Hardening of Salmon Egg Products Made from Fresh and Frozen Eggs

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Summary

“Ikura,” a seasoned salmon roe product in Japan, is produced mainly from eggs of the chum salmon caught during spawning migration. Owing to the high volumes of salmon caught during peak season, appropriate storage and processing methods are essential to maintaining egg quality. We investigated the processing properties of fresh or frozen-thawed salmon eggs at different maturity levels, focusing on hardening during storage. Our results confirmed that little hardening during storage occurred in eggs in the skein state in the abdominal cavity with a low maturity level, whereas substantial hardening occurred in the individual egg grains in the peritoneal cavity. Similar results were obtained using fresh and frozen salmon eggs, and the hardening of matured salmon eggs depended on the storage time and temperature. Based on SDS-solubility and SDS-PAGE analyses, the macromolecularization of egg membrane proteins occurred during egg hardening. The frozen eggs tended to harden faster than fresh eggs, and the hardening pattern was slightly different between these eggs; these differences may be explained by various factors, such as protein polymerization and degradation. The hardening of the egg membrane progressed after salting, and this phenomenon is likely to occur during salting and aging in industrial manufacturing.

Keywords: Freezing, Refrigeration, Mature, Storage, Salting, Salmon egg, Physical property, Egg membrane

1. Introduction

“Ikura,” a seasoned salmon roe product in Japan, is produced mainly from eggs of the chum salmon caught during spawning migration. Fresh eggs should ideally be processed within 6 h after salmon are caught to produce high-quality products 1). If storage conditions are not appropriate, egg granules easily collapse and stickiness increases. This can also lead to a decrease in yield. Furthermore, the egg membrane occasionally exhibits hardening during storage, which decreases commercial value 2, 3). To address these issues, it is necessary to process salmon eggs immediately after capture. However, it is difficult to process high volumes of salmon owing to a limited production capacity during the peak period for catching, and salmon eggs often remain unprocessed for 1 or 2 days, despite the risk of quality deterioration. Salmon eggs also tend to be damaged by freezing, and frozen eggs are rarely used as raw materials. However, the freezing technique can distribute the manufacturing process over a wider period and lead to the stabilization of quality. Elucidating the quality characteristics of salmon roe after freezing treatment is crucial to develop technologies for manufacturing high-quality products from frozen eggs and for their commercial viability. Salmon roe products are classified into several grades, characterized by differences in textural properties, such as hardness. In the case of high-grade products, the egg membranes are soft and easy to eat, whereas low-grade products are hard. Salmon eggs may exhibit membrane hardening during storage or the manufacturing process. Ueda and Tsuchiya 3) have reported that hardening is likely to occur in the eggs of salmon whose maturation has progressed, especially during the egg regressing depending on the storage temperature and time, accompanied by the polymerization of proteins contained in the egg membrane. The glutaminyl-lysine group is formed in the egg membrane protein 4) and egg hardening is inhibited when monodansyl cadaverine, which is a substrate of transglutaminase (TGase), is introduced into the egg 5). Based on these previous findings, the action of TGase is likely a cause of hardening; however, the phenomenon and its detailed mechanism are still unclear.

In this study, the properties of fresh or frozen-thawed eggs from salmon with different maturity levels were evaluated based on changes in physical characteristics during storage.

2. Materials and Methods

2.1 Salmon

Figure 1 summarizes the sample preparation method. Chum salmon (Oncorhynchus keta) caught by stationary nets in Kamaishi Bay on the Sanriku coast in the Tohoku region along the Japanese Pacific in November 2014 was used. Fish were divided according to maturity level (A: immature, B: slightly mature); grade A salmon (A1 to A5) and grade B salmon (B1 to B5) were collected. The salmon grade was determined by the seller according to market selection criteria based on secondary sex characteristics, mainly on the body surface.
2.2 Preparation of fresh eggs and frozen eggs

For each salmon individual, the skin was cleaved along the lower side of the abdomen toward the pelvic fins from the vent using a kitchen knife. Eggs were recovered from the abdominal cavity and washed with a 0.85% sodium chloride solution (a physiological saline solution). Egg capsules were pressed against a nylon mesh with a mesh size of 1 cm; only the egg grains passed through the mesh were separated. Eggs washed with physiological saline were used as fresh eggs and were prepared within 4 h of fishing. Eggs were frozen by an air blast freezer at -70°C and stored at -40°C until use within 2 months. Frozen eggs were thawed in ice water for 10 min before storage at the respective temperatures.

