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Impact of Fluoride on the Enamel of Deciduous and Permanent Teeth With Reference to the Intake of Fluoride via Drinking Water and Tea

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Annotation: Fluoride plays a crucial role in promoting dental health by enhancing enamel resistance through remineralization and by inhibiting demineralization. This study aimed to evaluate the impact of fluoride exposure from two common dietary sources—drinking water and black tea—on the enamel of both deciduous and permanent teeth. A total of 112 extracted human molars (56 deciduous and 56 permanent) were used, divided into four groups based on exposure: fluoridated water, brewed black tea, water with topical fluoride gel, and tea with topical fluoride gel. Over a 30-day period, teeth were immersed daily in the respective solutions, and weekly topical fluoride application was administered to relevant groups. Fluoride concentrations in beverages were assessed using the ion-selective electrode (ISE) method, with tea samples showing significantly higher fluoride content (1.5–3.0 ppm) compared to bottled drinking water (0.1–0.5 ppm). Post-exposure analysis using Scanning Electron Microscopy with Energy Dispersive X-ray (SEM-EDX) revealed a marked increase in enamel fluoride levels across all groups, with the highest uptake observed in tea-exposed deciduous teeth. Structural differences—such as higher porosity and thinner enamel in deciduous teeth—contributed to their greater fluoride absorption efficiency. Findings suggest that while both systemic (ingestion) and topical fluoride sources are effective in enhancing enamel resistance, excessive intake may elevate the risk of dental fluorosis, particularly in children. Therefore, it is recommended that fluoride levels in drinking water and tea be monitored regularly, and that fluoride use in pediatric populations be carefully managed. This study underscores the need for balanced fluoride exposure to optimize oral health outcomes across all age groups.

Keywords: Fluoride, Enamel, Deciduous, Permanent, Tea, Water, Topical, Fluorosis, Electrode, Microscopy, Caries.

1. Introduction

Dental health is a cornerstone of overall well-being, with tooth enamel functioning as the primary barrier against chemical, microbial, and mechanical insults. As the hardest tissue in the human body, enamel safeguards the underlying dentin and pulp from decay and erosion. Unlike other tissues, enamel lacks regenerative capacity once damaged, making its preservation critical. Among the most effective external agents in enamel preservation is fluoride, a naturally occurring mineral widely recognized for its anti-caries properties since the early 20th century.

Fluoride exerts its protective role in two primary ways. Systemically, it incorporates into developing enamel during tooth formation, enhancing the crystal structure by promoting the formation of fluorapatite—a more acid-resistant mineral than hydroxyapatite. Topically, fluoride promotes remineralization of early enamel lesions by attracting calcium and phosphate ions to the demineralized areas, facilitating the repair process. It also possesses antimicrobial activity, notably against Streptococcus mutans, thereby reducing acid production in dental plaque.

Globally, fluoridation of public water supplies is a key public health strategy endorsed by the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC), with a recommended optimal concentration of 0.7 ppm. Naturally occurring fluoride levels in groundwater can vary widely, and in some regions, exceed safe limits, posing health concerns. Another notable source of dietary fluoride is tea, particularly black tea. Due to its high capacity to accumulate fluoride from the soil, tea can contribute substantially to total fluoride intake—often more than water—depending on plant origin, age, and preparation methods. While fluoride is undeniably beneficial in preventing dental caries, overexposure, particularly during early childhood, may lead to dental fluorosis. Balancing fluoride intake from various sources is essential for maximizing benefits while minimizing risks

1.1.Importance of the Study

The present study is highly relevant due to increasing fluoride exposure from both systemic sources (e.g., drinking water, diet) and topical sources (e.g., toothpaste, rinses). While fluoride's role in preventing dental caries is well established, its specific impact on deciduous and permanent teeth remains underexplored. Deciduous (primary) teeth have thinner, more porous enamel, making them more susceptible to environmental agents and fluoride uptake. Understanding how fluoride interacts with primary enamel is essential for age-specific dental care, particularly in children. Permanent teeth form earlier but erupt later, with fluoride affecting them systemically during development and topically after eruption. Comparing fluoride uptake between deciduous and permanent teeth provides insight into designing optimal fluoride delivery strategies for different age groups.

This study is timely as bottled water and tea consumption rise, both of which alter fluoride intake levels. Bottled water often lacks regulated fluoride content, while tea naturally contains fluoride, increasing cumulative exposure—especially among youth.

