

Effect of Dehydrofreezing on the Quality of Frozen Walleye Pollock Ovaries Used as a Raw Material for Preparing *Tarako* Products

Yu UCHIUMI *

Toru SUZUKI

*Department of Food Science and Technology, Faculty of Fisheries, Tokyo University of Marine Science and Technology
(4-5-7 Konan Minato-ku Tokyo 108-8477)

Summary

Eating pickled roes of various fishes is a popular custom in Japan. *Tarako* is a salted or salt-seasoned product prepared from walleye pollock and/or cod ovary, and accounts for the majority of pickled roe products in the Japanese market. These products are prepared using 90% or more frozen ovaries as the raw material. Freezing damage, however, is a serious issue in *tarako* production. To prevent freezing damage of roes, cryoprotectants such as sugars are added before freezing. Further, it has recently been demonstrated that dehydrofreezing effectively prevents freezing damage in many foods. In this study, the use of high concentrations of NaCl solution in the dehydrofreezing process during *tarako* production was assessed. Walleye pollock ovaries were dehydrated using 3%, 10%, 17% and saturated NaCl solution before freezing and storage. The ovaries were evaluated after thawing by drip loss, alteration in color and microscopic observation. The results revealed that the freezing damage decreased with increase in the concentration of NaCl solution.

Keywords: Dehydration, Freezing, Ice crystal, Microscopic observation, NaCl concentration, *Tarako*, Walleye pollock ovary

1. Introduction

Eating pickled roes of fishes, known as caviar in markets worldwide, is a traditional custom in Japan. Some raw roes or ovaries are salted and/or seasoned and the market value of these products is usually higher than that of muscle meat. *Tarako* is the most popular and widely consumed pickled roe product; it is produced in much higher quantities than the roe and ovary products of salmon and herring [1].

Tarako is typically prepared using frozen ovaries of walleye pollock, sourced mainly from Russia and Alaska. The harvest season in these regions runs from October to the end of January. Optimal ovary harvesting for *tarako* products takes place between mid-November and mid-December [2]; therefore, it becomes necessary to freeze and store the raw materials. However, freezing damage of roes is a serious concern and may compromise the quality of the raw material.

The ovaries are packed in boxes (2 kg each) aboard the fishing vessel after removing blood and foreign substances using seawater. They are then frozen at -40°C using a contact freezer and stored at approximately -20°C before they are transported to Japan. To regulate the production amount, the ovaries are often stored from a few months to a few years before being supplied for the *tarako* manufacturing process.

During the *tarako* manufacturing process, the frozen ovaries are thawed overnight in about 3% NaCl solution under 10°C , subsequently salted with

15 - 17% NaCl solution, and seasoned with sugars, salt and amino acids. All immersion processes are usually carried out at under 10°C to be completed manufacturing *tarako* products. Then they are frozen at -40°C and stored at approximately -20°C in normal.

Previous research has demonstrated that freezing damage negatively affects taste as well as flavor of roes [3]. Therefore, several techniques such as high pressure treatment [4] or the addition of sodium aspartate, organic acids etc. [5, 6] have been proposed to prevent freezing damage of roes. However, these techniques have not yet been implemented in industries.

Hayabuchi reported that during the manufacturing process of *tarako*, the NaCl concentration of the pickle and the freeze/thaw process are the dominant factors that affect the microscopic structure of the roe membrane [7]. In particular, several roes incur significant damage when the ovary is frozen using 4 - 6% NaCl solution [5].

However, NaCl immersion treatment has been shown to be the most effective for the protection of egg membrane of salmon roe from freezing damage [8]. Thus, the effect of NaCl treatment on frozen ovaries is controversial. To the best of our knowledge, the dehydration effect of NaCl immersion treatment on roes has rarely been investigated in previous studies.

Dehydration before freezing, termed as dehydrofreezing, has recently been proposed to prevent freezing damage of perishable foods such as

fruits and vegetables [9, 10]. While a few studies have investigated the utilization of dehydrofreezing for marine products, Kojima found it to be effective for fish muscle meats as well [11]. However, no studies have reported the dehydrofreezing of fish roes. In this study, we investigated the effect of dehydration using various concentration of NaCl solution before freezing the roes of walleye pollock. The use of NaCl solution was justified by the ordinal use of NaCl in salting operations during the manufacturing process of *tarako*. Roes dehydrated with different concentrations of NaCl solutions prior to freezing at -40°C were stored at -25°C for up to 90 days. After thawing, the freezing damage of roes was evaluated by drip loss, alteration in color and microscopic observation.

