

X線回折法による鱗の灰分の成分分析

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X-ray Diffraction Analysis of Sardine Scale Ash

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The scale ash of the sardine *Sardinops melanosticta* was analyzed by an X-ray diffraction method in order to identify the compounds in the minerals of the ash. The diffraction pattern of the scale ash was compared with those of five authentic chemicals with a molar ratio of Ca to P or of Mg to P of about 3 to 2, based on our previous results by chemical analyses. The peaks of the diffraction pattern of the scale ash did not coincide with any of the α and β types of $\text{Ca}_3(\text{PO}_4)_2$, $\text{Ca}_3(\text{PO}_4)_2 \cdot n\text{H}_2\text{O}$, or $\text{Mg}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$. However, the peaks of the diffraction pattern of hydroxyapatite (HAP) coincided with those of scale ash except at 31.2° and 34.6° where HAP had no peaks. The chemical, among the five authentic chemicals, which showed the peaks at 31.2° and 34.6° was $\beta\text{-Ca}_3(\text{PO}_4)_2$. The X-ray diffraction pattern of the mixture of HAP and $\beta\text{-Ca}_3(\text{PO}_4)_2$ in a molar ratio of 5 to 5 showed almost the same diffraction pattern as that of scale ash. Consequently, the main compounds in the sardine scale ash were estimated to be HAP and $\beta\text{-Ca}_3(\text{PO}_4)_2$. The crystallization of HAP and $\beta\text{-Ca}_3(\text{PO}_4)_2$ in the scale progressed by ashing at 600°C . On the contrary, the bone mineral of the sardine was regarded as almost pure HAP and was practically different from the minerals of the scale.

The chemical composition of the scales of several fishes had been investigated by Shimada and Kaneda¹⁾ more than a half century ago. Subsequently, Takahashi *et al.*^{2,3)} made a chemical analysis of the placoid scale of roughskin dogfish (*Yumezame* in Japanese) *Centroscymnus owstoni* Garman. In those papers, chemical compounds neither of ash nor of nitrogen-containing substances of the scale were not identified. Recently, Hamada and Kumagai⁴⁾ investigated the chemical composition of the scales of the sardine, *Sardinops melanosticta*, and clarified that the major components of the scale were ash and collagen in the proportion of 53% and 43%, respectively. The chemical compounds of the scale ash, however, were not able to be identified from only the chemical analyses.⁴⁾

On the other hand, the major components of the minerals of the bones and teeth have been well known as hydroxyapatite (HAP).⁵⁻⁷⁾ Consequently, sardine scale ash was also assumed to contain HAP. To verify this assumption for its validity, scale-ash samples were submitted for X-ray diffraction analysis. The results of the examination proved this assumption to be correct.

Methods

Sample

The scale sample used in this examination was the same as that in the previous paper.⁴⁾ The lyophilized scale of the sardine was ashed at 600°C for about 6 hrs in an electric furnace, and the ash was powdered homogeneously. The ash powder was submitted for X-ray diffraction analysis. Besides this scale ash, bone ash of the sardine was also prepared under the same conditions as in the case of the scale and was submitted for X-ray diffraction analysis.

On the other hand, the lyophilized and pulverized scales were also submitted for X-ray diffraction analysis in order to examine whether the crystal structure of the minerals of the scales is changed by ashing at 600°C .

X-ray Diffraction Analysis

Copper K_α radiation was generated by a Rigaku diffractometer (Rigaku Electronic Instrument, type RAD-A). The X-ray beam was adjusted to 40 kV and 100 mA and the scanning rate to $2^\circ/\text{min}$ over the diffracted angle ranges of $5\text{--}80^\circ$. The measuring conditions in the slits were 0.5°

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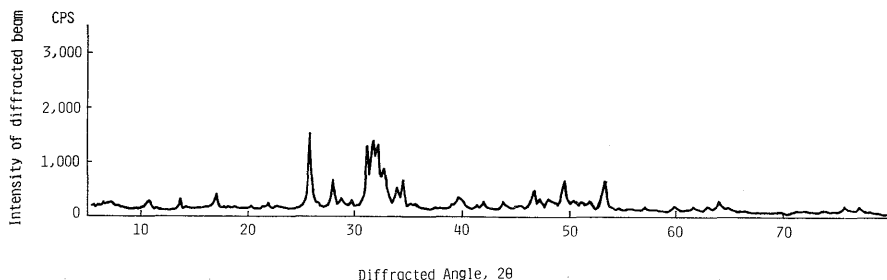


Fig. 1. X-ray diffraction pattern of the sardine scale ash.

for the divergence slit, 0.5° for the receiving slit, and 0.15 mm for the scatter slit.