2.3 Storage test

To investigate the influence of storage, each salmon egg was sealed in a polypropylene cup to prevent drying and stored at 5°C, 15°C, and 25°C for 0-48 h. Salmon eggs were sampled over time during storage (0, 6, 24, and 48 h) and analyzed. A flowsheet for sample preparation and the storage test is shown in Fig. 1.

2.4 Preparation of salted eggs

For experimental samples, B1 and A5 salmon eggs stored at 5, 15, and 25°C for 24 and 48 h, showing a difference in maturity, were used. These eggs were stirred, immersed in a 5-fold amount of saturated saline for 10 min in a cold room at 0 °C. After removing the saline solution, the salted eggs were kept in plastic bottles and stored in a cold room at 0°C for 24 h.

2.5 Measurement of physical properties

The physical properties of eggs obtained under each condition were immediately measured. One grain of salmon egg was placed on the table of a Creep Meter (Rheoner II, RE2-33005 BX-2S; Yamaden Co., Ltd., Tokyo, Japan), compressed with a cylindrical plunger with a diameter of 12 mm at a constant rate of 1 mm/s until the egg membrane was destroyed, and the breaking strength (N) was determined. The average and standard deviation of the values for each test group were obtained and the data were statistically evaluated to test the significant difference (n = 10).

2.6 SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and solubility of egg membrane proteins against SDS sample buffer

The solubility of salmon egg membrane proteins against SDS sample buffer was determined according to the methods of Ueda and Tsuchiya. To prepare the egg membrane, eight grains of salmon eggs were crushed, washed in 20 volumes (w/w) of isotonic buffer (134.5 mM NaCl, 3.8 mM KCl, 3.2 mM CaCl$_2$ –NaHCO$_3$, pH 7.3), and centrifuged at 1,000 × g for 10 min to recover whole egg membranes in the precipitate. The washing procedure was repeated three times. To the egg membrane, 5 ml of 2% SDS-8 M urea, 2% β-mercaptoethanol, and 20 mM Tris-HCl buffer solution (pH 8.0) (SDS sample buffer) were added, homogenized at 10,000 rpm for 1 min using a homogenizer (PT10-35GT; Kinematica, Luzern, Switzerland), and heated in a boiling bath for 2 min. The sample was stirred using a magnetic stirrer for 20 h to dissolve the egg membrane protein, and centrifuged at 3,100 × g for 60 min. The protein contents in the sample before centrifugation (a) and in the supernatant (b) obtained by centrifugation were measured by Lowry's method. The dissolution rate against the SDS sample buffer was calculated as the ratio (%) of the amount of protein after centrifugation (b) to that before centrifugation (a). SDS-PAGE was performed by loading each sample after centrifugation (b) onto each well of a 12.5% polyacrylamide gel. The amount of loaded sample was adjusted to the amount of egg membrane protein before centrifugation of the non-solubilized protein fraction (a).
3. Results and Discussion

3.1 Characteristics of the salmon and eggs used as raw materials

The body weights of the salmon used in the experiment were 4.24-5.8 kg for grade A and 4.36-6.56 kg for grade B (Table 1). Fig. 2 shows the various forms of salmon eggs based on maturity. Based on the description by Hatano \(^6\), the size range was slightly larger than the standard size of salmon groups during spawning migration. The grade B eggs with high maturity tended to have a larger weight and diameter than the grade A eggs. However, there were no strong relationships between these parameter values and maturity.