2. Materials and methods

The *Mako* [2] ovaries, which were thought to be the best maturity for the *tarako* material, produced in Hokkaido on the same day were used in this research. Written in detail, walleye pollock (*Theragra chalcogramma*) was harvested in the sea near Abashiri, Hokkaido. In order to retain freshness, catching walleye pollock and gathering the ovaries was done on the same day. The ovaries were transported by air under chilled conditions to our laboratory in Tokyo on next day from the catch in Hokkaido. The dehydration process of the ovaries is described below and a flow chart is shown in Fig. 1.

After removal of foreign substances as well as any excess water from the fresh ovaries using a paper towel, the samples were randomly divided into 5 groups and weighed. The samples in each group were individually packaged in commercial zippered plastic bags (273×268 mm; Ziploc, Asahi KASEI Home Products Corporation, Tokyo, Japan). NaCl (sodium chloride; Wako Pure Chemical Industries, Ltd., Osaka, Japan) solutions of 3%, 10%, 17% [w/w] and a saturated concentration were prepared at a room temperature of $18 - 20^{\circ}\text{C}$. A volume of 0.4 L NaCl solution per kg of ovaries was used for the immersion process. The NaCl solutions were added to each plastic bag and the bags were refrigerated at 10°C for 24 hours. A blank sample without immersion treatment was immediately frozen at -40°C .

After immersion in NaCl for 24 hours, the samples were removed from the solution and refrigerated at 10°C for 24 hours to drain solution well. After weighing, the samples were separated and placed in plastic bags (196×177 mm; Ziploc, Asahi KASEI Home Products Corporation, Tokyo, Japan) as shown in Fig. 2. And the actual measurement value of weight change was calculated to the value per 100 g of the samples, which was shown as weight change ratio in Fig. 3. The samples were then frozen at a -40°C freezer (MDF136;

SANYO Electric Co., Ltd, Osaka, Japan) for 24 hours before being frozen at -25°C freezer (SCR-R451G; SANYO Electric Co., Ltd, Osaka, Japan) up to 90 days. After 0, 10, 30 and 90 days period, drip loss measurement, light microscopy observation and determination of color alteration were performed at the room temperature of 24°C . Prior to measurements, the samples were thawed by holding them under running water at 17°C for 20 minutes.

2.1 Drip loss measurement

Drip loss was measured as the increase in the weight of the absorbent paper (Whatman 90 mm Diameter; Cat No 1002-090, Advantech Co., Ltd., Japan) and paper towel (S-200 mini; NIPPON PAPER ARECIA Co., Ltd, Tokyo, Japan). The pre-weighed paper was placed in the plastic bag containing the sample and allowed to absorb any drip from the sample for 5 min. The moisture around ovary and roes are wiped off with the pre-weighed paper towel. The paper and the paper towel were then weighed again and the amount of drip was calculated per 100 g of sample according to the following equation;

$$\text{Drip loss [g/100 g]} = 100 \times (\text{FP} - \text{IP}) / (\text{SW})$$

where FP is the final weight of the paper and the paper towel, IP is the initial weight of the paper and the paper towel, and SW is the sample weight.

2.2 Color measurement

The change in the color of the ovary was measured using a colorimeter (Chroma Meter CR-200; Konica Minolta, Inc., Osaka, Japan). Three different regions of each ovary were selected for monitoring and the average values of color parameters a^* and b^* were evaluated.

2.3 Light microscopy observation

The roes were separated from the ovary and carefully placed on the prepared glass slides using a small clean spatula. They were then microscopically observed (BX51TF; Olympus Corporation, Tokyo, Japan) and images were obtained using the digital camera for a microscope (DS-L2; Nikon Corporation, Tokyo, Japan).

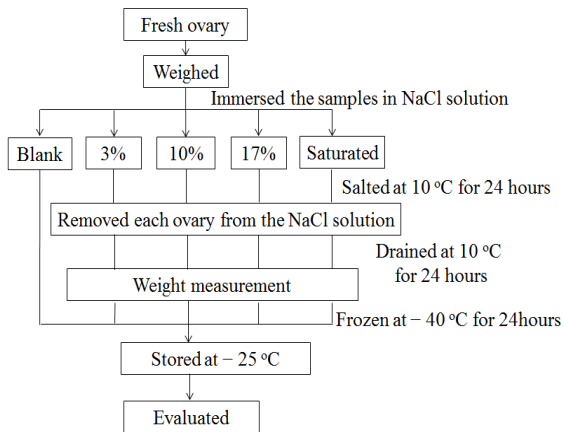


Fig. 1 Flow chart outlining the process from immersion treatment to sample freezing. The immersion treatment of fresh ovaries starts from the top of this flow chart. The blank sample was subjected to the same treatment as the other samples, except that it was not immersed in any solution before being placed in the plastic bag. Each NaCl solution used was prepared at a room temperature of 10°C. They were stored for 0 day (right after frozen), 10 days, 30 days and 90 days at maximum.

