressurization from 0.1 to 500 MPa under a temperature range from -20 to 100 °C, and hardness, cohesiveness and gumminess of gels formed were determined.

As a result, it was found that pressure-temperature dependency of protein gelation strongly depends on the protein. Apart from experiments carried out under - 20 °C, ovalbumin gelation showed a minimum at 25 °C whatever the pressure applied. However, egg yolk and soy protein did not show a minimum: gelation effectiveness continuously changed with the temperature. That is, soy protein gelation progressively decreased with lowering the temperature and egg yolk gelation showed even more drastic decrease. These unexpected behaviours may be attributed to the nature of these samples. Indeed, egg yolk contains a large amount of lipids (about 34 %) which may have a negative effect on the denaturation of proteins and/or on the interactions between the denatured side chains of proteins. Moreover, during the pre-incubation at low temperatures, aggregation among low density lipoprotein may have taken place and prevented them from pressure denaturation. In case of the soy protein, the high number of disulfide bridges within the fraction 11S may be responsible of this behaviour. Indeed, the extent of denaturation may be rather limited whatever the temperature of pressurization.

Experiments carried out at -20 °C have shown that the presence of ice seemed to affect the water holding capacity of the three dimensional network formed.

1. INTRODUCTION

In the search for new process to produce high quality food, high pressure has been found to be a promising technology. Potentially, high pressure can be used to inactivate vegetative cells of microorganism and some enzymes, or to induce the protein gelation without damaging the nutritional content or the freshness of the treated food.

The pressure-temperature diagram for protein denaturation has an elliptical shape [1]. Such a shape indicates that, under some conditions, heat can suppress pressure effects or pressure can suppress temperature effects. Apart from these extremities, it is also indicated that when the pressure increases, the temperature of cold denaturation increases and that of heat denaturation decreases. In case of egg white, it was found that a pressure up to 180 MPa can suppress heat-induced gelation [2]. Nevertheless, little is known about the pressure-temperature phase diagram of protein gelation in a wide range of temperatures and especially at subzero temperatures. Therefore, we carried out the pressure gelation process under a range of temperatures from -20 to 100 °C and a pressure range from 0.1 to 500 MPa for three typical food proteins, ovalbumin, egg yolk and soy protein, and we measured three basic textural properties, hardness, cohesiveness and gumminess.

2. MATERIAL AND METHODS

Crude ovalbumin obtained from Nacalai Tesque, Kyoto (lot M6A1932) was further purified by the procedure of Kekwick and Cannan [3] with modifications. The final purity was about 93% as judged by SDS-PAGE electrophoresis. Acid-precipitated soy protein was prepared from defatted soy flakes (Fujipro Co., Osaka) by the procedure of Bau *et al* [4]. Fresh eggs were obtained from a poultry.

Ovalbumin and soy protein were dissolved in distilled water by gentle mixing. The resultant solutions were introduced in polyethylene bottles (0.9 cm in diameter and 3.3 cm in height for ovalbumin and 1 cm in diameter and 2.6 cm in height for soy protein). Since gels of soy protein were particularly sticky and difficult to remove, bottles inside were coated with liquid paraffin before being filled with the protein solution. After being separated from the egg white, egg yolk was gently mixed and introduced in a polyethylene bottle (1.2 cm in diameter and 2.7 cm in height).

Pressure-induced gelation was carried out in a pressure bomb (inside size: 2.5 cm in diameter and 7.5 cm in depth) equipped with a hand-type oil pressure generator (Type KP5B, Hikari Koatsu Co., Hiroshima). Kerosene (Nacalai Tesque, Kyoto) was used as the pressure medium. The bomb was maintained at the desired temperature by immersing it in a water-ethanol bath. Before pressurization, the polyethylene bottles were incubated for 15 min at given temperatures.

Heat-induced gels were obtained by heating bottles for 10 min in a boiling water bath.

After the pressure or heat treatments, gels were kept for 30 min at room temperature and then carefully taken out of the bottle and cut in a disc of desired height (0.7 cm for ovalbumin gel, 0.8 cm for soy protein and 1 cm for egg yolk). Hardness, cohesiveness and gumminess were measured using a rheometer (RE 3305, Yamaden Co., Tokyo). The clearence was adjusted to 50 % of the initial height of the gel disc.

3. RESULTS

3.1 Ovalbumin

A 15% (w/w) solution of ovalbumin (pH 4.4) was submitted for 30 minutes to a pressure of 0.1, 100, 210, 300, 400 and 500 MPa under the following temperatures: -20, -5, 10, 25 and 50 °C. Gels formed under the following conditions exhibited enough firmness for the texture measurements: 210 MPa at -20 °C, 300 MPa at -5, 10 and 50 °C and 400 MPa at 25 °C. Table 1 summarizes the textural properties of gels formed under 500, 400 and 210 MPa.

Table 1
Textural properties of heat and pressure-induced gels of ovalbumin 15 % (w/w)
Disc size for texture measurement was 0.9 cm in diameter and 0.7 cm in height.
Clearance was adujsted to 0.35 cm.

