

Fluoride Concentration in Anchovy (*Stolephorus commersonnii*) Nanopowder and Its Potential in Dental Caries Prevention

Mutiara Dewi Pangaribuan*, Sondang Pintauli**, Saharman Gea***

*Master's Program in Dental Science, Faculty of Dentistry, Universitas Sumatera Utara, Indonesia

**Department of Dental Public Health, Faculty of Dentistry, Universitas Sumatera Utara, Indonesia

***Department of Chemistry, Faculty of Mathematics and Natural Sciences,
Universitas Sumatera Utara, Indonesia

Online Submission : 2 Juni 2025

Accept Submission : 17 Juli 2025

ABSTRACT

Background: Dental caries is a common infectious disease that leads to the demineralization and structural degradation of the tooth's hard tissues, caused by acid-producing bacteria such as *S. mutans* through carbohydrate fermentation. One of the essential strategies for caries prevention is using fluoride to inhibit bacterial activity and enhance enamel remineralization. Anchovy *Stolephorus commersonnii*, widely found in Indonesian waters, were rich in calcium and fluoride, particularly in the form of calcium fluoride (CaF_2), which may offer potential benefits in preventing dental caries. **Objective:** This study aimed to assess the fluoride concentration in *S. commersonnii* nanopowder to evaluate its potential as a natural source of fluoride for dental caries prevention. **Materials and Methods:** This research was a descriptive laboratory-based experimental study. The sample was *Stolephorus commersonnii* (*S. commersonnii*). The sample was prepared through drying, acid maceration, and high-energy milling to produce nanoparticle powder. Fluoride content was measured using two methods: a colorimetric method with a spectrophotometer and a portable fluoridimeter based on the SPADNS method. **Results:** The results showed that the fluoride concentration was 21.05 mg/100 g (21.05 ppm) by spectrophotometry and 20.0 ppm using the SPADNS method. The *S. commersonnii* nanopowder contained 1,305.80 mg/100 g calcium, 281.60 mg/100 g phosphorus, and 91.5% of particles were in the nano size. **Conclusion:** *S. commersonnii* nanopowder contains a considerable amount of fluoride and minerals, suggesting its potential as a natural dietary source of fluoride in supporting dental caries prevention strategies.

Keywords: anchovy, dental caries, fluoride, natural source, *Stolephorus commersonnii*

Correspondence: Sondang Pintauli, Department of Dental Public Health, Faculty of Dentistry, Universitas Sumatera Utara, Jl. Alumni No. 2, Medan 20155, Indonesia, Phone 061-8216131, Email: sondangp@yahoo.com

INTRODUCTION

Dental caries is a common infectious disease that leads to the demineralization and structural degradation of the tooth's hard tissues. Among the key pathogens in dental caries is *Streptococcus mutans* (*S. mutans*), which metabolizes sugars into acid that demineralizes tooth enamel.¹⁻³ Effective caries prevention involves reducing sugar intake and inhibiting the activity of cariogenic bacteria, especially *S. mutans*.^{4,5} In addition to commonly used synthetic agents such as sodium fluoride or stannous fluoride, researchers are increasingly exploring natural sources, such as plants, marine organisms, and minerals, for their potential in preventing dental caries.

Fluoride plays a crucial role in caries prevention through three primary mechanisms. First, fluoride ions in the oral cavity help regulate calcium and phosphate ions within demineralized enamel, promoting remineralization. Second, fluoride can replace hydroxyl ions in hydroxyapatite crystals, forming fluorapatite, which is more resistant to acid and thus protects the teeth from demineralization.⁶ Third, fluoride acts therapeutically on bacterial cells by penetrating their membranes and disrupting carbohydrate metabolism and the production of adhesive polysaccharides, which are essential for bacterial attachment to dental plaque.⁷ Fluoride inhibits the metabolism of plaque bacteria that ferment carbohydrates, and it enhances the transformation of hydroxyapatite ($\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$) into fluorapatite ($\text{Ca}_{10}(\text{PO}_4)_6\text{F}_2$), which is more resistant to acid attacks at pH levels below.^{4,5,8} The formation of fluorapatite reduces enamel solubility in acidic conditions, accelerates the remineralization process, and exhibits antimicrobial activity by inhibiting bacterial enzymes such as enolase, thereby preventing acid production within the biofilm.^{9,10} However, the antibacterial effect of fluoride is relatively limited. The growth and metabolism of cariogenic bacteria are influenced only when the fluoride concentration exceeds 10

ppm. In the context of caries prevention, fluoride is primarily effective by promoting remineralization of initial carious lesions and preventing the demineralization that leads to the initiation and progression of caries. Fluoride is effective when present in the oral cavity and is not effective when ingested systemically.¹¹