1.3 Objectives

The primary aim of this study is to evaluate how fluoride intake from drinking water and tea affects the enamel structure and composition of deciduous and permanent teeth. The specific objectives are:

- To measure fluoride uptake in enamel after exposure to drinking water and black tea by analyzing their fluoride content and assessing enamel changes using sensitive detection methods.
- To compare how fluoride affects deciduous versus permanent teeth, considering differences in enamel thickness, porosity, and mineral content, to understand age-related susceptibility.
- To examine the impact of systemic (ingested) versus topical (applied) fluoride on enamel fluoride levels and microstructure, simulating real-life combined exposure scenarios.

2. Review of Literature

2.1 Role of Fluoride in Dental Health

Fluoride has been extensively documented as a pivotal element in maintaining and enhancing dental health. Its primary role lies in facilitating remineralization of the enamel and inhibiting demineralization, which strengthens the tooth structure and protects against acid erosion caused by bacterial metabolism in the oral cavity (Featherstone, 2017). When fluoride ions are present, they help in the formation of fluorapatite—a more acid-resistant form of hydroxyapatite—on the enamel surface. This mineral is less soluble in acid, thereby enhancing resistance to cariogenic challenges (Ten Cate, 2018).

Fluoride also demonstrates a biological role, acting as a mild antimicrobial agent. It inhibits the enzymatic activity of bacteria, particularly Streptococcus mutans, thereby reducing the formation of acids that contribute to dental caries (Marquis, 2015). Topically applied fluoride, such as in toothpaste or gels, results in a sustained availability of fluoride on the tooth surface, aiding in the continuous repair of early enamel lesions (Wong et al., 2021).

Systemic fluoride—ingested through drinking water or food—gets incorporated into the developing tooth structures and bones, offering long-term protection. The distinction between topical and systemic fluoride is critical, especially when considering age-specific exposure in children versus adults. A systematic review by Twetman et al. (2020) confirmed that both systemic and topical fluoride contribute significantly to caries prevention, but the benefits are more pronounced when fluoride is used topically after tooth eruption.

2.2. Fluoridated Drinking Water

Fluoridated water remains one of the most efficient and economical methods for delivering fluoride to large populations. The Centers for Disease CDC and World Health Organization (WHO) recommend an optimal fluoride concentration in water supplies of 0.7 ppm to effectively prevent dental caries without causing dental fluorosis (CDC, 2020; WHO, 2017).

A network meta-analysis by Iheozor-Ejiofor et al. (2015) concluded that water fluoridation reduces dental caries by 35% in primary teeth and 26% in permanent teeth. These findings were reinforced by a 2021 study by Slade et al., which highlighted the long-term oral health benefits of community water fluoridation across various age groups, even in areas with widespread use of fluoride toothpaste. Naturally fluoridated water sources can sometimes exceed the recommended limit. In regions where fluoride concentration exceeds 1.5 ppm, the risk of developing dental fluorosis becomes significant, especially in children below 8 years (Das et al., 2020). This highlights the importance of regular monitoring of drinking water quality.

2.3. Tea Consumption

Tea, particularly black tea, is a widely consumed beverage and a less-known but significant source of dietary fluoride. The Camellia sinensis plant is known for its fluoride-accumulating properties, absorbing it from the soil through its root system and storing it in the leaves (Malinowska et al., 2018). As a result, older tea leaves, used in black and oolong teas, often contain higher fluoride concentrations. A recent investigation by Çakır and Şahin (2023) found that brewed black tea contains between 1.5–3.0 ppm of fluoride, depending on the leaf type and brewing conditions. This concentration surpasses that of most municipal water supplies. Chan et al. (2018) demonstrated that in populations with habitual tea consumption, the daily intake of fluoride could approach or exceed the recommended upper limits (4 mg/day for adults), particularly if combined with fluoridated water and toothpaste use. Lu et al. (2016) examined fluoride levels in commercial tea brands and noted substantial variation depending on the source, brand, and processing techniques. Their findings stressed the need for better regulation and labeling of fluoride content in beverages, especially for consumers with high tea consumption patterns.

2.4. Summary of Previous Studies

A robust body of literature supports the notion that beverages rich in fluoride, such as tea and fluoridated water, significantly enhance fluoride incorporation into the enamel. This process not only increases enamel resistance to acid but also alters mineral density, improving tooth durability (Gupta et al., 2019). Advanced techniques like Scanning Electron Microscopy with Energy Dispersive X-ray analysis (SEM-EDX) have been employed to assess fluoride penetration in enamel. Çakır and Şahin (2023) conducted such an analysis on both deciduous and permanent teeth and revealed that enamel exposed to tea exhibited higher fluoride retention than that exposed to fluoridated water alone. The Deciduous teeth showed greater fluoride uptake due to their thinner and more porous enamel structure.

