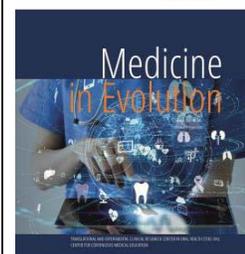


# Remineralization of Enamel White Spot Lesions Using Plant-Based Agents

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## Abstract

**1. Background/Objectives:** Dental caries is one of the most prevalent chronic diseases worldwide and results from the demineralization of dental hard tissues caused by acids produced by bacterial metabolism of fermentable carbohydrates. Early enamel lesions, known as white spot lesions (WSLs), represent the initial and potentially reversible stage of the caries process. Although fluoride is considered the gold standard for remineralization therapy, increasing attention has been directed toward natural plant-derived agents with antimicrobial and bioactive properties. The aim of this *in vitro* study was to evaluate the remineralization potential of ginger (*Zingiber officinale*) and rosemary (*Rosmarinus officinalis*) extracts compared with sodium fluoride on artificially induced enamel white spot lesions using laser fluorescence. **2. Methods:** Thirty extracted human teeth were randomly assigned to three groups (n = 10): ginger extract, rosemary extract, and sodium fluoride varnish. Artificial enamel lesions were induced using 37.5% orthophosphoric acid. Laser fluorescence values were measured with a DIAGNOdent device at baseline (T0), after demineralization (T1), and after 10 days of remineralization treatment (T2). **3. Results:** DIAGNOdent values increased after demineralization, confirming successful lesion formation, and decreased after treatment in all groups. The greatest reduction was observed in the ginger extract group. **4. Conclusion:** Plant-based agents demonstrated remineralizing potential on early enamel lesions, with ginger extract showing the most promising effect as a possible complementary alternative to fluoride-based therapies

**Keywords:** dental caries, enamel remineralization, white spot lesions, plant extracts, laser fluorescence, DIAGNOdent

## INTRODUCTION

Dental caries remains one of the most prevalent chronic diseases worldwide and represents a major public health concern. The disease is characterized by the progressive demineralization of dental hard tissues caused by acids produced during the bacterial metabolism of fermentable carbohydrates within the dental biofilm [1,6]. When the balance between demineralization and remineralization shifts toward mineral loss, structural damage to enamel occurs, eventually leading to cavitated lesions [5]. The earliest clinically detectable stage of the caries process is represented by enamel white spot lesions (WSLs), which are characterized by subsurface mineral loss while the superficial enamel layer remains relatively intact [6]. Clinically, these lesions appear as opaque white areas on the enamel surface and represent the initial stage of caries development. White spot lesions are potentially reversible if appropriate preventive strategies are implemented. Consequently, modern preventive dentistry increasingly focuses on non-invasive approaches aimed at enhancing enamel remineralization and preventing lesion progression [8]. Fluoride-based agents are widely considered the gold standard in the prevention and management of dental caries due to their ability to enhance enamel remineralization and increase resistance to acid dissolution [18]. Fluoride promotes the formation of fluorapatite and facilitates the deposition of calcium and phosphate ions in demineralized enamel areas. However, concerns regarding excessive fluoride exposure, particularly the risk of dental fluorosis and other potential adverse effects associated with chronic intake, have stimulated the search for alternative or complementary remineralizing agents [17].

In recent years, natural plant-derived compounds have attracted increasing attention in dental research due to their antimicrobial, antioxidant, and anti-inflammatory properties [19]. Several plant extracts have demonstrated the ability to inhibit the growth and metabolic activity of cariogenic microorganisms, particularly *Streptococcus mutans*, which plays a key role in the development of dental caries [16]. Among these natural agents, ginger (*Zingiber officinale*) and rosemary (*Rosmarinus officinalis*) have been investigated for their potential oral health benefits. Ginger contains biologically active compounds such as gingerols and shogaols that exhibit antimicrobial and anti-inflammatory properties [2,9], while rosemary contains phenolic compounds such as rosmarinic acid and carnosic acid with antibacterial and antioxidant activity [12]. These compounds may contribute to the inhibition of cariogenic bacteria and indirectly support enamel remineralization.