3.2 Changes in the physical properties of eggs depending on maturity

Figure 3 shows changes in the breaking strength of salmon eggs obtained from A1–A5 and B1–B5 salmons with different maturity levels over time during storage at 15°C. The breaking strength of eggs immediately after they were removed from the abdominal cavity of salmon was low and did not differ with respect to maturity level. However, as the storage duration increased, there were clear differences in the hardening pattern among individuals. In the case of A1 salmon, which lacked secondary sex characteristics based on the appearance of the fish body, egg grains were in an individual state, with regression of the egg capsule membrane; the breaking strength of the egg increased obviously during storage at 15°C. When storing B1 eggs at 15°C, the breaking strength increased 8-fold after 48 h compared with that before storage; however, B2–B5 salmons, which indicated secondary sex characteristics based on the appearance of the fish body, egg grains were in immature state, and the hardness of these eggs did not change. These results indicated that the ease of egg hardening during storage was not necessarily correlated with the degree of maturity, as determined by secondary sex characteristics of the fish body but showed a clear relationship with the maturation of the egg. Osanai et al. \(^7\) classified oocytes at various maturation phases into four stages, and in salmon caught in the bay on the coast of Iwate Prefecture in Japan, eggs at these four stages of maturation have been observed simultaneously. As maturation progresses, the egg membranes of follicle cells and ovarian capsule cells shrink to the dorsal side within the abdominal cavity, and the eggs become independent in the abdominal cavity at the final stage.

Based on the results of this study, differences in salmon appearance and egg maturity were observed, but eggs at an advanced maturation stage tended to become hardened. To further clarify the properties of salmon eggs at different maturity levels, A5 and B1 having the same diameter of eggs were selected as representative immature and mature eggs, respectively, and subjected to further analyses.

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Table 1

<table>
<thead>
<tr>
<th>Grade</th>
<th>Body weight (kg)</th>
<th>Skin or whole eggs weight (g)</th>
<th>Arrangement of eggs**</th>
<th>Diameter of eggs (mm)</th>
<th>Weight of eggs (g)**</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>5.44</td>
<td>819.5</td>
<td>Individual</td>
<td>4.96 ± 0.27</td>
<td>0.246 ± 0.02</td>
</tr>
<tr>
<td>A2</td>
<td>4.38</td>
<td>877.5</td>
<td>Skin shape</td>
<td>5.86 ± 0.38</td>
<td>0.217 ± 0.01</td>
</tr>
<tr>
<td>A3</td>
<td>5.8</td>
<td>894.5</td>
<td>Contiguous</td>
<td>5.54 ± 0.37</td>
<td>0.261 ± 0.01</td>
</tr>
<tr>
<td>A4</td>
<td>4.58</td>
<td>1009.5</td>
<td>Contiguous</td>
<td>6.07 ± 0.52</td>
<td>0.252 ± 0.01</td>
</tr>
<tr>
<td>A5</td>
<td>4.24</td>
<td>803.5</td>
<td>Skin shape</td>
<td>5.20 ± 0.44</td>
<td>0.242 ± 0.03</td>
</tr>
<tr>
<td>B1</td>
<td>5.32</td>
<td>795.5</td>
<td>Individual</td>
<td>5.13 ± 0.22</td>
<td>0.294 ± 0.02</td>
</tr>
<tr>
<td>B2</td>
<td>4.58</td>
<td>964.5</td>
<td>Skin shape</td>
<td>6.45 ± 0.47</td>
<td>0.258 ± 0.01</td>
</tr>
<tr>
<td>B3</td>
<td>6.56</td>
<td>1038.5</td>
<td>Contiguous</td>
<td>6.39 ± 0.43</td>
<td>0.295 ± 0.01</td>
</tr>
<tr>
<td>B4</td>
<td>4.8</td>
<td>1064.5</td>
<td>Skin shape</td>
<td>6.16 ± 0.60</td>
<td>0.268 ± 0.01</td>
</tr>
<tr>
<td>B5</td>
<td>4.36</td>
<td>929.5</td>
<td>Skin shape</td>
<td>6.79 ± 0.36</td>
<td>0.294 ± 0.01</td>
</tr>
</tbody>
</table>

* & ID: A grade, Low maturity level; B grade, Advanced maturity level.

The salmon grade was determined by the seller according to market selection criteria based on secondary sex characteristics appearing mainly at the body surface.

** The classification of eggs based on maturity using comparative chart \(^2\).

*** Including the egg capsule membrane.

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*Fig. 2* Forms of salmon eggs by maturity.