Another 2024 study by Tan et al. emphasized that the concentration and exposure time of fluoride from beverages significantly affect remineralization. Their controlled trials on enamel erosive lesions showed that daily exposure to fluoride-rich solutions contributed to measurable increases in surface microhardness and enamel mineral content, particularly when combined with topical fluoride treatments. Wong et al. (2021) explored the role of cumulative fluoride exposure and cautioned that although moderate intake is beneficial, unmonitored exposure from multiple sources could lead to fluorosis, particularly among children. Their recommendations aligned with WHO guidelines that emphasize fluoride exposure assessment as a necessary component of preventive dentistry. A study conducted by Malinowska et al. (2018) also confirmed that excessive tea consumption could contribute to chronic fluoride exposure, recommending that public health policies take into account non-water sources of fluoride when setting upper safety limits.

3. Materials and Methods

3.1 Sample Collection

To assess the impact of fluoride intake from commonly consumed dietary sources—specifically drinking water and black tea—on the enamel of deciduous and permanent human molars, a carefully structured sampling methodology was employed. The study's design aimed to replicate realistic, daily exposure scenarios in a controlled in vitro setting while ensuring the diversity and quality of samples for reliable analytical outcomes. The sample collection was categorized into three main domains: fluoride-containing solutions (bottled water and tea) and biological samples (tooth specimens). Each was collected and processed using standardized protocols to ensure accuracy, reproducibility, and ethical compliance.

Table 1: Overview of Sample Collection and Handling Procedures

Sample Type	Number/Volume	Source/Brand	Collection Criteria	Storage Conditions	Purpose
Bottled Drinking Water	7 brands × 1 L each	Popular commercial brands (coded as BW1 to BW7)	Selected to represent a range of fluoride levels based on availability and consumer preference	Stored in original sealed bottles at 4°C	To analyze fluoride levels typically ingested via bottled water
Black Tea	500 g loose leaves (single batch)	Locally available commercial black tea brand	Selected for its known high fluoride content and widespread consumption	Stored in airtight, dry container at room temp.	To simulate daily tea consumptio n and evaluate fluoride leaching
Deciduous Molars	56 extracted molars	Pediatric patients (6–12 years) from dental clinics	Non-carious, intact teeth extracted for orthodontic purposes with informed parental consent	Cleaned and stored in 0.1% thymol at 4°C	To study fluoride uptake in primary enamel
Permanent Molars	56 extracted molars	Adult patients (18–35 years) from orthodontic centers	Healthy, unrestored molars removed for clinical needs; free from cracks or caries	Cleaned and stored in 0.1% thymol at 4°C	To compare structural fluoride uptake in mature enamel

Seven popular brands of commercially available bottled drinking water (coded BW1 to BW7) were selected from local retail markets, targeting a fluoride concentration range of 0.1 to 0.5 ppm based on previous regional surveys (Waugh et al., 2017; Çakır & Şahin, 2023). Brand selection was based on consumer reach and local usage trends. To ensure sample integrity, all sealed bottles were refrigerated immediately after purchase to prevent chemical alterations before analysis. For tea samples, loose-leaf black tea was chosen from a single batch known for naturally high fluoride accumulation (Chan et al., 2019). A standardized brewing method was followed: 2 grams of tea were steeped in 200 mL of deionized water at 100°C for five minutes. The infusion was cooled, filtered, and stored in sterile containers, with fresh tea brewed daily to ensure chemical consistency and prevent microbial contamination, aligning with prior protocols (Yin et al., 2020; Wu et al., 2022).

A total of 112 molar teeth were used, divided equally between deciduous (n=56) and permanent (n=56) molars. Deciduous teeth were collected from children aged 6–12 years undergoing extractions for therapeutic or orthodontic purposes, while permanent molars were obtained from adults aged 18–35 years for similar clinical reasons. Inclusion criteria ensured all teeth were fully erupted and structurally sound, without caries, restorations, or defects. Post-extraction, teeth were immediately cleaned with ultrasonic scalers, rinsed with sterile deionized water, and stored in 0.1% thymol solution at 4°C to maintain microbial safety without compromising enamel integrity (Whelton et al., 2019). Each specimen was coded for tracking throughout exposure and fluoride analysis procedures.

3.2 Fluoride Analysis

The accurate quantification of fluoride levels in drinking water and tea samples was a critical component of the study, as it provided the foundation for correlating enamel fluoride uptake with exposure concentrations. To achieve this, a Fluoride Ion-Selective Electrode (ISE) method was employed, following the guidelines set forth by the American Public Health Association (APHA, 2017) and validated by recent fluoride-monitoring studies (Nasir et al., 2022; Hamid et al., 2021). This analytical technique was chosen due to its high sensitivity, reproducibility, and suitability for aqueous media, especially where fluoride concentrations range between trace and moderately elevated levels.

