An Analysis of Brick-colored Pearls Harvested from Japanese Pearl Oysters Infected with Akoya Oyster Disease

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Abstract: Pearl oysters, infected by Akoya Oyster Disease, have a high likelihood of producing brick-colored pearls, known as Nigori Tama. NMR analysis of Nigori Tama showed the presence of melanin and fatty acid peroxides. Normally these organic matters can be disintegrated and removed from the pearls by bleaching with hydrogen peroxide solution and methanol under fluorescent lamp irradiation by the professional bleaching companies. However, even slightly brick-colored Nigori Tama was not bleached by this process. Additionally scanning electron microscopic observations revealed that the prismatic layer of Nigori Tama was significantly thicker than that of the normal pearls. Therefore we conclude that the thick prismatic layer is the cause of the brick-colored pearls.

Key words: Brick-colored pearl; Organic matter; Crystal structure; Akoya Oyster Disease

In Japan, historically, all harvested pearls are sorted by color. The competitive pricing of pearls is decided by buyers with salability like the color, sheen, blotches, size and shape. The economic value of the pearl is influenced especially by sheen and color. Almost all of the pearl oysters are bleached in hydrogen peroxide solution and methanol under fluorescent lamp irradiation by professional bleaching companies. This “bleaching” process removes organic matters in pearls and helps move the raw pearls higher on the economic scale (Wada 1999).

There are several studies on the pearl color variations, including the black matter (Mizumoto 1964), dark brown matter (Yano 1975), yellow pigment (Koizumi 1970), melanin (Ishizu et al. 1986) and porphyrin (Halime et al. 2007). In recent years, the pearl oyster was attacked by an infectious disease, Akoya Oyster Disease (A.O.D.). (Kurokawa et al. 1999). Since then the frequency of discoloration of pearls beyond the desirable “normal” level has increased, and these low economic value pearl-producing phenomena have been attributed to A.O.D. These pearls were brick-colored and are called “Nigori Tama” in the pearl market. To date, these have not been any reports on Nigori Tama, making this report the first of its kind. Of our 120 pearls obtained in 1991, only 3 Nigori Tama with slight discoloration were found. The frequency of occurrence in severely discolored Nigori Tama was 7%–82% in northern Uwa Sea, 6%–92% in central Uwa Sea, and 21%–100% in southern Uwa Sea in 2004.

Nigori Tama’s resistance to the bleaching process makes them low in marketing value; therefore, reducing the number of Nigori Tama occurrences increases the income of the pearl farmer (Fig. 1).

Nigori Tama may be coloring of the unknown
organic matter. The NMR is used as means of analysis and the identification of the material. Therefore, Nigori Tama extract was analyzed by NMR method to detect the material cause of the coloring. When Mn (II) is included in the nacreous layer or prismatic layer, the color of both layer are different (Ishizu et al. 1986). Therefore, we observed the crystal structure by the scanning electron microscopy (SEM).

This paper investigates the major cause of Nigori Tama’s resistance to the bleaching process, which we suggest is due to the thicker prismatic layer than that of normal pearls.

**Materials and Methods**

**Determination of the organic compounds in Nigori Tama by NMR method**

Nigori Tama was donated by pearl farmers in Uwajima City. The mantle piece was obtained from local-bred Japanese pearl oyster (donor pearl oyster). The mantle piece was implanted to the recipient pearl oyster, from which Nigori Tama was later obtained.

One kg of the gross weight of the crushed Nigori Tama was placed in a 50:50 ethanol:water solution containing 5% acetic acid, and stored in dark for three months. The solution was neutralized with saturated Na₂CO₃ solution and dried with an evaporator. Ethanol was added to dried sample, forming a solid residue which was discarded by filtration. The filtrate was concentrated before it was reconstituted in ion-exchanged water. An open chromatograph column (30 cm × 6 cm i.d.) packed with ODS resin (20 cm) was used to separate the final solution. Salts were removed by eluting 400 ml distilled water through the column. Organic matters were then eluted from the resin by passing 400 ml methanol through the column. The eluent was fractionated and dissolved in d₄-methanol prior to nuclear magnetic resonance (NMR) analysis (JNM EX-400, JEOL) (Preths and Bühlman 2000) to identify the organic matters.

The price of normal pearls weighting about 1 kg costs over half a million yen, therefore we only analyzed the Nigori Tama pigments for comparison with the pigments reported before the onset of A.O.D.

**Observation of the crystal layer by the scanning electron microscopy**

Normal pearl (Fig. 1-1, n = 15), slightly brick-colored Nigori Tama (Fig. 1-2, n = 16) and extremely brick-colored Nigori Tama (Fig. 1-3, n = 32) were observed under the scanning electron microscope (SEM). Enough pressure was applied to the pearl with pliers such that the pearl was broken partially and the crystal layer was exposed. The broken side of the pearl was coated with a gold film by vacuum deposition (IB 3, Eiko Co., Ltd.) (Ennos 1957). The target surface was observed by SEM (S-2250N, Hitachi Co., Ltd.) (Goldstein et al. 2003).