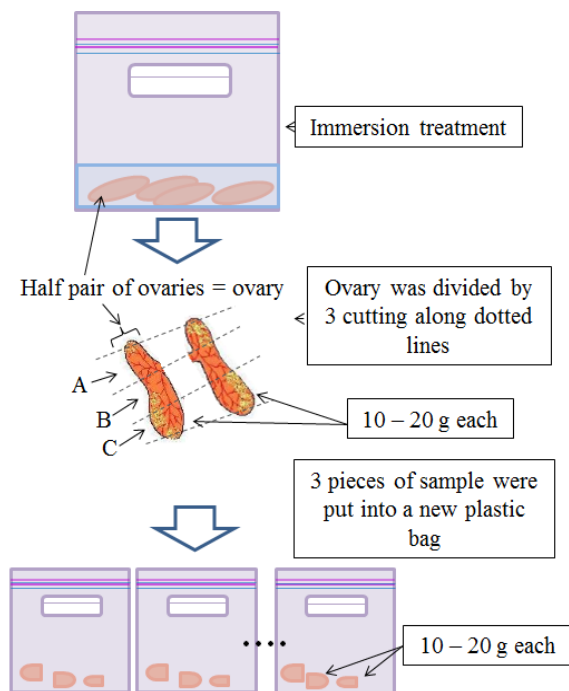


Fig. 2 The parting method of the samples after immersion treatment. The size and weight of the divided sample was approximately 25 × 30 mm and 10 - 20 g. Three pieces of sample were obtained from one ovary as A, B and C, and each sample was randomly parted into a new plastic bag.

3. Results and discussion

The change in the weight ratio before and after the immersion process for all samples except the blank is shown in Fig. 3.

Although the sample ovaries immersed in 10% or more concentrated NaCl solutions showed up to 20% increase of weight, their weight remained independent of the increase in the concentration of NaCl at 24 hours immersion. In contrast Fig. 4 (A), shows the shrunken appearance of roes after immersion in NaCl at increased concentrations. Thus, the results presented in Figs. 3 and 4 (A) appear contradictory. We attribute the increase in the weight of the ovary to the absorption of moisture by the theca cells or connective tissue during the immersion process since it is one of the distinctive characters for those tissue and cells to hold much moisture [12].

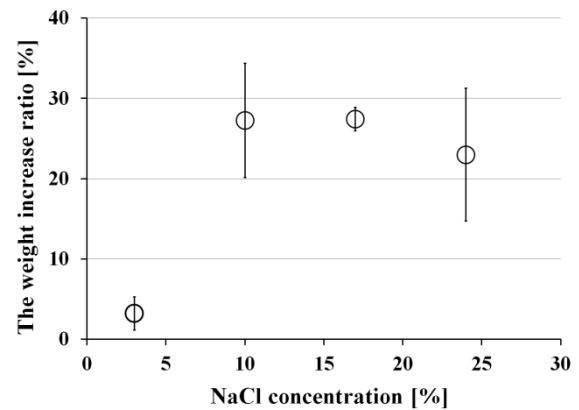


Fig. 3 Change in the weight of the sample ovaries after 24-hour immersion treatment (n = 3). This shows the ratio of weight change of the samples treated with different concentrations of NaCl solution. All samples put on weight as they got all positive value. It was increased up to 25% for the samples treated with 10% or more NaCl solution and it looked final value of weight increase.

Thus, it is notable that NaCl immersion treatment led to the dehydration of roes despite the increase in ovarian weight.

