

IN VITRO COMPARISON OF WHITE SPOT ONSET TIME AFTER ACIDIC BEVERAGE IMMERSION

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ABSTRACT

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Background: Beverages made from apple cider vinegar, pineapple vinegar, kombucha, and lemon juice are consumed for perceived health benefits. Acid content causes enamel demineralization and white spot lesions. This condition increases the risk of tooth decay when consumed continuously with poor oral hygiene habits. The study aimed to compare the onset time of white spot lesions on enamel immersed in apple cider vinegar, pineapple vinegar, kombucha, and lemon juice under in vitro conditions. Method : The study used 40 caries-free permanent premolars divided into four groups: apple cider vinegar (ACV), pineapple vinegar (PV), kombucha (KBC), and lemon juice (LJ), with 10 samples per group. Observations were conducted every minute from the start of immersion until white spots formed on the enamel surface under standardized lighting. Result : Kruskal-Wallis test showed significant differences among groups ($p=0.000$). Mann-Whitney tests showed significant differences between the AC and LS groups ($p=0.000$), the PN and LS groups ($p=0.000$), and the KBC and LS groups ($p=0.000$). Conclusion : The study concludes that there are differences in the effects of apple cider vinegar, pineapple vinegar, kombucha, and lemon juice on the onset of white spot formation in enamel. White spot lesion formation is influenced not only by pH but also by the chemical characteristics and acid composition of each beverage, including the types of organic acids and buffering properties. Lemon juice most rapidly accelerates white spot formation compared to apple cider vinegar, pineapple vinegar, and kombucha.

1. INTRODUCTION

Oral health is an important part of overall physical health. Tooth enamel plays a vital role in protecting teeth from chemical, mechanical, and microbiological damage. Once enamel is formed, this tissue cannot regenerate; therefore, it can undergo progressive damage over time, leading to a permanent reduction in the strength of the tooth structure (Lile et al, 2026). Tooth enamel consists of calcium hydroxyapatite molecules, which are the primary component of the tooth's hard tissue. Calcium hydroxyapatite crystals are reactive to acidic environments and begin to dissolve when the oral cavity pH falls below the critical pH of enamel, which is approximately 5.5. These conditions cause structural changes in the enamel that can progress to damage to the hard tissues of the teeth if they persist over time.(Xuedong, 2016; Garg, 2015).

Demineralization is the loss of minerals from enamel due to the dissolution of hydroxyapatite crystals in the hard tissues of the teeth, triggered by an acidic environment, leading to porosity in the enamel structure (Lopes et al, 2024). Acidic conditions can result from the consumption of foods and beverages with a low pH or from the metabolic byproducts of cariogenic bacteria in the oral cavity.

Exposure to acid dissolves the inorganic components of enamel. The mechanism of demineralization in teeth involves the release of calcium and phosphate ions from hydroxyapatite crystals due to the penetration of hydrogen ions into the enamel structure. This process reduces the integrity and strength of the enamel structure and marks the initial stage in the development of dental caries. In the oral cavity, demineralization and remineralization occur dynamically and in opposition to one another. Remineralization occurs when

mineral ions, primarily calcium and phosphate, are redeposited on the enamel surface, thereby helping to repair the mineral loss that has occurred. Conversely, if demineralization is more dominant than remineralization, mineral loss will continue and may progress to carious lesions. (Abou neel et al, 2016; Mitthra et al., 2020). The balance between demineralization and remineralization is influenced by various factors, including oral pH, saliva's buffer capacity, saliva's mineral content, microbial activity, and dietary and beverage consumption patterns. Carvalho and Lussi explain that repeated exposure to acid can shift this balance toward demineralization.⁷ The lower the pH of a beverage and the longer its contact with the tooth surface, the greater the potential for the release of calcium and phosphate ions from hydroxyapatite crystals, thereby increasing the risk of damage to the hard tissues of the teeth. (Saads and Lussi, 2020).