Conditions for gel formation		Textural properties		
Temperature °C	Pressure[b] MPa	Hardness g/cm^2	Cohesiveness T.U.[c]	Gumminess T.U.[c]
50	500	647.5	0.804	520.77
50	400	275.2	0.745	204.93
25	500	290.2	0.771	222.36
25	400	158.1	0.746	117.85
10	500	379.1	0.746	282.42
10	400	412.9	0.753	321.83
-5	500	485.6	0.694	336.99
-5	400	476.2	0.738	367.18
-20	500	67.6	0.727	49.23
-20	400	102.2	0.752	76.76
-20	210	366.5	0.695	254.11
100[a]	0.1	580.3	0.800	464.30

[a] Heated for 10 min in a boiling water bath. [b] Pressurized for 30 min. [c] Texture unit.

For temperatures ranging from -5 to 50 °C, hardness showed a minimum at 25 ° C. This result is consistent with the usual elliptical diagram described for the pressure denaturation of protein which is minimum around room temperature [1]. This suggests that, in this range of temperatures, hardness of gel globally reflects the protein denaturation. Under 10, 25 and 50 °C, hardness of gels increased with increasing the pressure. This may be due to a greater protein denaturation induced by higher pressures and/or to more effective intramolecular interactions between denatured protein through their side chains. On the contrary, at - 5 °C, gels with the same high hardness were formed independently of the pressure applied, even under 300 MPa (data not shown). Nevertheless, at this temperature, no gel were formed under 210 MPa. This suggests that the minimum pressure needed to induce the gelation is not

dramatically reduced, but it seems that once the minimal pressure is applied, further increase in pressure does not improve the protein denaturation and/or the formation of the three-dimensional network. Pressure-induced gels, except those obtained under 300 MPa at 10 and 50 °C (data not shown), were more elastic and more difficult to break than heat-induced gels even if some of them were as hard as heat-induced gels.

At -20 °C, hard gels were obtained at 210 MPa, indicating that operating at this temperature allowed to reduce the minimal pressure needed for the protein gelation. Nevertheless, gels obtained were significantly different from those obtained under other temperatures: they were less white and less homogeneous and their structure was slightly porous with a slight syneresis. At 300, 400 and 500 MPa, beige- and sponge-like soft gels were obtained. Since, under -20 °C, water is frozen under 300 MPa (Type III ice) or 400 and 500 MPa (Type V ice), whereas it is still liquid under 210 MPa [5], the differences observed with gel texture depending on the pressure applied were probably due to those different states of water. This suggests that the ability of the three-dimensional network to hold water is affected by the presence of ice.

In general, it was found that both pressure and temperature only slightly affected the cohesiveness (Table 1). Nevertheless, all pressure-induced gels were slightly less cohesive than heat-induced gels. It is of our interest to note that gels formed at -20 °C under 300, 400 and 500 MPa exhibited a cohesiveness similar to other gels despite of the syneresis. This may be explained by the water re-entry into the gel after the first compression-decompression cycle.

Gumminess followed the same tendency as hardness.

3.2 Egg yolk

Egg yolk was pressurized for 30 min at 0.1, 100, 210, 300, 400, 450 and 500 MPa at -20, -5, 3, 10, 25, and 50 °C. At 10, 25 and 50 °C, gels being firm enough for texture measurements were formed respectively at 500, 400 and 300 MPa. The hardness of gels increased as the pressure increased at a given temperature. Similarly, at a given pressure, hardness increased with increasing the temperature. All pressure-induced gels were more yellow and considerably softer than heat-induced gels; and they were unbreakable by compressing between fingers. Concerning the cohesiveness, no special differences were noted among all the gels formed including heat-induced gels.

No gels were formed at temperatures below 10 °C, including subzero temperatures, even when egg yolk was pressurized at 500 MPa, although highly viscous solutions were obtained. Egg yolk consists of about 16% of protein [6]. It is a mixture of lipoproteins (about 57% of the total protein) which are phospholipids-protein complexes, and water soluble proteins (about 43 % of the protein content) [7]. It was shown that when egg yolk is frozen and thawed, a marked change in fluidity is observed. This change has been attributed to the aggregation of the low density lipoprotein [8]. Therefore, during the pre-incubation time at low temperatures and especially at subzero temperatures, such an aggregation may have taken place and prevented further pressure denaturation to occur. Moreover, egg yolk contains a large

amount of lipids (about 34 %) [6] which may have a negative effect on the denaturation of proteins and/or on the interactions between the denatured side chains of proteins.

3.3 Soy protein

A 17% (w/w) soy protein solution (pH 6.8) was pressurized under 0.1, 100, 210, 300, 400 and 500 MPa at -20, -5, 10, 25 and 50 °C for 30 min. Gels having enough firmness for texture measurements were formed under 210 MPa at -20 °C and under 300 MPa at -5, 10, 25 and 50 °C. Table 2 shows the textural properties of gels obtained under 210, 400 and 500 MPa.

