

# REVIEW ARTICLE

## Utilization of Anchovy in Dentistry

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### ABSTRACT

Anchovy (*Stolephorus sp*) is a fish that usually consumed by most people in Indonesia. It is served as a famous dish in Indonesian cuisine. It is a natural source of calcium, phosphor, and fluoride. In dentistry, anchovy had been studied due to its bioactive content as bone stimulator and teeth development agent, topical fluoridative agent, and pulp capping agent. There are several methods in preparing anchovy extract that leads to different bioactive content. These variations provide several roles as biomaterial in dentistry. The latest issue regarding anchovy was its hydroxyapatite content. The previous study showed promising result and become a base of future exploration to obtain safe and effective treatment in dentistry.

**Keywords:** Anchovy extract, Topical fluoridative agent, Reparative dentin, Pulp capping agent, Hydroxyapatite.

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### INTRODUCTION

Our earth is dominated by the ocean, which cover approximately 70.8%. (1) Marine biodiversity may provides raw materials that used in pharmaceuticals need. One of the marine fisheries production is anchovy (*Stolephorus sp*). It is one of the most abundant resources in Indonesia and easily obtained. (2) In contrast to the economically price, anchovy has great nutritional benefits and all of the part of anchovy could be consumed. The fish bone is the source of protein and calcium, while in each 100 grams of fresh anchovy contains 77 kcal energy, 16 grams protein, 1.0 gram fat, 500 mg calcium, 500 mg phosphor, 1.0 miligram iron, vitamin A and B. (3)

Due to environmental pollution, people aware of the mercury toxicity of fish origin. Previous study, held in Ankara and Istanbul, Turki, showed mercury content in anchovies is 0.5 mg/kg (ppm). It was below the permitted limit. It is known that mercury accumulate more on the large fish. (4)

### Anchovy In Dentistry

In dentistry, anchovy extract is studied for its potential in stimulating bone and teeth development. It is studied also as a preventive agent of tooth caries and a stimulative agent of dentin reparative. The potential bioactivity of anchovy that have been studied is due to its mineral content, calcium, phosphor and fluoride.

Calcium content in anchovies may prevent bone loss and provide bone and tooth growth. A study was designed to evaluated effect of anchovies intake on the amount of osteocyte cells of lower alveolar bone of white Wistar rat. Result of the study showed that anchovies intake influenced osteocyte cell numbers. It raise the amount of osteocyte cells of the lower alveolar bone compared to group without anchovies feeding. The study also report that the anchovies intake increase the density of the lower alveolar bone of white Wistar rat. The calcium and phosphor in anchovy presumed raising extracellular calcium concentration that trigger mobilization and proliferation of osteocytes as bone forming cells. (5)

Anticaries activity of anchovy is provided by the fluoride content in calcium fluoride form ( $\text{CaF}_2$ ). Dental enamel formation is structured by enamel prisms which formed by apatite crystals. Most apatite crystal founded is

$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$  or known as hydroxyapatite (HAp). Twice a day topical application of 5% anchovy solution on rat's enamel surface showed reduction of the size of apatite crystal significantly in seven days. It increased enamel surface microhardness and reduced enamel demineralization. (6) The reduction of enamel solubility is an impact of hydroxyl ion replacement with the fluor ion. Chemical reaction of fluor ion and hydroxyapatite produce fluorapatite (FA) which is more acid resistant. (7) Administration of feeding or smearing anchovy solution in tooth enamel of Sprague Dawley rats also results in increasing fluoride retention on tooth enamel. There were no significantly difference between two treatments. (8)

Antimicrobial effect of anchovy extract was exhibited by another study. Dental caries are caused by bacteria, specifically *Streptococcus mutans*. (9) Fluoride influences the metabolic behavior of microbes and inhibit metabolic enzymes including phosphatase. (10) Phosphatase is an intracellular hydrolase enzyme which is participate in metabolic process of the cells. As an example enzyme alkaline phosphatase. This enzyme occurs in many organisms, ranging from bacteria to man. Its function is to catalyze the hydrolysis of monoesters of phosphoric acid. It also catalyze the trans phosphorylation reaction with the presence of large concentrations of phosphate acceptors. (11) The previous study showed the antimicrobial potential of anchovy extract against the growth of *Streptococcus mutans* bacteria at 3%, 6% and 12% concentration. The 12% concentration of anchovy extract has more power to inhibit *S. mutans* growth than natrium fluoride as positive control. (12)

Anchovy extract was also investigated for its capability in stimulating reparative dentin. Differ from reactionary dentin that is formed by odontoblast cells, reparative dentin was formed by odontoblast like cells due to the death of primary odontoblast cells triggered by injury. The morphology of dentin reparative is atubular and bone alike. (13-15) Calcium and phosphate in anchovy may form calcium phosphate complex which stimulate a stem cell differentiation into odontoblast or odontoblast like cell. Research found out that anchovy extract increase odontoblast cell numbers in molar tooth of Wistar rat. (16) Odontoblast initiates ion nucleation to start the mineralization process. It activates the intramembrane transport mechanism of calcium and phosphate ions. This activation result in balance of intracellular ion to increase the mineralization of the reparative dentin matrix. As shown in another study, whereas application of anchovy cream as pulp capping agent showed more reparative dentin formation compared to calcium hydroxide in molar tooth of Wistar rats. (17-18)

### **Anchovy Extract Preparation Method**

There are several methods to prepare the anchovies

extract that used in previous experiments. These variations lead to different bioactivity of anchovy extract. They are in accordance with the role of anchovy that was designed in research, either it was as a fluoridative agent, pulp capping agent or else.