X-ray diffraction patterns of the scale ash were compared with those of authentic samples quoted from the Powder Diffraction File,⁸⁾ and the crystal structure of the minerals was examined for identification. As authentic samples, five chemicals {HAP, α - $\text{Ca}_3(\text{PO}_4)_2$, β - $\text{Ca}_3(\text{PO}_4)_2$, $\text{Ca}_3(\text{PO}_4)_2 \cdot n\text{H}_2\text{O}$ and $\text{Mg}_3(\text{PO}_4)_2 \cdot n\text{H}_2\text{O}$ } were selected from the Powder Diffraction File based on our previous results.⁴⁾ That is, ash minerals were presumed to have the molar ratio of the sum of Ca and Mg to P of about 3 to 2. On the other hand, commercially available HAP {Merck, approx. $\text{Ca}_{10}(\text{PO}_4)_6 \cdot \text{H}_2\text{O}$ } and β - $\text{Ca}_3(\text{PO}_4)_2$ (Nacalai Tesque Inc., Japan) were submitted for X-ray diffraction analysis. Prior to the experiments, Merck's HAP was ashed once at 600°C .

Results and Discussion

X-ray Diffraction Pattern of the Scale Ash

The relation of the intensity of the diffracted beams in counts per second to the diffracted angle in degrees, or the X-ray diffraction pattern of the sardine scale, is shown in Fig. 1.

As shown in the figure, major peaks were observed at 26° , 31 – 33° (adjacent four peaks), 49.5° and 53.2° . However, those results alone do not provide any information for the identification of the compounds in the ash. Consequently, the diffraction pattern of the scale ash was compared with those of five authentic chemicals as shown in Fig. 2. The Powder Diffraction File, however, offers only the relation of the intensity to the diffraction angle, and hence those relations were superimposed on the diffraction pattern of the scale ash for each authentic chemical.

As shown in Fig. 2, the peak angles of the scale ash did not coincide with any of those of the α or β type of $\text{Ca}_3(\text{PO}_4)_2$, $\text{Ca}_3(\text{PO}_4)_2 \cdot n\text{H}_2\text{O}$, or $\text{Mg}_3(\text{PO}_4)_2 \cdot 8\text{H}_2\text{O}$. However, the peak angles

of the diffraction pattern of HAP coincided with those of the scale ash with a few exceptions. Accordingly, the main component was presumed to be HAP. That is, the components of the scale ash were presumed to have a crystalline structure similar to that of HAP, having the molecular formula of $\text{Ca}_{10}(\text{PO}_4)_6 \cdot (\text{OH})_2$, but the scale ash contains some compounds besides HAP, according to the following reasons. The intensity of the peak of scale ash at 31.7° was low; whereas, that of HAP at the same angle was the largest of all the peaks. Similarly, large peaks at 31.2° and at 34.6° were observed for the scale ash, though no such peaks appeared for any of the authentic chemicals.

As shown in Fig. 2, the compound which has large peaks at 31.2° and 34.6° was β - $\text{Ca}_3(\text{PO}_4)_2$. Accordingly, β - $\text{Ca}_3(\text{PO}_4)_2$ was assumed to coexist with HAP as a component of the scale ash. If this estimation is correct, the diffraction pattern of the mixture of β - $\text{Ca}_3(\text{PO}_4)_2$ and HAP would be assumed to coincide with that of the scale ash. To make sure of the validity of this estimation, some mixtures in various ratios of β - $\text{Ca}_3(\text{PO}_4)_2$ and HAP were prepared and submitted for X-ray diffraction analysis. Those results are shown in Fig. 3.

As the ratio of β - $\text{Ca}_3(\text{PO}_4)_2$ increased, the peaks at 31.0° and 34.3° apparently became large. On the contrary, the inverse was observed at 31.6° . That is, as the ratio of β - $\text{Ca}_3(\text{PO}_4)_2$ increased in the mixture, the peak intensity at 31.6° decreased, and the diffraction pattern came to resemble that of the scale ash.