The search for natural sources of fluoride has gained interest. One such potential source is anchovy (*Stolephorus* sp.), a small marine fish commonly found in Indonesian waters.¹⁰ According to data from FishBase, there are 19 species of anchovy identified in Indonesian waters. *Ikan teri nasi* (*Stolephorus commersonni*) has known locally as *ikan teri Medan* or Medan anchovy, is a well-known traditional food product from Medan city, which is often brought home by visitors as a signature local delicacy. It is known for its small size and rich mineral content, making it a potential natural source of fluoride.¹²

Anchovies are considered high-quality food because the entire body—including the bones—is edible, making them a rich source of protein, calcium, and micronutrients. Anchovy bones are particularly valuable for their calcium and fluoride content, essential for tooth development in children. Every 100 grams of fresh anchovy provides approximately 77 kilocalories of energy, along with 16 grams of protein, 1 gram of fat, and essential minerals such as 500 milligrams of calcium, 500 milligrams of phosphorus, and 1 milligram of iron. Moreover, anchovies contain various essential vitamins, particularly vitamin A and B-complex groups.¹⁰

A study by Agustanti et al. using an ion-selective electrode method found that fresh *Stolephorus insularis* contained 12.935–13.381 ppm of fluoride, while the dried form had higher concentrations ranging from 15.416 to 24.914 ppm.¹³ A recent study by Prahasti and Yuanita demonstrated the successful fabrication of nanoscale *Stolephorus* sp. powder using a top-down approach through high-energy milling. The process involved drying the fish, sieving the

resulting powder, and subjecting it to milling durations of up to 24 hours. The resulting powder was found to be rich in bioactive components, including 28,912 mg/kg calcium and 1,924 mg/kg magnesium, indicating its potential application as a natural source of bioactive minerals.¹⁴ These findings suggest that anchovy could serve as a natural and bioavailable source of fluoride for dental applications, making it a promising candidate for further exploration in caries prevention strategies.

Despite these findings, no specific studies have been conducted to quantify the fluoride content in *S. commersonnii*. Therefore, this study aimed to evaluate the fluoride content of *S. commersonnii* processed into nanopowder and to explore its potential use in dental caries prevention.

MATERIALS AND METHODS

This research was a descriptive laboratory-based experimental study conducted in April 2025. The anchovy samples were obtained from local fishermen in Tanjung Tiram Village, Batubara Regency, North Sumatra Province (3.2361° N, 99.5902° E). It was then sent to the Animal Systematics Laboratory, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, for taxonomic identification. Based on the official identification letter No. 25/UN5.2.1.11/KRK/ 2024, the local fish known as ikan teri Medan was taxonomically confirmed as *Stolephorus commersonnii*. This study received ethical approval from the Ethics Committee of Sumatera Utara, Medan-Indonesia, with reference number 327/KEPK/USU/2025.

A total of 1 kg of anchovy was used in this study, selected through purposive sampling based on freshness and absence of strong odor. The sample size was determined based on the minimum quantity required to perform the necessary laboratory analyses. It was boiled at 80°C for one hour to eliminate pathogens and

reduce odor, and then washed and sun-dried for one day. After drying, 100 g of anchovy was macerated in 1 M hydrochloric acid (HCl) for 2 hours to help dissolve certain components, followed by rinsing with distilled water until the pH was neutral. The sample was then oven-dried at 50°C for 36 hours to ensure complete dehydration. The dried anchovy was ground using a mortar and sieved through a 60-mesh filter to obtain a uniform powder. The powder was further processed using High Energy Milling (HEM) for 7 hours at 600 rpm with 4 mm-diameter stainless steel grinding balls to produce nanoparticle-sized anchovy powder, and was measured using a Particle Size Analyzer (PSA) with a Fritsch Analysette 22 NanoTec.