The evaluation of enamel remineralization requires reliable diagnostic methods capable of detecting early structural changes in dental tissues. Laser fluorescence devices, such as DIAGNOdent, are widely used for the detection and monitoring of early enamel lesions due to their ability to quantify changes in tooth structure associated with demineralization [20]. Therefore, the aim of the present in vitro study was to evaluate the remineralization potential of ginger (*Zingiber officinale*) and rosemary (*Rosmarinus officinalis*) extracts compared with sodium fluoride on artificially induced enamel white spot lesions using laser fluorescence measurements obtained with the DIAGNOdent system. The working hypothesis was that plant-based extracts may demonstrate measurable remineralizing effects on early enamel lesions and could represent potential complementary alternatives to conventional fluoride-based therapies.

### *Aim and objectives*

The aim of the present study was to evaluate the remineralization potential of plant-based agents on artificially induced enamel white spot lesions using laser fluorescence analysis. Specifically, the study investigated the effectiveness of ginger (*Zingiber officinale*)

and rosemary (*Rosmarinus officinalis*) extracts compared with sodium fluoride, a widely used remineralizing agent in preventive dentistry.

The primary objective of the study was to determine the capacity of the tested plant extracts to promote enamel remineralization, assessed through changes in DIAGNOdent values before and after treatment. Secondary objectives included comparing the remineralizing effectiveness of ginger and rosemary extracts with sodium fluoride, as well as evaluating the influence of the duration of agent application on the remineralization process. Additionally, the study aimed to explore the potential of plant-derived agents as complementary or alternative preventive strategies in the management of early enamel lesions.

## MATERIAL AND METHODS

This in vitro experimental study was designed to evaluate the remineralization potential of plant-based agents on artificially induced enamel white spot lesions. The remineralizing effects of ginger (*Zingiber officinale*) extract and rosemary (*Rosmarinus officinalis*) extract were compared with those of sodium fluoride varnish using laser fluorescence measurements obtained with the DIAGNOdent device. A total of 30 extracted human teeth were collected from patients who required dental extraction for therapeutic reasons. All patients were informed about the use of the extracted teeth for scientific and educational purposes, and written informed consent was obtained prior to their inclusion in the study. The study protocol followed the ethical principles outlined in the Declaration of Helsinki (2013 revision). Teeth were selected according to strict inclusion criteria in order to ensure sample homogeneity. Only intact teeth without primary or secondary caries, restorative treatments, endodontic therapy, structural defects, or enamel developmental abnormalities were included. Teeth presenting fractures caused during extraction, visible enamel hypoplasia, fluorosis, structural discolorations, or prior bleaching treatments were excluded from the study. After extraction, the teeth were mechanically cleaned to remove calculus deposits and stored in 0.1% thymol solution for five days at room temperature in order to prevent bacterial contamination. To ensure specimen stability during the experimental procedures, the roots of the teeth were embedded in addition silicone impression material (V-Posil Putty Fast, VOCO, Germany). The lingual surfaces of the teeth were covered with two layers of transparent nail varnish, leaving a standardized enamel area measuring approximately 4 × 4 mm exposed on the vestibular surface. This exposed area represented the experimental window used for the induction of artificial enamel lesions and for subsequent remineralization procedures.

After preparation, the specimens were allowed to dry at room temperature for 24 hours and were then stored in distilled water until further use.

Artificial white spot lesions were induced by applying 37.5% orthophosphoric acid gel to the exposed enamel area for 30 seconds. This protocol is frequently used in in vitro studies to simulate early enamel demineralization without causing irreversible structural damage [7,12]. The specimens were then rinsed with water for 30 seconds and air-dried for 10 seconds until the characteristic chalky white appearance of early enamel demineralization became visible.