Table 2
Textural properties of heat and pressure-induced gels of soy protein 17 % (w/w)
Disc size for texture measurement was 1 cm in diameter and 0.8 cm in height.
Clearance was adujsted to 0.40 cm.

Conditions for gel formation		Textural properties		
Temperature °C	Pressure [b] MPa	Hardness g/cm^2	Cohesiveness T.U. [c]	Gumminess T.U. [c]
50	500	156.9	0.820	139.98
50	400	136.7	0.896	122.07
25	500	113.4	0.950	107.77
25	400	108.1	0.942	102.90
10	500	84.0	0.930	78.27
10	400	70.4	0.870	61.26
-5	500	78.5	0.887	69.58
-5	400	59.7	0.829	49.37
-20	500	0.0	0.000	0.00
-20	400	0.0	0.000	0.00
-20	210	13.58	0.802	10.95
100[a]	0.1	317.2	0.912	289.26

[a] Heated for 10 min in a boiling water bath. [b] Pressurized for 30 min. [c] Texture unit.

All the pressure-induced gels were softer and more deformable without breaking and less white than heat-induced gels. Except for gels obtained at -20 °C, the gel hardness continuously increased as the pressure increased at given temperatures. Similarly, the hardness continuously increased with the temperature at given pressures except at 210 MPa. Therefore, the usual elliptical behaviour for protein denaturation was not reflected by the gel hardness.

Soy protein isolate is a mixture of proteins essentially composed of globulins, conglycinin (7S) and glycinin (11S), both having a complex oligomeric structure [9,10]. The extensive disulfide bridgind within the major globulin protein 11S, about

18-20 per molecule, which leads to a compactly folded molecule [11,12], may be responsible of the unexpected results obtained. Indeed, it can be speculated that, whatever is the temperature of pressurization, the extent of denaturation of the protein 11S may be rather limited. Nevertheless, the effects of the pressure to the disulfide bonds remain to be study. Moreover, it has been shown that the gelling properties of soy protein depend on the preferential interactions which occur between the different subunits [13]. Therefore, studies of the aggregation behaviour of the protein subunits upon pressurization, may give a new insight to explain the result obtained.

At -20 °C, a soft gel was formed at 210 MPa. It was smoother than gels obtained by pressurization at other temperatures. At higher pressures under - 20 °C, where water is under ice state, no gels were formed, confirming the hypothesis exposed for ovalbumin gelation.

4. CONCLUSION

It can be concluded from the data presented in this paper that pressure-induced gelation can be improved by operating at low or high temperature. Nevertheless, pressure-temperature dependency of protein gelation strongly depends on the protein. Apart from experiments carried out under - 20 °C, ovalbumin gelation showed a minimum at 25 °C whatever is the pressure applied, this result being consistent with the usual elliptical diagram described for protein denaturation; suggesting that hardness of gel globally refelects the the extent of protein denaturation.

However, egg yolk and soy protein did not show a minimum: gelation effectiveness continuously changed with the temperature. That is, soy protein gelation progressively decreased with lowering the temperature and egg yolk gelation showed even more drastic decrease. These unexpected behaviours may be attributed to the nature of these samples. Indeed, egg yolk contains a large amount of lipids (about 34 %) which may have a negative effect on the denaturation of proteins and/or on the interactions between the denatured side chains of proteins. Moreover, during the pre-incubation at low temperatures, aggregation among low density lipoprotein may have taken place and prevented them from pressure denaturation. In case of the soy protein, the high number of disulfide bridges within the fraction 11S may be responsible of this behaviour.

Experiments carried out at -20 °C have shown that the presence of ice seemed to affect the water holding capacity of the three dimensional network formed.

From a practical point of view gelation under low temperatures and especially under subzero temperatures may be of interest for food industries since depending on the nature of the protein :

- gels with new textures can be obtained when gelation is carried out under conditions where water is in ice state;
- gels can be conveniently formed at low pressure and low temperature;
- moderate pressures and low temperatures could be used to sterilize protein solutions without strongly modifing their texture.

Nevertheless, further experiments are needed for a better understanding the gelation process and reasons of the different pressure-temperature dependency observed among proteins.

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QUESTION and ANSWER

Q: Why egg yolk does not make a gel under pressure treatment at low temperature

A: The egg yolk consists of about 16% of protein among them about 57% are involved in phospholipids-protein complexes, the other ones being water soluble proteins. The lipidic content is near 34 % therefore, this high content of lipid may have a negative effect on the pressure-induced gelation under low temperature. They may protect proteins from denaturation or inhibit the intramolecular interactions between the denatured protein.

Then, the three main lipoproteins of egg yolk (lipovitellin, lipovitellinin and livetin) or the water soluble proteins may have a different pressure-temperature diagram of denaturation. If at least one of them is relatively pressure-stable under low temperatures, it may result in the inhibition of the three-dimensional network formation.

Finally, during the preincubation time at low temperatures and especially at subzero temperatures, low density lipoprotein may have aggregated. This aggregation may have protected them from pressure denaturation and then prevented the gelation.