The fluoride content in *S. commersonnii* nanopowder was measured using two different analytical methods. The first method was conducted at the Industrial Research and Consultation Agency (BPKI) Surabaya using a colorimetric method with a spectro-photometer. The second method used a portable fluorimeter (Hanna Instrument HI739) based on the SPADNS method (sodium 2-(parasulfophenylazo)-1,8-dihydroxy-3,6-naphthalene disulfonate). SPADNS reacts with zirconyl chloride solution to form a red complex, which is then decolorized in the presence of fluoride ions. This color change is measured using a colorimeter.

The procedure using the portable fluoridometer was as follows: The instrument was turned on by pressing the ON/OFF button. When "Add C.1" appeared with a blinking "Press" icon, 2 mL of HI739AS reagent was added to a cuvette using a syringe, followed by filling to the mark with HI739BS reagent (Figure 1). The cuvette was sealed, inverted several times to mix, and inserted into the checker for zeroing by holding the ON/OFF button. After zeroing, the cuvette was removed, and 1 mL of sample was added, mixed, and reinserted. The measurement was then initiated by pressing and holding the button again, and the fluoride concentration was displayed in ppm.



Figure 1. Step-by-step fluoride analysis procedure using Hanna Checker Fluoride HR. (A) Addition of 2 mL Reagent A (HI739AS) into a 10 mL cuvette. (B) Addition of Reagent B (HI739BS) until the cuvette reaches the 10 mL mark, resulting in a red-colored solution. (C) *S. commersonnii* was added to the reagent solution using syringe. (D) The cuvette containing the test mixture is inserted into the Hanna Checker Fluoride HR device for fluoride concentration measurement.

RESULTS

According to The Animals Systematics Laboratory USU, *S. commersonnii* is characterized by a maximum body length of 7-8 cm and a body height of 1-2 cm. The fish has a light brown translucent body, a slightly compressed shape with a somewhat rounded abdomen, no anal spines, and 21-22 soft anal fin rays. The belly bears 0-5 small prepelvic scutes resembling needles. The upper jaw is pointed, reaching or slightly exceeding the posterior margin of the preopercle. The lower gill rakers number 23-28. Small teeth present on the upper edge of the hyoid bone. The isthmus

musculature tapers evenly forward to the posterior edge of the branchial membrane. The tips of the pelvic fins reach below the anterior dorsal fin, and the anal fin is short, with three unbranched rays and 18-19 branched rays.

The Particle Size Analyzer (PSA) measurement revealed that the nanoparticle powder of *S. commersonnii* had a mean particle size of 43.88 nm. The median particle size was recorded at 26.20 nm, while the mode (the most frequently occurring particle size) was 11.36 nm. The mean-to-median ratio of 1.67449 indicates a relatively homogeneous distribution within the nanoparticle size range. The particle size distribution graph shows that the majority of particles fall below 100 nm, confirming the success of the nanoparticulation process (Figure 1).

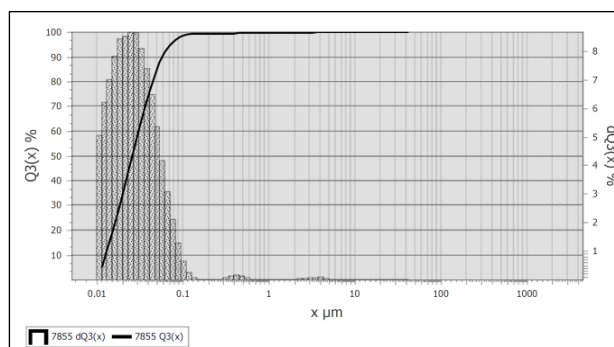


Figure 2. Particle Size Analyzer of *S. commersonnii* Nanopowder

The analysis revealed that the fluoride concentration in the anchovy was 21.05 mg/100 g, or 21.05 ppm, indicating a significant natural fluoride content suitable for potential use in caries prevention, particularly in topical applications. Alongside fluoride, the chemical composition analysis also indicated the presence of 1,305.80 mg/100 g of calcium, 281.60 mg/100 g of phosphorus, and 3.14 mg/100 g of iron. Furthermore, 91.50% of the particles were confirmed to be in the nano range, supporting the suitability of the powder for nanoparticle-based applications (Table 1).