The drip loss of each sample after storage is shown in Fig. 5. After 24 hours, all samples showed minimal (< 0.5 [g/100 g]) drip loss. Drip loss of the blank sample increased after 10 days, while that of the sample immersed in 3% NaCl sample increased up to 11 [g/100 g] after 90 days. Drip loss of the sample immersed in 10% NaCl increased, reaching 6 [g/100 g] at 90 days, however samples immersed in saturated and 17% NaCl solutions showed less than 0.1 [g/100 g] drip loss after 24 hours, which showed no change after immersion for 90 days. Figure 3 (B)

shows the microscopic images of each roe after the 90-day storage. Ruptured roes and small fragments were observed in the samples treated with 3% and 10% NaCl solutions and in the blank sample. In contrast, the roes treated with 17% and saturated NaCl solutions were not ruptured. This result supports the observation that samples treated with 17% or more concentrated NaCl solutions showed little drip loss; it is thought that these samples were dehydrated, preventing the growth of ice crystals inside the roe and their subsequent rupture. On the contrary, formation of ice crystals was observed during storage in the samples treated with 10% or less concentrated NaCl solutions, resulting in the rupture of the roe. The images of each sample at each storage time was correlated with the amount of drip loss; ruptured roes exhibited high drip loss. This result corresponds with the results of a previous study, in which drip loss was associated with both storage time and freezing damage [3]. In the previous study, the initial size of ice crystals formed in the roes was thought to be related to the speed of freezing as the size of ice crystals were determined by freezing speed [13]. Drip loss in the sample frozen slowly at -20°C was higher even at the initial stage. The drip loss of the commercially frozen sample increased over the 30-day storage duration, whereas the sample quick-frozen by liquid nitrogen showed minimal drip loss even after 90-day storage [3]. And since it was investigated that freezing was not the main cause of destroying the roe membrane of fish [7], it was supposed that there was the other external agents to rupture roes. Thus this result could be explained as follows: when the size of ice crystals formed inside the roe exceeded the diameter of the roe during storage, membrane rupture occurred, resulting in increased drip loss. Moreover, the microscopic images of roes frozen at different freezing speeds support the result of drip loss in this research.

In this study, it was speculated that the formation of ice crystals in samples treated with 3% and 10% NaCl solutions, and the blank sample increased with the duration of storage, resulting in rupturing of the roe. On the other hand, samples treated with 17% or more concentrated NaCl solution were sufficiently dehydrated, preventing the formation of ice crystals exceeding the diameter of the roe; therefore, ice crystal formation in these samples did not proceed to the point of rupture, even after 90-day storage. Moreover, it has previously been reported that the membrane structure of the roes of walleye pollock is altered to increase its elasticity during the manufacturing and salting processes [7], in which the concentration of the salting solution is generally 16 - 17%. Thus, we propose that the elasticity of the membrane of samples treated with 17% or more concentrated NaCl solutions increased, resulting in reduced susceptibility to rupture.

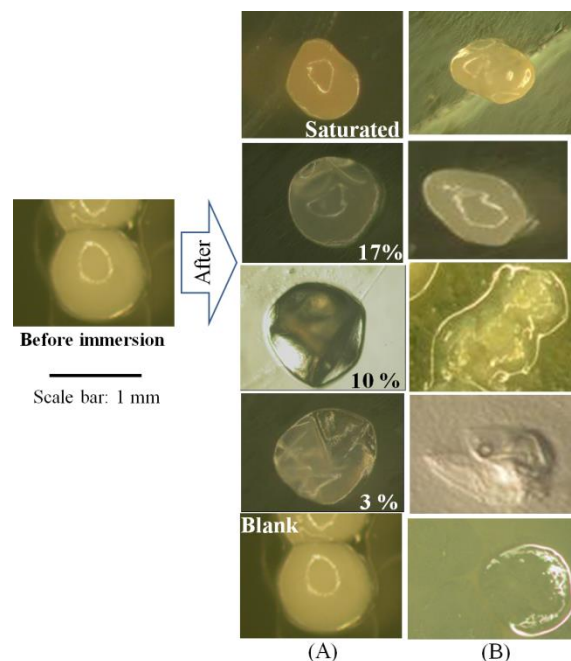


Fig. 4 Appearance of roes before and after immersion treatment. Pictured on the left side is an intact roe before immersion treatment. Images of roes (A) immediately after immersion treatment with various concentrations of NaCl solution and (B) stored at -25°C for 90 days after pretreatment as described in Materials and methods. The images are presented at the same magnification.