![Fig. 1. Normal pearl (1) and the abnormal pearl (Nigori Tama; 2, 3). 2, slightly brick-colored pearl; 3, extremely brick-colored pearl.](image-url)
Results

**Detection of organic matters from Nigori Tama**

We have successfully separated the Nigori Tama extract using column chromatography with ODS resin. Effective separation was achieved using water and methanol as developing solvents. The two main yellow fractions were eluted with two different times at room temperature. Each fraction was concentrated to give an oily yellow residue and NMR spectra were recorded in CD3OD, as shown in Fig. 2. Fig 2-1 shows typical aromatic compounds (melanin) signal around 7–8 ppm. On the other hand, long-chain aliphatic moiety (solid line) and oxidized moiety (dotted line) were also confirmed as shown in Fig. 2–2.

**Observation of the nacreous layer using the scanning electron microscopy**

Only in the broken side of the Nigori Tama, the dark brown layer was observed on the nuclear and normal colored layer was observed on the dark brown layer by gross pathology. Prismatic layer thickness of normal pearls was below 5 μm on the nucleus (Fig. 3-1). On the other hand, abnormalities were observed on the broken side of the extremely brick-colored Nigori Tama. Formation of the extremely thick prismatic layer (>200 μm) was commonly observed in extremely brick-colored Nigori Tama (Fig. 3–2). The prismatic layer of slightly brick-colored Nigori Tama averaged approximately 20 μm, which was significantly thicker than that of the normal pearl (Fig. 4).

![Fig. 3. A scanning electron micrograph of the section of normal pearl (1) and extremely brick-colored peal (2). a, nacreous layer; b, prismatic layer; c, nuclear surface.](image)

![Fig. 4. Thickness of the mineral layer formed on the nuclear surface. 1, whole layer; 2, prismatic layer. Mean with the asterisk (*, P<0.05; **, P<0.01; ***, P<0.005) are significantly different by Aspin-Welch t - test.](image)
Discussion

Melanin pigmentation in the pearl has previously been reported (Ishizu et al. 1986). It seems that when the nucleus and the mantle piece from a pearl oyster is implanted into the soft tissues of another pearl oyster, they are recognized as foreign substances by the leukocyte of the recipient pearl oyster. In pearl oysters infected with diseases like A.O.D., it has been suggested that a radical damages had occurred (Uchimura et al. 2003, 2006), resulting in the oxidation of tyrosine in the ligament (Tsujii 1963). This oxidation results in the generation of melanin that colors the pearl. Detection of oxidized fatty acids in pearls was the first confirmed data of the radical-damaged products in pearls (Uchimura et al. 2003, 2006), further indicating the adverse effects of the infectious disease, A.O.D. on pearls.

Melanin was also detected by NMR method and our study detected existence of the oxidative fatty acids in pearls for the first time. Organic matters, like melanin and the oxidized fatty acids, should be disintegrated by hydrogen peroxide solution and methanol under fluorescent lamp irradiation (Wada 1999). However, even Nigori Tama with only slight discoloration could not be bleached by professional bleaching companies. This indicates the presence of matters other than the described organic matters in Nigori Tama that are resistant to the bleaching process.

Normal pearls had the same crystal structure as that reported by Wada (Wada 1978). On the other hand, the prismatic layer of Nigori Tama was significantly thicker than that of normal pearls. Furthermore, the dark brown layer was observed on the nucleus in Nigori Tama. Therefore, we conclude that the main cause of the brick-colored pearls is the thickened prismatic layer.

It has not been made clear why the thick prismatic layer forms in the Nigori Tama; nor is it clear why the discoloration in the prismatic layer does not respond to the bleaching process. It is necessary to analyze metals, such as Mn (II) ESR of the prismatic layer for the identification of the pigments (Ishizu et al. 1986).

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References

An Analysis of Brick-colored Pearls


**アコヤガイ赤変病に罹病した貝で多発するレンガ色真珠の分析**

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アコヤガイ赤変病が発生以来、収穫された真珠にレンガ色の真珠（濁玉）の割合が増加した。この真珠は収穫された真珠の有機物を除去する脱色作業でも除去されないことが多い、真珠生産者では商品価値のある真珠の収量を激減した。そこで、レンガ色の正体を明らかにするため、濁り珠を破砕し抽出した真珠の色素の NMR 分析と、電子顕微鏡による正常な真珠との結晶構造を比較した。色素の NMR 分析では、これまで報告されたメラニンのほかに、アコヤガイ体内のラジカル損傷産物である過酸化脂質が検出された。一方、電子顕微鏡観察では、濁り珠は正常な真珠に比べ稜柱層が有意に厚いことがわかった。抽出された色素は、真珠加工会社の脱色過程で除去されることから、レンガ色の正体は厚く形成された稜柱層にあると考えられた。