White spot lesions are the earliest clinical manifestation of the carious process, characterized by mineral loss in the enamel surface layer without visible surface damage. White spot lesions indicate demineralization beneath the intact, highly mineralized enamel layer, which may or may not progress to caries. (Lopes et al., 2024). These lesions result from an imbalance between the processes of demineralization and remineralization occurring over a specific period of time. According to modern cariology, white spot lesions are still in a reversible stage because the superficial enamel layer is generally intact, allowing remineralization to occur if the causative factors are controlled. Therefore, identifying lesions at this stage is of great clinical importance in preventing the progression of caries to cavities. (Xeudong, 2016)/ Clinically, white spot lesions appear as

milky-white, opaque areas that have lost the normal translucency of enamel due to increased porosity in the subsurface enamel layer. These lesions generally have an intact surface and show no signs of cavitation, but appear duller than the surrounding healthy enamel. When dry, the lesion's white color becomes more apparent as air fills the pores formed by mineral loss. Conversely, when the tooth surface is wet with saliva, the lesion may become less visible because water's refractive index is closer to that of healthy enamel. If demineralization continues without remineralization, the lesion can progress to an irreversible cavity. (Garg, 2015)

Dietary habits are one of the main factors determining dental enamel health. Acidic foods and beverages have the greatest impact on the occurrence of chemical erosion of dental enamel. Various studies show that consuming high-acid beverages is associated with an increased risk of demineralization and enamel erosion. The extent of enamel damage is influenced by several factors, including the beverage's pH, the type of acid it contains, its buffering capacity, the duration of exposure, and the frequency of consumption. Certain organic acids, particularly citric acid, are known to have a higher erosive potential due to their ability to chelate calcium ions from the hydroxyapatite structure, thereby accelerating the process of mineral loss in tooth enamel. (Klimek et al., 2020).

In recent years, there has been an increase in the consumption of health drinks as part of the healthy-lifestyle trend across various countries. Health drinks are widely consumed because they are believed to offer various physiological benefits, including antioxidant, antimicrobial, and anti-inflammatory properties, as well as support for digestive health and metabolism. Among

the various types of health drinks popular with the public, apple cider vinegar, pineapple vinegar, lemon juice, and kombucha are widely consumed for their bioactive compounds, which may offer health benefits (Garg, 2015).

According to Hadi et al., apple cider vinegar is a product of apple juice fermentation that involves two stages: alcoholic fermentation by yeast and acetic acid fermentation by acetic acid bacteria. (Hadi et al., 2021). In addition to containing acetic acid as its main component, apple cider vinegar also contains polyphenols, flavonoids, vitamins, and various organic acids that contribute to its biological activity. However, its high acetic acid content gives apple cider vinegar a low pH of 2.5 to 3.0, which can accelerate the demineralization of tooth enamel when consumed repeatedly. Pineapple vinegar is a fermented product derived from pineapple juice (*Ananas comosus*) through alcoholic fermentation by *Saccharomyces cerevisiae*, followed by acetic acid fermentation by acetic acid bacteria. In addition to producing acetic acid as its main component, pineapple vinegar also contains various phenolic compounds and antioxidants derived from the pineapple fruit.

Research findings indicate that pineapple vinegar has a pH of 2.6 to 2.9, placing it among highly acidic beverages that can affect the mineral stability of tooth enamel. (Saragih et al., 2025). Kombucha is a fermented beverage produced by the fermentation of a tea and sugar solution using a Symbiotic Culture of Bacteria and Yeast (SCOBY). During fermentation, the SCOBY produces various organic acids, such as acetic acid, gluconic acid, and glucuronic acid, as well as other bioactive compounds, including polyphenols, vitamins, and flavonoids. Although known for its various health benefits, kombucha is acidic, with a

pH ranging from 2.5 to 3.5, which can lower the oral cavity's pH and increase the risk of tooth enamel demineralization. (Morales, 2020).

Lemon juice is a liquid extracted from the Citrus limon fruit that is rich in vitamin C, flavonoids, essential oils, and various other phytochemicals. Citric acid is the dominant acid that gives lemons their characteristic sour taste. Its high citric acid content results in a pH of approximately 2.0–2.6. In addition to lowering the pH of the oral environment, citric acid can chelate calcium ions, potentially accelerating tooth enamel demineralization. (Klimek et al., 2020).

Various studies have shown that beverages with high acidity can cause demineralization and erosion of tooth enamel. A study by Mitic et al. reported that immersing teeth in orange juice, energy drinks, vinegar, and carbonated beverages caused morphological changes in the enamel and the cemento-enamel junction, indicating mineral loss (Mitic et al., 2020). A study by Anderson et al. also reported that regular vinegar consumption is associated with an increased incidence of erosive tooth wear due to prolonged exposure of tooth surfaces to acetic acid. (Anderson et al., 2021) A study by Kolumban et al. showed that low-pH solutions can cause significant mineral loss in enamel and promote the formation of early demineralization lesions.¹⁴ Lile et al. reported that lemon juice caused the most severe enamel damage compared to orange juice, coffee, tea, and milk, based on scanning electron microscope (SEM) analysis, as evidenced by the loss of interprismatic substance, the opening of enamel prisms, and increased surface porosity of the enamel (Lile et al., 2025).