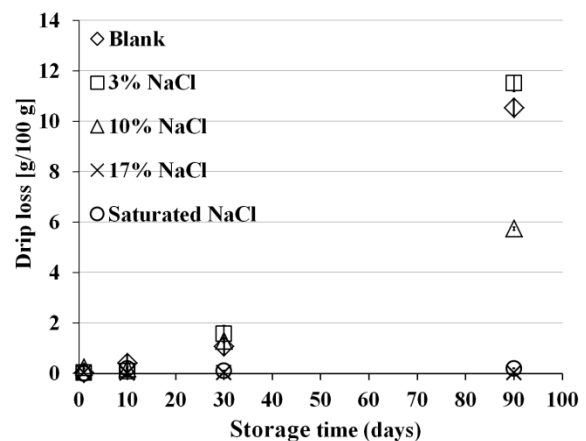
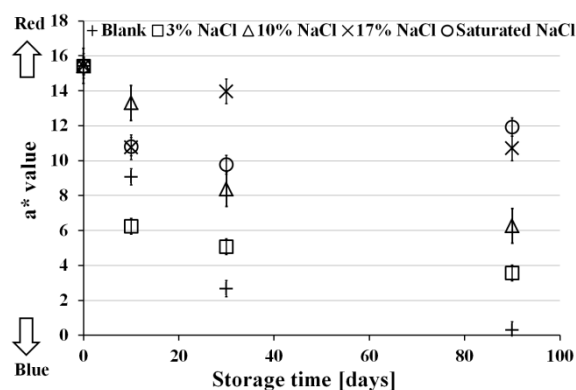


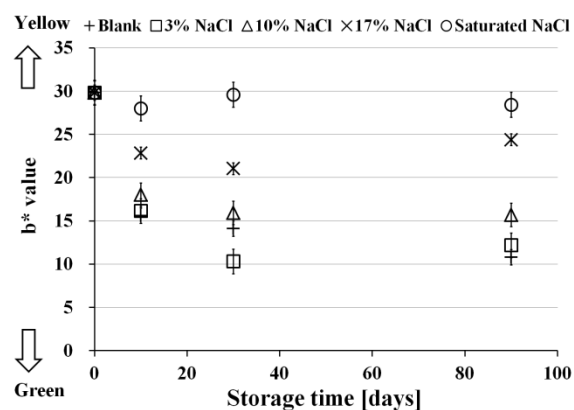
Fig. 5 Drip loss in frozen ovaries pretreated with various concentrations of NaCl after storage at -25°C . (n = 3). Drip loss was measured at 0, 10, 30 and 90 days after storage. The storage time for 0 day meant that the measurement was carried out right after frozen at -40°C . Drip loss gradually increased for the blank sample and the samples treated with 3% or 10% NaCl immersion treatment. Especially it was significantly increased about the blank sample and the sample treated with 3% NaCl solution.

The color parameters a^* and b^* are shown in Fig. 6 (A) and (B), respectively.

The a^* and b^* values of the 3% and 10% NaCl samples and the blank sample tended to decrease with storage, i.e., the red and yellow colors of the samples faded. However, a^* and b^* values of the 17% and saturated NaCl samples showed a small decrease compared to the other samples. In general, the a^* value is regarded as an important characteristic parameter of the color of *tarako* products; the greater the a^* value grows, the better the quality is [14, 15].



(A)



(B)

Fig. 6 Color parameter (a^* and b^*) values of ovaries pretreated with various concentrated NaCl after storage at -25°C . ($n = 3$). The samples treated with 17% or more concentrated NaCl solution kept the both value of a^* and b^* higher even after stored for 90 days.

For walleye pollock ovaries, its main red color is composed of carotenoid pigments [16], which are known as discolored by oxidation [17]. Then the techniques such as blanching, salting or adding food additives before freezing are conducted to foods

which have carotenoids. Thus it was thought that the effectiveness of NaCl in preventing the discoloration of *tarako* could be attributed to the anti-oxidative effect of NaCl [18], which is commonly used in preserving the color of foods such as pickles, sliced apples and corned meats.

Color fixatives and food color are often added to express vivid *tarako* color in order to keep commodity value. On the other hand, those food additives give a negative impression to consumers [19]. Thus the color preserving ability of NaCl for walleye pollock roes may lead another additional value of *tarako* products.

4. Conclusion

Our results indicated that pretreatment with $\geq 17\%$ NaCl solutions remarkably prevented freezing damage in roes by reducing the volume of frozen water within them. In addition, it was observed that this effect is independent of the freezing speed, as the ice crystals did not exceed the diameter of the roe; that is, dehydrofreezing the ovaries of walleye pollock does not require a quick freezing process to ensure a high quality product. In conclusion, this study proposes an innovative method for the freezing of walleye Pollock ovaries, which are used as raw materials for preparing *tarako* products, as an alternative to conventional freezing.

5. References

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