Table 1. Chemical Composition of *S. commersonnii* nanopowder

Parameter	Unit	Result
Calcium (Ca)	mg/100 g	1,305.80
Fluoride (F ⁻)	mg/100 g	21.05
Phosphorus (P)	mg/100 g	281.60
Iron (Fe)	mg/100 g	3.14
Particle size (nano)	%	91.50

Source: Certificate of Analysis, BPKI Surabaya, Report No. 09315/KI/V-2025

Although calcium was found to be the most abundant mineral in *S. commersonnii*, this study specifically focused on fluoride due to its well-established role in the prevention of dental caries. Fluoride contributes directly to the inhibition of cariogenic bacteria and the enhancement of enamel remineralization. While calcium is also essential for tooth structure, its effect is largely supportive, whereas fluoride exerts a more active, targeted role in caries prevention. Therefore, quantifying the fluoride content was prioritized to assess the potential of *S. commersonnii* as a natural source for dental caries preventive topical agent.

The analysis using a portable fluoridimeter (Hanna Instruments HI739), based on the SPADNS method, showed a fluoride concentration of 20.0 ppm in the anchovy sample. This result aligned well with the standard laboratory method, confirming the accuracy and reliability of both approaches in determining fluoride levels (Figure 3).

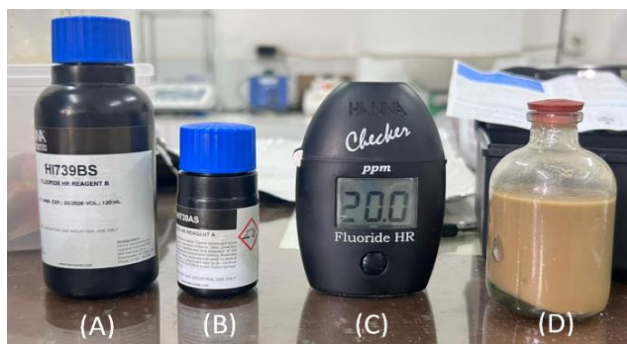


Figure 3. Fluoride analysis setup using the Portable Fluoridimeter (Hanna Checker Fluoride High Range). (A) HI739BS Fluoride HR Reagent B, (B)

HI739AS Fluoride HR Reagent A, (C) Fluoride HR digital reader showing 20.0 ppm, and (D) anchovy (*Stolephorus commersonnii*) extract sample used for fluoride detection.

DISCUSSION

The morphological identification of *S. commersonnii* confirmed characteristic features consistent with existing taxonomic descriptions. These morphological traits validate the accurate identification of the species used in this study, ensuring the reliability of the sample source for further chemical and nanoparticle analysis. Accurate species identification is crucial, as mineral composition, including fluoride content, may vary significantly among different anchovy species.

This study demonstrated that *S. commersonnii* contains a relatively high fluoride concentration, measured at 21.05 ppm. This value is notable when compared to other commonly consumed natural sources of fluoride, such as tea leaves, seafood, or drinking water in fluoridated areas. The presence of fluoride in anchovies is likely associated with their habitat in marine environments, where seawater and sediment can influence fluoride accumulation in fish tissues and bones. The relatively high fluoride level suggests that *S. commersonnii* could serve as a viable source of natural fluoride, particularly for the development of alternative or supplementary topical agents for dental caries prevention.

In addition to fluoride, the powder was found to contain high levels of calcium (1,305.80 mg/100 g) and phosphorus (281.60 mg/100 g), both of which are essential minerals for maintaining enamel integrity and facilitating remineralization processes. While calcium is abundant, its bioavailability and direct role in caries prevention are not as pronounced as fluoride's antimicrobial and remineralizing effects. Therefore, fluoride was selected as the primary focus of this study, despite calcium being more dominant in quantity.

An important finding in this study is that over 91% of the anchovy nanopowder within the nanoparticle range. Nanoparticles are known to enhance the bioavailability and surface reactivity of bioactive compounds. In the context of fluoride delivery, nano-sized particles may offer improved penetration into enamel micropores and increased contact surface area, which could lead to enhanced remineralization potential.