The above results indicate that the main components of the scale ash are both HAP and β - $\text{Ca}_3(\text{PO}_4)_2$, and their ratio in the scale ash is assumed to be almost the same, or the latter is a little higher. On the contrary, chemically synthesized HAP from CaO and H_3PO_4 is reported to change to β - $\text{Ca}_3(\text{PO}_4)_2$ at above 860°C .⁹⁾ Accordingly, a part of the HAP in the scale ash

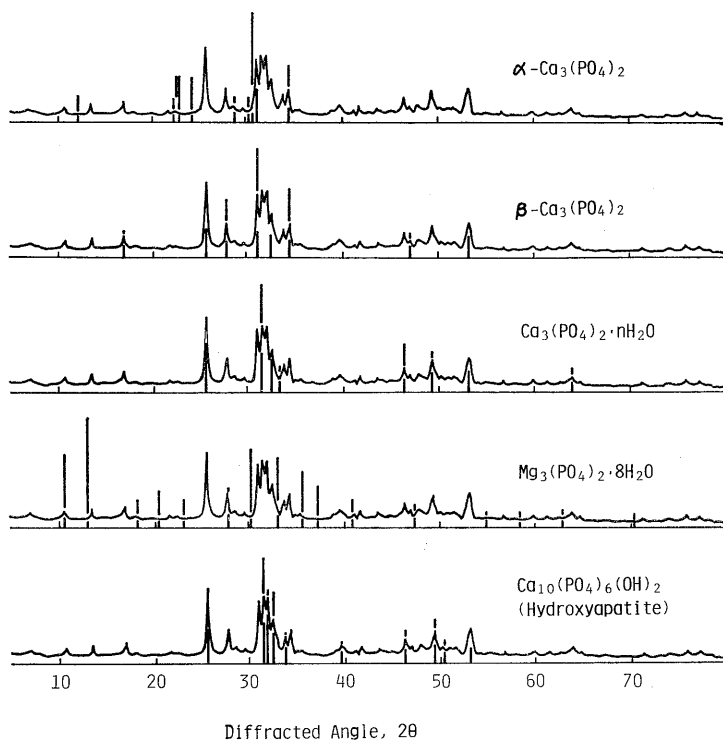


Fig. 2. Comparison of the X-ray diffraction patterns between sardine scale ash (solid line) and authentic samples (a set of vertical lines).

The vertical lines indicate the major peaks of the diffraction pattern of an authentic sample; that is, the height of the vertical line indicates the intensity at its corresponding diffracted angle. The height of the largest peak of each authentic sample was made uniform here. The X-ray diffraction pattern of the authentic sample was obtained from the Powder Diffraction File.

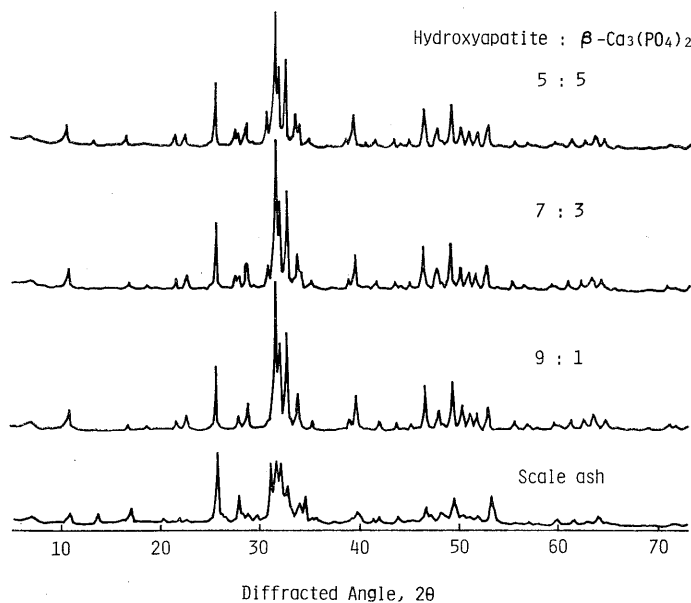


Fig. 3. Comparison of the X-ray diffraction patterns between scale ash and the mixture of hydroxyapatite and $\beta\text{-Ca}_3(\text{PO}_4)_2$. Hydroxyapatite and $\beta\text{-Ca}_3(\text{PO}_4)_2$ were mixed in molar ratios of 9 : 1, 7 : 3, and 5 : 5 as shown in the figure.