Following the creation of artificial enamel lesions, the specimens were randomly divided into three groups of equal size (n = 10 per group):

**Group 1:** treatment with ginger extract (0.5% glyceric extract; AdNatura, Romania)

**Group 2:** treatment with rosemary extract (0.5% extract; PlantExtract, Romania)

**Group 3:** treatment with sodium fluoride varnish (Fluorodose, 5% NaF; Centrix, USA)

The plant-based extracts were applied to the exposed enamel surfaces using sterile dental applicators for 60 seconds twice daily over a period of 10 consecutive days. A new applicator was used for each specimen in order to prevent cross-contamination. For the control group, the sodium fluoride varnish was applied according to the manufacturer's instructions. After application, the materials were gently removed from the enamel surface without rinsing, and the specimens were returned to distilled water for storage throughout the experimental period.

Enamel mineralization was evaluated using a laser fluorescence device (DIAGNOdent, KaVo, Biberach, Germany), a diagnostic method widely used for the detection and monitoring of early enamel lesions [15,20]. The device operates by emitting laser light with a wavelength of approximately 655 nm onto the tooth surface. The fluorescence emitted by bacterial metabolites and altered enamel structures is detected and displayed as numerical values ranging from 0 to 99, corresponding to the degree of enamel demineralization.

Measurements were performed at three time points during the experiment:

- **T0:** baseline measurement before artificial demineralization
- **T1:** after the induction of enamel white spot lesions
- **T2:** after 10 days of remineralization treatment

Prior to each measurement session, the device was calibrated using the ceramic calibration standard supplied by the manufacturer. Each measurement was performed three times on the same area of the enamel surface under standardized conditions, and the mean value of the three readings was recorded for statistical analysis.



Figure 1. DIAGNOdent device used for laser fluorescence evaluation of enamel lesions

All measurements were recorded manually in a data collection table organized according to experimental group and measurement time (T0, T1, and T2). The recorded data were subsequently transferred to a Microsoft Excel spreadsheet for data organization and preparation for statistical analysis.

Statistical analysis was performed using IBM SPSS Statistics software (version 23; IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated as means and standard deviations for each group and measurement time. The normality of the data distribution was assessed using the Shapiro-Wilk test due to the relatively small sample size. Differences between experimental groups were analyzed using one-way analysis of variance (ANOVA). Within-group comparisons across different time points were evaluated using paired t-tests. Non-parametric comparisons of percentage changes were performed using the Kruskal-Wallis test. Additionally, the influence of contact time (1, 3, and 6 minutes) on the remineralization process was analyzed using the Friedman test. The level of statistical significance was set at  $p < 0.05$ .

## RESULTS

The laser fluorescence measurements obtained with the DIAGNOdent device were analyzed at three different time points: baseline (T0), after artificial demineralization (T1), and

after remineralization treatment (T2). These measurements allowed the evaluation of enamel mineralization changes throughout the experimental procedure.

The descriptive statistical analysis showed a clear variation in DIAGNOdent values across the three evaluation stages. The mean value increased markedly from 1.99 at baseline (T0) to 10.97 after demineralization (T1), confirming the successful induction of artificial white spot lesions. After the remineralization phase, the mean value decreased to 2.25 (T2), indicating a partial restoration of enamel mineral content. Although the mean value at T2 did not return completely to the baseline level, the difference between T0 and T2 was relatively small, suggesting a substantial remineralization effect.

Table 1 Descriptive statistics and comparison of overall DIAGNOdent values (repeated measures ANOVA)

Time point	Mean	Standard deviation	95% CI lower	95% CI upper	Minimum	Maximum
T0	1.99	1.03	1.60	2.38	0.55	4
T1	10.97	2.77	9.93	12	6.3	15
T2	2.25	0.64	2.01	2.49	0.66	3.66

Repeated measures ANOVA did not reveal statistically significant differences between the three measurement moments ( $p = 0.65$ ).