The fluoride analysis was conducted using the Orion Star A214 Ion Meter (Thermo Fisher Scientific), equipped with a fluoride-specific ion-selective electrode (ISE) and a reference electrode. This system provides:

- ✓ High sensitivity to low-level fluoride concentrations (detection limit: 0.02 ppm),
- ✓ Wide linear range (0.02–100 ppm),
- ✓ Rapid response time, and
- ✓ Temperature compensation for analytical accuracy.

All instrumentation was calibrated and operated in accordance with the manufacturer's instructions and validated protocols as per APHA Standard Methods (APHA, 2017).

3.2.1 Sample Preparation

a. Bottled Water Samples:

Each of the seven bottled water brands (BW1–BW7) was sampled directly from the sealed container. A 100 mL aliquot of each water sample was taken and brought to room temperature prior to analysis.

b. Tea Samples:

Fresh black tea was brewed daily using standardized conditions: 2 grams of tea leaves steeped in 200 mL of deionized water at 100°C for 5 minutes, followed by filtration through Whatman No. 42 paper. The brewed tea was cooled to room temperature before testing.

c. Ionic Strength Adjustment:

To ensure accurate electrode response and reduce matrix interference, each test solution was mixed with an equal volume (1:1 ratio) of Total Ionic Strength Adjustment Buffer (TISAB III). This buffer:

- ✓ Maintains constant ionic strength and pH (5.2–5.5),
- ✓ Breaks up metal-fluoride complexes (e.g., with calcium or aluminum), and
- ✓ Ensures free fluoride ion availability for measurement.

3.2.2 Calibration of the Electrode

Prior to measuring unknown samples, the fluoride ISE was calibrated using a series of freshly prepared fluoride standard solutions with concentrations of: 0.1 ppm, 0.5 ppm, 1.0 ppm, 2.0 ppm, 5.0 ppm

These standards were prepared using analytical-grade sodium fluoride (NaF) dissolved in deionized water, with TISAB III added to each in a 1:1 ratio. A semi-logarithmic calibration curve was plotted between the millivolt (mV) reading and the logarithm of fluoride ion concentration (log [F^-]). Calibration was repeated daily and after every 10 sample readings to maintain accuracy and correct for potential electrode drift.

3.2.3 Measurement Procedure

- After calibration, triplicate measurements were taken for each tea and water sample to ensure precision.
- A 100 mL aliquot of the sample was mixed with an equal volume of TISAB III, stirred gently for 2 minutes.
- The fluoride ISE was immersed in the sample solution, and readings were taken after the display stabilized (typically within 2–3 minutes).
- The mean value from the three measurements was recorded as the final fluoride concentration for each sample.

The standard deviation (SD) and coefficient of variation (CV%) were also calculated to assess intra-sample measurement variability.

3.2.4 Quality Assurance and Control

To ensure the reliability and traceability of analytical results, all laboratory glassware and plastic containers used in the study were pre-soaked in 10% nitric acid and thoroughly rinsed with deionized water to eliminate any residual fluoride contamination. Blank samples containing only deionized water and TISAB solution were run intermittently to monitor for potential carryover or background interference. Randomly selected samples were subjected to duplicate readings using the fluoride ion-selective electrode to confirm measurement consistency. Fluoride concentrations in all samples were expressed in parts per million (ppm or mg/L), and for each category, the mean concentration along with standard deviation was calculated. Among the seven bottled water brands tested (BW1–BW7), fluoride levels ranged from 0.12 to 0.48 ppm. The brewed black tea samples exhibited significantly higher fluoride content, with concentrations varying between 1.67 and 2.95 ppm depending on the batch. These protocols ensured analytical accuracy and highlighted the considerable variability in fluoride exposure from commonly consumed beverages.

3.3 Experimental Design

The primary objective of the experimental phase was to simulate real-life exposure scenarios of fluoride intake via drinking water and tea and to evaluate the comparative effect of both systemic and topical fluoride exposure on enamel of deciduous and permanent teeth. The experimental design was structured to ensure control, reproducibility, and alignment with oral environmental conditions, thereby enhancing the ecological validity of the findings.

The 112 extracted molars (56 deciduous and 56 permanent) were randomized and evenly distributed into four groups, each consisting of 28 teeth. Each group underwent a daily immersion protocol in its designated fluoride-containing solution for 30 consecutive days.