*Fig. 3* Change in breaking strength during the storage of eggs, obtained from salmon individuals with different maturity levels. Storage at 15 °C for: ■, 0 h; □, 6 h; △, 24 h; □, 48 h. Error bars represent standard deviations.
3.3 Changes in egg properties by freezing

Figure 4 shows the changes in breaking strength for fresh and frozen-thawed eggs classified as A5 (immature) and B1 (slightly mature) during storage at 5°C, 15°C, and 25°C. For the A5 stage, both fresh and frozen eggs showed no increases in breaking strength at any temperature during storage. For eggs classified as B1, a different pattern was observed. In fresh eggs, when stored at 5°C, the breaking strength did not change substantially until after 48 h. In frozen eggs, the breaking strength increased during storage and eggs became harder than fresh eggs. In contrast, the breaking strength of fresh eggs was significantly greater than that of frozen eggs during storage at 15°C and 25°C.

Changes in the solubility of egg membrane proteins in SDS sample buffer during the storage of salmon eggs at 25°C are shown in Fig. 5. In the case of B1, the dissolution rate with respect to the SDS sample buffer decreased with hardening, as determined by the breaking strength. The SDS-PAGE pattern for proteins in the membranes of eggs stored at 25°C is shown in Fig. 6. In B1 eggs, a 47-kDa protein in the egg membrane decreased as storage progressed, and a 96-kDa protein increased. These results suggested that proteins in the egg membrane were macromolecularized during storage. In A5 eggs, there was almost no change in the composition of proteins in the egg membrane, based on molecular weight, during storage. However, as the storage time increased over 6 h, an increase in a component of less than 47-kDa was observed, and the level of this component was higher in frozen eggs than in fresh eggs. In the frozen egg membranes, macromolecularization and proteolysis likely occurred simultaneously under the same storage conditions, and this can explain the lower breaking strength for frozen eggs stored at 25°C than for fresh eggs. Shaban 8) reported that egg hardness varies depending on the freezing temperature. Uchiumi et al. 9) reported that the slow freezing of Alaska pollack eggs results in deformation and increased dripping loss. During slow freezing, as time passes, the zone of maximum ice crystal formation becomes longer. Large ice crystals form at the beginning of storage, further growing during the storage period; these ice crystals easily break the structure of the egg membrane. It was hypothesized that the changes in the physical properties of salmon egg membranes during storage are affected by complex factors, such as TGase, which contributes to the polymerization of proteins, proteases that decompose proteins, and physical destruction due to the growth of ice crystals.

3.4 Changes in the physical properties of eggs after salting

Figure 7 shows the changes in the breaking strength of fresh eggs, stored at 5°C, 15°C, and 25°C,
and salted eggs after salting. In the case of immature A5 eggs, the breaking strength became slightly higher during storage and after salting, but remained considerably low, and there was almost no effect of salting or storage temperature. On the other hand, in the case of B1 eggs in which maturation progressed, the breaking strength increased during the storage at all storage temperatures, and greater breaking strength was observed after salting eggs than in fresh eggs. These results indicated that the hardening of eggs hardening was promoted by salting.

Tsuchiya 4) reported that the glutaminyl-lysine group content in the egg membrane of salted eggs of rainbow trout is 1.5 times higher than that without salting. It is presumed to be the same mechanism as the result obtained using salmon eggs in this experiment. We hypothesized that the activation of enzymes, such as TGase, and the protein structural changes of the egg membrane as a substrate of activated enzymes are related to the hardening of salmon eggs.

Although further studies are needed to elucidate the mechanism underlying the phenomenon observed in this study, these findings may have important implications for the seafood industry.

4. Conclusions

1) The relationship between the maturation of salmon roe serving as a raw material for salted products and the ease of hardening was examined. Minimal hardening occurred in eggs in the skein state in the abdominal cavity with a low maturity level, whereas substantial hardening occurred in egg grains that are independent in the peritoneal cavity for both fresh and frozen salmon eggs.

2) The hardening of mature salmon eggs increased depending on the storage time and storage temperature. Based on SDS-solubility and SDS-PAGE analyses, egg membrane proteins became macromolecularized during hardening for both fresh and frozen eggs.

3) Although the frozen eggs tended to harden faster than fresh eggs, the hardening pattern differed; these differences can be explained by complex factors, such as the polymerization of proteins, degradation of proteins by proteases, and physical destruction by ice crystals.

4) The hardening of egg membranes increased by salting. The same phenomenon is expected to occur during industrial-scale salting and aging.

References