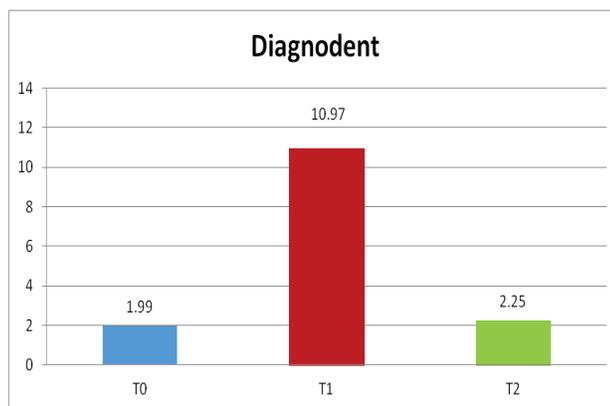


Figure 2. DIAGNOdent values recorded at different observation times (T0, T1, T2)

The comparison of DIAGNOdent values between the experimental groups showed no statistically significant differences at baseline ( $p = 0.82$ ) or after demineralization ( $p = 0.48$ ), indicating that the specimens were comparable prior to treatment.

However, at the end of the remineralization period (T2), differences between groups became evident. The lowest mean DIAGNOdent value was recorded in the ginger extract group ( $1.7 \pm 0.59$ ), followed by the sodium fluoride group ( $2.29 \pm 0.39$ ), while the rosemary extract group presented the highest mean value ( $2.76 \pm 0.41$ ).

The ANOVA test revealed a statistically significant difference between the groups at T2 ( $p = 0.0001$ ).

Table 2. Descriptive statistics and comparison of DIAGNOdent values between groups

Group	T0 mean $\pm$ SD	T1 mean $\pm$ SD	T2 mean $\pm$ SD
Ginger extract	1.81 $\pm$ 0.89	10.25 $\pm$ 2.74	1.70 $\pm$ 0.59
Rosemary extract	2.08 $\pm$ 1.18	11.76 $\pm$ 2.45	2.76 $\pm$ 0.41
Sodium fluoride	2.07 $\pm$ 1.11	10.89 $\pm$ 3.15	2.29 $\pm$ 0.39

Post-hoc analysis using the Bonferroni test demonstrated a statistically significant difference between the ginger and rosemary groups ( $p = 0.0002$ ), while no significant differences were observed between the other comparisons.

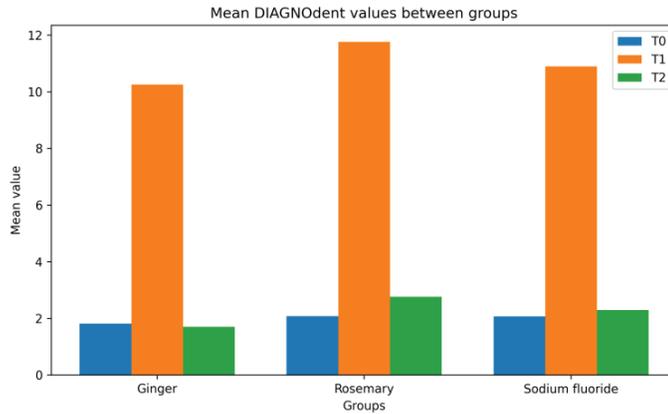


Figure 3. Mean DIAGNOdent values in the experimental groups at the three observation times

The intragroup analysis revealed a significant increase in DIAGNOdent values between T0 and T1 in all groups ( $p < 0.001$ ), confirming the effectiveness of the artificial demineralization protocol. Subsequently, a significant reduction in DIAGNOdent values was observed between T1 and T2 in all groups, indicating the remineralization effect of the applied agents. However, no statistically significant differences were detected between T0 and T2, suggesting a partial recovery of enamel mineralization toward baseline conditions.

Additional analysis evaluated the absolute differences and percentage changes between the experimental stages.

The greatest median increase from T0 to T1 was observed in the rosemary group, followed by the ginger group, while the smallest increase was recorded in the sodium fluoride group. However, these differences were not statistically significant ( $p > 0.05$ ).