These findings align with previous studies, Agustanti et al. reported that fluoride concentrations ranging from 15.7 to 38.33 ppm in anchovy species such as *Stolephorus insularis*,¹³ while Gunawan et al., and Sakinah et al. demonstrated the enamel-strengthening effects of anchovy-derived fluoride in animal models.^{15,16} Supporting this, a study by Yuanita analyzed nano brown anchovy powder and found a fluoride content of 19.81 ppm, calcium at 1,310 mg/100 g, iron at 8.96 mg/100 g, and nano particle distribution at 81.80%.¹⁷ These results further confirmed the potential of anchovies as a reliable natural fluoride resource. Moreover, the study also emphasized that the antibacterial activity of the nano brown anchovy powder, particularly its effect against oral pathogens, complements its fluoride content in caries prevention.¹⁸ The slightly higher values in this study may result from differences in species, sample preparation, or milling processes.

Further supporting evidence comes from a series of studies by Wardani and colleagues that explored the antibacterial effects of *Stolephorus insularis* extract. The effectiveness observed in these studies can be partially attributed to the fluoride content naturally present in anchovies. Fa'izah et al. found that anchovy extract significantly inhibited the growth of *S. mutans*, a major contributor to dental caries. The mechanism likely involves fluoride's ability to disrupt bacterial metabolism and inhibit the enolase enzyme, thereby reducing acid production and biofilm formation.¹⁹ Similarly, Mahendra et al. demonstrated antibacterial activity against *Enterococcus faecalis*, a pathogen commonly involved in persistent root

canal infections. The presence of fluoride may contribute to bacterial membrane disruption and impaired energy metabolism in this species.^{20,21} Additionally, Wardani and Listya observed the extract's inhibitory effects on *Pseudomonas aeruginosa*, a resilient bacterium associated with oral infections and antibiotic resistance. These findings suggest that the natural fluoride content in anchovy plays a crucial role not only in enamel remineralization but also in exerting broad-spectrum antimicrobial effects against pathogenic oral bacteria.²²

The high calcium content (1,305.80 mg/100 g) observed in this study is consistent with the nutritional profile reported by Syah Putri and Zakaria, who found anchovy to be a rich source of calcium and protein. These nutrients are crucial for dental development, especially in children, making anchovy-based products a promising candidate for pediatric oral health applications.¹⁰

The high nano-sized particle distribution (91.5%) observed in this study is particularly relevant to modern dental applications. Nanotechnology has been shown to improve fluoride uptake by enamel, prolong fluoride retention, and enhance penetration into enamel microstructures.²³ This opens potential for anchovy-derived products to be formulated as advanced fluoride delivery systems, such as varnishes, gels, or even nano-coating agents for tooth surfaces.

Natural sources of fluoride, like anchovy, present several advantages: they are cost-effective, biocompatible, and culturally acceptable in fluoride-deficient or low-resource regions. Marine fish bones, especially from anchovy, have been identified as bioavailable sources of fluoride and calcium, supporting the formulation of sustainable and accessible caries-preventive agents.

This study adds to the growing body of literature that supports the use of marine-derived ingredients in oral health. Given the high fluoride content, nano-scale particle size, and supportive evidence from previous studies, *S.*

commersonii anchovy powder holds significant promise for use in preventive dentistry. Comparatively, the fluoride level found in this study is within the range of fluoride concentrations reported in some fish species used for fluoridated supplements, indicating that *ikan teri Medan* could serve as a base material for the development of natural fluoride

CONCLUSION

This study identified that *S. commersonii* (anchovy) contains a notable concentration of natural fluoride (21.05 ppm) alongside high levels of calcium and phosphorus. The majority of the processed powder particles were within the nano range, indicating its potential suitability for further development in dental applications. Fluoride plays critical role in inhibiting cariogenic bacteria and enhancing enamel remineralization. The presence of fluoride in anchovy nanopowder suggests its promise as a natural source in caries prevention strategies. Further research is needed to explore its bioavailability, safety, and efficacy in clinical settings, as well as formulation studies to develop anchovy-based fluoride agents that meet safety and regulatory standards.

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