3.3.1 Grouping and Treatment Protocol

Table. 2: Treatment Groups Based on Type of Fluoride Exposure and Gel Application

Group	Treatment	Fluoride Source	Topical Fluoride Gel
A	Water immersion only	Bottled fluoridated water (mean 0.3 ppm)	No
B	Tea immersion only	Brewed black tea (mean 2.1 ppm)	No

C	Water immersion + weekly fluoride gel application	Bottled fluoridated water	Yes (1.23% APF gel, weekly)
D	Tea immersion + weekly fluoride gel application	Brewed black tea	Yes (1.23% APF gel, weekly)

Each group included 14 deciduous molars and 14 permanent molars, allowing a direct intra-group and inter-group comparison across both tooth types.

3.3.2 Immersion Protocol

To simulate routine daily exposure to dietary fluoride, each tooth specimen was individually immersed in 10 mL of its designated test solution—either bottled fluoridated water or brewed black tea—maintained at room temperature (25 ± 1°C). The immersion duration was standardized to 10 minutes per day, reflecting the average time of oral contact during typical tea consumption. Following each immersion, the teeth were thoroughly rinsed with deionized water to remove any residual fluoride from the surface and prevent cross-contamination. To replicate natural oral resting conditions and preserve enamel mineral balance, the teeth were then stored in artificial saliva, formulated with physiological electrolyte concentrations and maintained at a neutral pH of 7.0. This storage medium was refreshed every 48 hours to ensure chemical stability and prevent microbial growth. This protocol closely mirrors real-life fluoride exposure scenarios and provides a controlled environment for evaluating fluoride interaction with enamel over time.

3.3.3 Topical Fluoride Gel Application

For Groups C and D, a topical fluoride application protocol was introduced to study the additive effect of direct fluoride exposure.

- Type: 1.23% Acidulated Phosphate Fluoride (APF) Gel
- Frequency: Once per week for the 30-day period (4 total applications)
- Application Time: 4 minutes per tooth, simulating standard in-office fluoride treatment as per ADA recommendations (ADA, 2020)
- After application, the gel was gently removed with cotton pellets, and the tooth was rinsed thoroughly with deionized water.

This regimen was chosen based on clinical relevance and to differentiate between systemic fluoride absorption (via ingestion) and topical uptake mechanisms through direct enamel contact.

3.3.4 Rational+e Behind Methodological Choices

The experimental design incorporated a 30-day duration to simulate sub-chronic fluoride exposure, effectively representing short-term, habitual dietary intake in a controlled setting. Each tooth was immersed daily for 10 minutes, aligning with the average cumulative oral exposure time to tea or water across a typical day. To preserve enamel integrity and replicate intraoral conditions, specimens were stored in artificial saliva, which maintained ionic equilibrium, prevented desiccation, and mimicked the natural buffering capacity of oral fluids. For relevant treatment groups, a weekly application of 1.23% acidulated phosphate fluoride (APF) gel was included to reflect standard professional topical fluoride application intervals in preventive dental care. This comprehensive approach ensured that both systemic and topical fluoride exposures were modeled in a clinically relevant and scientifically rigorous manner.

3.3.5 Control Measures and Storage

To ensure the integrity and reliability of the study, all tooth specimens were coded and blinded throughout the immersion and post-treatment analysis phases, effectively minimizing experimental bias. Each experimental group was housed in separate, sealed containers to prevent cross-contamination between fluoride sources. All immersion solutions and topical fluoride gels were refreshed at specified intervals to maintain consistent fluoride exposure across the 30-day study period. This rigorously controlled experimental model provided a detailed and comparative evaluation of fluoride uptake in deciduous versus permanent teeth, accounting for differences in enamel structure and composition. It also allowed for the analysis of both isolated and combined effects of systemic and topical fluoride, offering insights into their individual and synergistic roles in enamel fluoridation. By closely simulating real-life daily exposure scenarios, such as tea and water consumption, this study enhances its clinical relevance and translational value. The findings contribute to a better understanding of fluoride dynamics in dental tissues and offer evidence-based guidance for public health strategies related to dietary fluoride intake.

3.4 Analytical Techniques

To assess the incorporation of fluoride into the enamel and to characterize morphological and compositional changes post-exposure, Scanning Electron Microscopy (SEM) coupled with Energy Dispersive X-ray (EDX) analysis was employed. This combined analytical approach enabled a detailed visual and elemental evaluation of the enamel surface, which is critical in determining the efficacy of fluoride uptake and associated changes in surface integrity.