Similarly, the analysis of percentage changes showed the highest increase from T0 to T1 in the ginger group, followed by rosemary and sodium fluoride, without statistically significant differences between groups.

To evaluate the influence of the duration of contact with remineralizing agents, additional measurements were performed at 1, 3, and 6 minutes on days 1, 5, and 10 of the treatment protocol. A consistent decreasing trend in DIAGNOdent values was observed with increasing contact time in all groups. The lowest values were generally recorded at 6 minutes, suggesting a stronger remineralization effect with prolonged exposure to the tested agents. In the final evaluation performed on day 10, the lowest mean value was observed at 6 minutes (2.18), compared with 2.25 at 1 minute and 2.26 at 3 minutes. The Friedman test demonstrated a statistically significant difference between the three contact times ( $p < 0.000001$ ).

Table 3. Mean DIAGNOdent values (T2, day 10) according to contact time

Contact time	Mean DIAGNOdent value
1 minute	2.25
3 minutes	2.26
6 minutes	2.18

Overall, the results indicate that all tested agents produced measurable remineralization effects on artificially induced enamel lesions. Among the tested agents, ginger extract demonstrated the most favorable results, followed by sodium fluoride and rosemary extract. Additionally, increasing the duration of contact with the remineralizing agents enhanced the effectiveness of the treatment.

## DISCUSSIONS

The present in vitro study evaluated the remineralization potential of two plant-based agents, ginger (*Zingiber officinale*) and rosemary (*Rosmarinus officinalis*), compared with sodium fluoride on artificially induced enamel white spot lesions using laser fluorescence analysis. The results demonstrated a clear increase in DIAGNOdent values following the demineralization procedure, confirming the successful creation of early enamel lesions. After the remineralization phase, a decrease in fluorescence values was observed in all experimental groups, indicating a partial recovery of enamel mineralization. White spot lesions represent the earliest clinically detectable stage of the caries process and are characterized by subsurface enamel demineralization while the outer enamel layer remains relatively intact [6]. At this stage, the lesion is still reversible if the balance between demineralization and remineralization is shifted toward mineral gain. For this reason, contemporary preventive dentistry increasingly focuses on non-invasive strategies aimed at promoting enamel remineralization and preventing lesion progression [8].

Fluoride remains the most widely used remineralizing agent in clinical dentistry due to its ability to enhance the deposition of calcium and phosphate ions in demineralized enamel and to promote the formation of fluorapatite, a mineral phase that is more resistant to acidic dissolution [18]. However, concerns related to excessive fluoride exposure and the potential risk of dental fluorosis have stimulated interest in alternative or complementary remineralizing agents [17].

In recent years, plant-derived bioactive compounds have attracted considerable attention in dental research due to their antimicrobial, antioxidant, and anti-inflammatory properties [19]. Several plant extracts have demonstrated the ability to inhibit the growth and metabolic activity of cariogenic microorganisms, particularly *Streptococcus mutans*, which plays a key role in the development of dental caries [16]. By reducing bacterial colonization and acid production within dental biofilms, plant-derived compounds may indirectly contribute to the prevention of enamel demineralization and support remineralization processes. Among the tested agents, the ginger extract group showed the lowest DIAGNOdent values after treatment, suggesting a higher remineralization potential compared with rosemary extract and sodium fluoride. Similar findings have been reported in previous studies that investigated the remineralizing effects of herbal extracts on early enamel lesions [2,9]. Ginger contains several biologically active compounds, such as gingerols and shogaols, which have demonstrated antimicrobial and anti-inflammatory properties. These compounds may inhibit the growth of cariogenic bacteria and reduce acid production within dental biofilms, thereby creating more favorable conditions for enamel remineralization [19]. Additionally, some studies suggest that ginger may contain naturally occurring minerals such as calcium and fluoride, which could further contribute to enamel remineralization processes [9]. The antibacterial activity of ginger against *Streptococcus mutans* may also play an important role in reducing the cariogenic potential of dental biofilms [16].