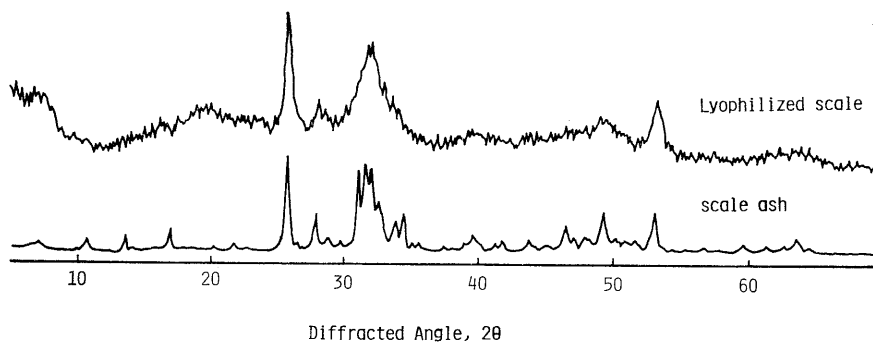


Fig. 4. Comparison of the X-ray diffraction patterns between lyophilized sardine scale and that of ash.

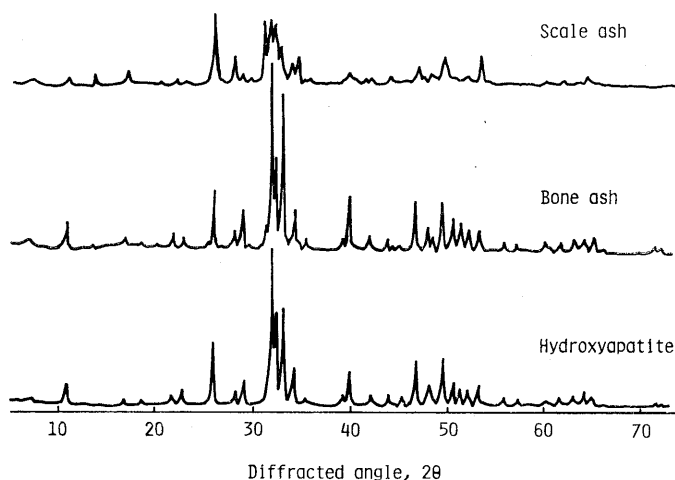


Fig. 5. Comparison of the X-ray diffraction patterns among sardine scale ash, bone ash, and authentic hydroxyapatite.

might change to $\beta\text{-Ca}_3(\text{PO}_4)_2$ by heating at 600°C . However, the detailed reason for the appearance of the peaks corresponding to those of $\beta\text{-Ca}_3(\text{PO}_4)_2$ in the scale ash was not made clear in the present experiment.

Effect of Ashing on the Crystal Structure of the Scale Minerals

The scale sample was ashed at 600°C in the present experiment, but the crystal structure of the scale minerals was suspected to have been changed by ashing at 600°C . Thereafter, the effect of ashing on the crystal structure of the minerals of scales was examined. In Fig. 4, the diffraction pattern of the ashed scale was compared with that of the lyophilized and pulverized scales.

As shown in the figure, the peaks of the lyophilized and pulverized scale changed to a sharp

peak after ashing, without the alteration of the diffraction angles. That is, blunt peaks of the lyophilized and pulverized scales in the diffraction angle range of $31\text{--}34^\circ$ separated to four sharp peaks by heating at 600°C . As a consequence, crystallization of HAP in the scale was found to progress by ashing.

Difference of the X-ray Diffraction Patterns between Scale Ash and Bone Ash

The bone mineral of vertebrates has been accepted to be HAP⁵⁻⁷⁾. Consequently, the bone minerals of sardine were also assumed to be HAP. To clarify this assumption and also to clarify the difference in the crystal structure between bone minerals and scale minerals, the X-ray diffraction pattern of the scale ash was compared with that of bone ash. As shown in Fig. 5, the X-ray diffraction pattern of the scale

ash was slightly different from that of HAP. On the contrary, the X-ray diffraction pattern of the bone ash was exactly comparable with that of HAP.

As a result, the main component of the bone minerals of sardine was almost pure HAP and was different from that of scale minerals.

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