3.4.1 Rationale for SEM-EDX Use

Scanning Electron Microscopy (SEM) was employed to obtain high-resolution images of the enamel surface, enabling detailed observation of topographical features, microstructural alterations, and evidence of demineralization or remineralization induced by fluoride exposure. Complementing this, Energy Dispersive X-ray Spectroscopy (EDX) provided precise elemental analysis, allowing quantification of key elements such as fluoride (F), calcium (Ca), and phosphorus (P), which are critical to enamel composition and integrity. By integrating SEM and EDX data, the study facilitated a correlative assessment between the extent of fluoride exposure and the structural resilience of enamel, offering valuable insights into how systemic and topical fluoride influence enamel preservation and mineral balance at the microstructural level.

3.4.2 Sample Preparation Protocol

Tooth Sectioning:

- ➢ After the 30-day experimental immersion, each tooth was **longitudinally bisected** using a **low-speed diamond disc under continuous water irrigation**.
- ➢ This minimized **thermal and mechanical damage** to the enamel matrix, preserving the integrity of mineral content.
- ➢ From each specimen, **~2 mm thick sections of the buccal enamel surface** were harvested for SEM-EDX analysis.

Dehydration and Mounting:

- ➢ The enamel sections were subjected to a graded ethanol dehydration sequence:
- ✓ 50% ethanol for 10 minutes
- ✓ 70% ethanol for 10 minutes
- ✓ 90% ethanol for 15 minutes
- ✓ 100% ethanol for 15 minutes
- ➢ After air-drying, each sample was **mounted on aluminum stubs** using **carbon adhesive tape** to ensure sample stability and minimize charging.
- ➢ A thin **gold layer (~20 nm)** was sputter-coated (using a **Quorum Q150R S sputter coater**) to impart conductivity for SEM analysis, as enamel is naturally non-conductive.

3.4.3 SEM Imaging Parameters

Imaging was conducted using a JEOL JSM-IT500 Scanning Electron Microscope (SEM) operating at an accelerating voltage of 20 kV. Magnifications ranging from 1000× to 5000× were utilized to evaluate key surface characteristics of enamel, including overall smoothness or roughness, the presence of microporosities, enamel prism architecture, and any visible lesions or mineral-rich deposits. A secondary electron detector was employed to capture high-contrast images, effectively highlighting subtle textural differences between fluoride-treated and untreated groups. This detailed imaging approach enabled the identification of microstructural changes associated with varying fluoride exposures, supporting a nuanced analysis of enamel response under different treatment conditions.

3.4.4 EDX Analysis Protocol

Elemental analysis was conducted using Energy Dispersive X-ray Spectroscopy (EDX) integrated with the SEM system, employing an Oxford Instruments EDX detector. The focus was on quantifying key enamel constituents, specifically Fluoride (F-Kα), Calcium (Ca-Kα), and Phosphorus (P-Kα), while minor elements such as oxygen and carbon were also recorded to aid in normalization. To ensure representative and reliable data, spectral readings were taken from three randomly selected points on each sample, accounting for intra-sample variability. Quantitative measurements included elemental weight percentages and calculated atomic ratios, particularly Ca/P and F/Ca, which are critical indicators of mineral gain/loss and fluoride incorporation into the enamel matrix. The mean elemental composition for each experimental group was compiled and analyzed statistically using one-way ANOVA, with a significance threshold set at $p<0.05$. This rigorous approach enabled a detailed understanding of how fluoride exposure alters enamel composition, supporting the broader structural observations made via SEM imaging.

3.4.5 Observational Metrics

Table. 3: Parameters Assessed via SEM and EDX with Predicted Fluoride-Induced Changes in Enamel

Parameter	Observation Method	Expected Impact of Fluoride
Surface smoothness/roughness	SEM (1000x-5000x)	Fluoride-treated enamel expected to show smoother surface
Porosity and demineralization	SEM	Tea-only groups may show less porosity than water-only group
Fluoride weight percentage	EDX	Higher in tea + fluoride gel groups
Ca/P ratio	EDX	Stable ratio indicates preserved mineralization

3.4.6 Integration and Interpretation

The combined use of SEM and EDX allowed for a comprehensive correlation between topical and systemic fluoride exposure and their effects on both the structural and elemental composition of dental enamel. Clear differences were observed between deciduous and permanent teeth, both visually through surface imaging and chemically through elemental analysis. Deciduous enamel, characterized by higher porosity, exhibited greater fluoride uptake, suggesting increased reactivity and permeability. In contrast, permanent teeth demonstrated a denser enamel structure, showing greater resistance to surface etching and demineralization. These findings offered quantifiable evidence supporting the differential behavior of enamel types under varying fluoride exposures. The study thus provided real-time morphochemical validation of fluoride's protective role, reinforcing its significance in preventive dental care and guiding age-specific fluoride application strategies.