The rosemary extract group also demonstrated a reduction in DIAGNOdent values following treatment, although the remineralization effect appeared to be less pronounced than that observed in the ginger group. Rosemary contains phenolic compounds such as rosmarinic acid and carnosic acid, which have been shown to exhibit antibacterial and antioxidant properties [12]. These compounds may inhibit the growth of cariogenic bacteria and reduce oxidative stress in the oral environment, indirectly supporting the remineralization process. The results obtained in the present study are partially consistent with previous investigations that explored the potential of natural plant extracts in the prevention of dental caries. Several studies have demonstrated that herbal compounds can reduce bacterial adhesion, inhibit biofilm formation, and decrease the metabolic activity of

cariogenic microorganisms [11,12]. However, the magnitude of the remineralization effect may vary depending on factors such as extract concentration, treatment duration, and experimental conditions.

In the present study, although descriptive analysis suggested a more pronounced remineralization effect in the ginger group, some comparisons between experimental groups did not reach statistical significance. This may be attributed to the relatively small sample size and the controlled conditions of the *in vitro* experimental design. While *in vitro* studies provide a controlled environment for evaluating the direct effects of therapeutic agents, they cannot fully replicate the complex biological conditions of the oral cavity, where saliva composition, dietary habits, oral hygiene practices, and microbial diversity play important roles in the caries process. Another important aspect investigated in this study was the influence of contact time between the remineralizing agents and the enamel surface. The results indicated that longer exposure times were associated with lower DIAGNOdent values, suggesting enhanced remineralization. The lowest values were generally observed after six minutes of contact, and statistical analysis confirmed that contact duration significantly influenced the outcome of the remineralization process. These findings highlight the importance of adequate exposure time when applying remineralizing agents, particularly those based on natural extracts.

Despite the promising findings, several limitations should be considered when interpreting the results of this study. First, the relatively small sample size may limit the statistical power of the analysis and reduce the ability to detect subtle differences between treatment groups. Second, the *in vitro* design does not fully replicate the dynamic conditions of the oral environment, where saliva, biofilm interactions, and dietary factors significantly influence remineralization processes. Additionally, the experimental protocol lasted only ten days, which may not fully reflect the long-term effects of remineralizing agents. Future research should aim to validate the findings of the present study through *in vivo* investigations involving larger sample sizes and longer observation periods. Further studies could also explore the remineralization potential of other plant-derived compounds such as green tea, honey, or aloe vera, as well as combinations of natural extracts that may produce synergistic effects. Moreover, incorporating plant-based remineralizing agents into oral hygiene products such as toothpastes, mouth rinses, or topical gels could represent a promising direction for preventive dentistry.

Overall, the findings of the present study suggest that plant-derived agents, particularly ginger extract, may exhibit measurable remineralization effects on early enamel lesions. These results support the potential role of natural compounds as complementary strategies in the prevention and management of early dental caries.

## CONCLUSIONS

Within the limitations of this *in vitro* study, the tested remineralizing agents demonstrated the ability to reduce DIAGNOdent values after the induction of artificial enamel lesions, indicating a measurable remineralization effect. Among the evaluated agents, ginger extract showed the most favorable results, presenting the lowest DIAGNOdent values after treatment compared with rosemary extract and sodium fluoride. These findings suggest that plant-derived compounds may contribute to the remineralization of early enamel lesions. The results also highlighted the importance of the duration of contact between the remineralizing agents and the enamel surface, with longer application times leading to improved remineralization outcomes. This observation may have practical implications for the clinical use of topical remineralizing treatments. Although the differences between some experimental groups were not statistically significant, the overall trend indicates that natural

plant extracts may represent promising complementary agents in preventive dentistry. Further in vivo studies with larger sample sizes and longer observation periods are necessary to confirm these findings and to better understand the clinical applicability of plant-based remineralizing therapies.

### *Conflicts of Interest*

The authors declare no conflict of interest.

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