As solution to be smeared on rat's tooth or to be mixed in feeding, anchovies were heated at 80°C for 20 minutes and powdered to 100 Mesh. About five grams of the powder was diluted into 100 milliliter of demineralized distilled water to obtain anchovy solution. Followed by adjusted the solution to pH 5.5 using phosphoric acid. (6,8) Phosphoric acid was used to initiate ion exchange. Enamel surface and fluoride have negative charge. In acidic environment, which has enough  $\text{H}^+$  ion, fluoride could intrude into enamel and replace the phosphor ion in HAp. (6) Previous study had compared different concentration of anchovy extract and it was concluded that pH 5.5 result in biggest changes of crystal size particles and acid retention. (17)

To investigate anchovy extract's capability as pulp capping agent, author found out there were different methods that had been used. One study, sun-dried the fresh anchovies over tempe for two weeks. The dried anchovies were blended and sifted with number 1.000 sieve. Anchovies powder mixed into saline solution in 1:1 ratio. In this method, anchovies powder still contain organic compound, such as protein. This protein may contributed in pulp repair and result of the research. (18) The other study, heated anchovy at 50°C for 6 hours and using blender to obtain powder. About ten milligrams diluted into one liter of aquadest and extracted for 24 hours. Anchovy solution filtered, and mixed with 12.85 milligrams natrium hydroxide and centrifuged at 250 rpm for 15 minutes. The sediment was heated at 50°C for 5 hours. (16) Natrium hydroxide was used to hydrolize collagen and other organic moieties, as the researcher tried to investigate the role of calcium and phosphor effect in the odontoblast cell numbers.

To obtain the purity of calcium from anchovies, there is another method that could be held. It is extracted the HAp from the anchovies. HAp is the most stable form of calcium phosphate and molar ratio of Ca/P is 1.67. HAp could be extracted from natural source, such as bovine bone, fish bone, marine shells and egg shells. (20) Fish bone is rich in calcium, phosphate and carbonate which make it a great source of HAp extract. The method used to extract HAp from fish bone and shells are calcination, alkaline hydrolysis or combination. (21) Previous study showed that HAp could be extracted from anchovies. (22)

Synthesis of HAp was obtain by washing the anchovies bone thoroughly, dried at 60°C and ground in mortar pestle. Alkaline hydrolysis method was followed. Every 20 grams of anchovy bone was treated with 1 M hydrochloric with constant stirring for 12 hours. Then

the sample was treated with 1 M sodium hydroxide with stirring for 12 hours. The mixture filtered in suction pump with continuous water washing and dried in oven at 100°C. One gram of powder was put into crucible silica and calcined at 900°C for 12 hours. Sample characterization showed this method exhibit the promising chemical composition and structure. Ratio Ca/P of HAp synthesized from anchovy was closed to 1.67. (22)

HAp from natural source showed ratio Ca/P higher than 1.67. Higher ratio will impact on its calcium release, whereas the higher ratio will release more calcium in longer time. (23-24) HAp from natural resources, such as fish bone have metabolic activity and provide a suitable response compared to synthetic HAp. Recent studies showed application of HAp from fish bone increase Dentin matrix Protein (DMP)-1 of odontoblast like cell in 3, 7 and 14 days of observation. (17) Bioactivity of HAp from fish bone formed more apatite than synthetic HAp, has positive effect on viability, and alkaline phosphate (ALP) activity of osteoblast cell. (23)

### Anchovy In Future Dentistry

Anchovy is natural source of bioactives. Fluoride content showed great achievement act as topical fluoridation. As pulp capping agent, anchovy showed great potential as calcium and phosphor natural source. Research showed increasing extracellular calcium induce raising of fibroblast growth factor 2 gene expression via extracellular signal regulated kinase and protein kinase A. It showed calcium ion role in dentin regeneration. (24) In biomolecular level, extracellular calcium level is detected by the calcium sensing receptor (CaSR), that belongs to the C family of the G-protein coupled receptor. An activated CaSR elicits intracellular signaling pathways that lead to migration, proliferation and differentiation of cells. (25) It will activate cell signaling that influenced in DMP-1 expression as shown on the recent studies. (17) DMP-1 was one of dentinogenesis marker on early stage. (26) Successful therapy was counted by variable parameter, and DMP-1 was one of those parameters.

### CONCLUSION

There are so many improvement and possibilities in researches based on anchovies as biomaterial in dentistry. Preparation of anchovies will effect on bioactive material contain in its extraction and in return will give impact on the results. Promising results from recent studies made exploration is widely open.

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