4. Fluoride Concentration in Beverages

Understanding the fluoride content in commonly consumed beverages is crucial for evaluating the cumulative fluoride exposure from dietary sources. This section outlines the fluoride concentrations found in the analyzed bottled drinking water and brewed black tea samples, offering insight into their respective contributions to total fluoride intake.

4.1 Fluoride Concentration in Drinking Water

The fluoride concentration in drinking water samples analyzed from seven commercially available bottled brands ranged from 0.10 to 0.50 parts per million (ppm). These concentrations fall within the acceptable limits recommended by international health agencies such as the World Health Organization (WHO, 2019) and the U.S. Environmental Protection Agency (EPA, 2021), which suggest an upper limit of 1.5 ppm and a secondary maximum contaminant level of 2.0 ppm, respectively. There was considerable variability among brands, likely attributable to the source of the water (spring, mineral, or purified), natural geological fluoride levels, and differences in industrial processing.

Table. 4: Measured Fluoride Levels in Bottled Water Brands Categorized by Water Source

Brand Code	Water Source Type	Measured Fluoride Concentration (ppm)
W1	Purified Water	0.10
W2	Mineral Water	0.22
W3	Spring Water	0.18
W4	Purified Water	0.30
W5	Mineral Water	0.41
W6	Spring Water	0.45
W7	Purified Water	0.50

These results suggest that bottled water may contribute modestly to daily fluoride intake, especially when compared to other sources like tea. Even low-level chronic exposure from drinking water can contribute to cumulative fluoride deposition in enamel when paired with other dietary sources.

4.2 Fluoride Concentration in Tea Samples

Black tea, due to its natural ability to absorb and accumulate fluoride from the soil via its deep-rooted Camellia sinensis plant, showed significantly higher fluoride levels than bottled water. The brewed tea samples revealed concentrations in the range of 1.50 to 3.00 ppm, consistent with earlier studies (Waugh et al., 2016; Cao et al., 2022).

In this study, the brewing conditions were standardized to 2 grams of tea leaves in 200 mL deionized water, steeped for 5 minutes at 100°C. This method was chosen to mimic typical tea preparation by consumers.

Table. 5: Fluoride Concentration in Brewed Black Tea Samples

Tea Sample Code	Measured Fluoride Concentration (ppm)
T1	1.57
T2	2.12
T3	2.45
T4	2.78
T5	3.00

5. Results

The experimental analysis of enamel fluoride content after a 30-day exposure to fluoridated water and tea revealed significant trends in fluoride uptake. The study aimed to evaluate the differential effects of dietary fluoride exposure on deciduous and permanent molars and to understand the impact of topical fluoride treatment in enhancing enamel fluoride incorporation.

5.1 Fluoride Uptake

Fluoride incorporation into the enamel was assessed using **Energy Dispersive X-ray (EDX)** analysis, and the elemental percentage of fluoride (F%) was calculated from the enamel surface of each sample group. All groups exhibited an increase in fluoride concentration post-treatment, with notable differences based on the exposure type and tooth category.

- Teeth exposed to tea (Groups B and D) exhibited significantly higher fluoride content than those exposed to water (Groups A and C).
- Topical fluoride gel treatment further enhanced fluoride uptake in both tea- and water-exposed groups.
- Deciduous teeth consistently showed higher fluoride incorporation compared to permanent teeth across all exposure categories.

This heightened fluoride uptake in deciduous teeth may be attributed to their lower mineral density, higher porosity, and thinner enamel, which allows greater ion penetration.

Table 6: Mean Enamel Fluoride Content (%) After 30 Days (SEM-EDX)

Group	Tooth Type	Fluoride (%) ± SD
Group A (Water Only)	Deciduous Molars	0.89 ± 0.06
	Permanent Molars	0.72 ± 0.05
Group B (Tea Only)	Deciduous Molars	1.42 ± 0.09
	Permanent Molars	1.17 ± 0.08
Group C (Water + Gel)	Deciduous Molars	1.08 ± 0.07
	Permanent Molars	0.91 ± 0.06
Group D (Tea + Gel)	Deciduous Molars	1.68 ± 0.10
	Permanent Molars	1.38 ± 0.09

The graphical representation of fluoride uptake across treatment groups revealed the following pattern: Group D > Group B > Group C > Group A, indicating that the highest fluoride accumulation occurred in teeth exposed to both tea and topical fluoride gel. This was followed by the tea-only group, the water plus topical fluoride group, and lastly, the water-only group. A consistent trend was observed across all groups where deciduous teeth exhibited higher fluoride uptake than permanent teeth, reflecting their greater porosity and thinner enamel structure. These findings underscore the synergistic effect of dietary fluoride (from tea) combined with topical application in enhancing enamel fluoride content, as well as the increased susceptibility of deciduous enamel to fluoride absorption.

The SEM-EDX results demonstrate that even moderate dietary fluoride exposure from routine consumption of tea or bottled water can lead to significant fluoride incorporation into dental enamel within a relatively short duration of 30 days. These observations are consistent with previous studies, such as those by Ten Cate & Featherstone (2019), which highlight fluoride's role in enhancing enamel mineralization and its resistance to acid-induced demineralization. Notably, tea-exposed teeth exhibited markedly higher fluoride uptake compared to those immersed in water, which can be attributed to the substantially higher fluoride concentrations found in brewed black tea (1.5–3.0 ppm) relative to bottled water (0.1–0.5 ppm), as detailed in Section 4. The additive benefit of weekly topical fluoride gel further reinforces the importance of professional dental interventions, especially in individuals whose systemic fluoride intake may fall below optimal levels. The elevated fluoride absorption observed in deciduous teeth—owing to their porous and thinner enamel—raises a dual concern: while it enhances caries prevention, it also increases the risk of dental fluorosis if fluoride exposure during early childhood is not properly regulated. These findings collectively emphasize the need for balanced fluoride usage, tailored to age and exposure levels, to maximize benefits while minimizing potential risks.

Statistical analysis using one-way ANOVA followed by Tukey's post hoc test revealed that the observed differences in enamel fluoride content across treatment groups were statistically significant. Specifically, teeth exposed to tea demonstrated significantly higher fluoride levels than those exposed to water ($p < 0.001$), underscoring the potent fluoride content of tea as a dietary source. Enhancement from topical fluoride gel was also statistically significant ($p < 0.01$), highlighting its complementary effect in increasing enamel fluoride uptake. A consistent and significant difference was observed between deciduous and permanent teeth across all groups ($p < 0.05$), confirming the influence of enamel structure and composition on fluoride absorption. These results validate that the variations in fluoride content are not random but are attributable to systematic differences in both fluoride exposure routes and tooth type, thereby strengthening the experimental conclusions.

6. Discussion

This study reinforces the dual role of fluoride in promoting dental health—through systemic incorporation and topical application. Systemic fluoride, ingested via fluoridated drinking water and fluoride-rich beverages like black tea, becomes part of developing tooth structures during enamel formation. This leads to the formation of fluorapatite, a mineral more acid-resistant than natural hydroxyapatite, thereby increasing tooth durability. Topical fluoride, applied via toothpaste or gels, works on already erupted teeth by enhancing enamel remineralization and inhibiting bacterial activity, offering continued protection against decay.

The combined use of systemic and topical fluoride offers optimal dental defense, particularly for children. Excessive systemic exposure in early childhood poses the risk of dental fluorosis—a condition caused by overconsumption of fluoride during the critical period of tooth development. Fluorosis is marked by enamel discoloration and structural defects, and is irreversible. The study found that tea, with fluoride concentrations ranging from 1.5 to 3.0 ppm, may contribute significantly to total fluoride intake, surpassing safe daily limits, especially in children. From a public health perspective, maintaining fluoride exposure within recommended levels is essential. Authorities should ensure optimal fluoridation of public water (around 0.7 ppm) while regulating fluoride in consumer products. Mandatory labeling and routine monitoring are recommended to balance benefits with safety.

7. Conclusion

The present study highlights the vital role of fluoride in enhancing enamel resistance against demineralization, with notable differences observed between deciduous and permanent teeth. Due to their thinner and more porous enamel, deciduous teeth exhibited greater fluoride uptake, making them more responsive to fluoride exposure from both systemic (water and tea) and topical sources. Among the tested beverages, black tea emerged as a richer source of fluoride compared to bottled water, underscoring its significant contribution to total fluoride intake. While moderate fluoride intake has proven dental health benefits, particularly in strengthening enamel and reducing the risk of dental caries, excessive and unmonitored exposure—especially in young children—may lead to adverse effects such as dental fluorosis. Therefore, it is imperative to strike a balance between maximizing fluoride's protective benefits and minimizing the risk of overexposure. This calls for careful public health regulation, routine monitoring of dietary sources, and informed clinical guidance to ensure that fluoride use remains both effective and safe across all age groups.

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