2. METHOD

The method is a laboratory-based study that compared the effects of several treatments—namely, immersion in acidic beverages—on changes in the surface condition of tooth enamel. The study was conducted in the laboratory of the Faculty of Dentistry at Kadiiri University in May 2026.

The study sample consisted of 40 natural premolar teeth extracted, selected based on the following criteria: free of caries, white spot lesions, and calculus; intact; and free of crown or root fractures. All samples were cleaned of debris, calculus, stains, and residual soft tissue adhering to the tooth surfaces. The samples were divided into four treatment groups, with each group consisting of 10 samples. The CA group was soaked in apple cider vinegar, the CN group in pineapple vinegar, the KBC group in kombucha, and the SL group in lemon juice.

The pH of each acidic beverage was measured with a pH meter, yielding the following results: apple cider vinegar, 2.45; pineapple vinegar, 2.36; kombucha, 2.27; and lemon juice, 1.9. All samples were embedded in red dental wax and arranged in rows according to treatment group. Next, initial documentation was performed prior to treatment to serve as baseline data for comparison with the samples' condition after treatment. Documentation was performed using a digital camera with standardized shooting position, distance, and lighting under wet tooth conditions to ensure consistent and comparable results across samples.

After all samples were documented, each group was immersed in a solution corresponding to its treatment: apple cider vinegar (ACV), pineapple vinegar (PV), kombucha (KBC), or lemon juice (LJ). Observations to determine the onset of white spot lesions were made on the palatal surface

of the premolar crowns. The onset of white spot lesions was defined as the time required from the start of soaking until the first visual appearance of white spot lesions on the tooth enamel surface.

Visual observations were conducted every minute until white spot lesions appeared under wet conditions. Measurements were taken in minutes using a stopwatch and confirmed through digital photographic documentation. Once the white spot lesions were visible through direct visual observation, post-treatment photographic documentation was performed. Pre- and post-treatment photos of the samples were then compared to confirm the formation of white spot lesions on the tooth enamel surface. The time at which white spot lesions first appeared on each sample was recorded on the research observation sheet for subsequent statistical analysis.

The data obtained were analyzed using SPSS software. Descriptive analysis was performed to determine the mean, standard deviation, minimum, and maximum values for each treatment group. A normality test was conducted using the Shapiro–Wilk test. If the data were not normally distributed ($p < 0.05$), comparisons between groups were analyzed using the Kruskal–Wallis test. If significant results were obtained ($p < 0.05$), the analysis continued with the Mann–Whitney U post hoc test to identify pairs of groups that differed significantly. The Bonferroni correction was applied in the post hoc analysis to reduce the risk of Type I errors resulting from multiple comparisons.

3. RESULTS AND DISCUSSION

The results of the study showed that soaking teeth in lemon juice (SL) resulted in the fastest formation of white spot lesions,

with an average time of 3.00 minutes. Soaking teeth in kombucha (KBC) led to the slowest formation of white spot lesions, with an average duration of 10.70 minutes. The results of the normality test using the Shapiro –Wilk test showed that the data across all groups were not normally distributed ($p < 0.05$), specifically Apple Cider Vinegar (ACV) with a p-value of 0.025, Pineapple Vinegar (PV) with a p-value of 0.022, Lemon Juice (LJ) with a p-value of 0.022, and Kombucha (KBC) with a p-value of 0.015. Therefore, the data across all groups are not normally distributed; consequently, the subsequent analysis uses a nonparametric test, namely the Kruskal–Wallis test.

A Kruskal–Wallis statistical test was conducted to determine differences in the time variable among treatment groups. The results of the Kruskal–Wallis analysis are shown in Table 1, which indicates a p-value of <0.001 ($p=0.000$), indicating a statistically significant difference in the time variable among the study groups.

The Mann–Whitney U test was performed as a follow-up analysis to identify pairs of groups with statistically significant differences. In this study, six pairwise comparisons were conducted; therefore, to determine the significance level, a Bonferroni correction was applied, yielding a result of 0.0083, meaning that pairs of groups were considered significantly different if $p < 0.0083$.

The results of the Mann–Whitney U test are shown in Table 2, which indicates comparisons between the SL group and the CA ($p = 0.000$), CN ($p = 0.000$), and KBC ($p = 0.000$) groups—all with p-values < 0.0083 . This means the SL group exhibited a statistically significant difference in time compared to the CA, CN, and KBC groups. Comparisons between the CA and CN groups ($p = 0.510$), CA and KBC groups ($p = 0.188$),

and CN and KBC groups ($p = 0.038$) all yielded p -values greater than 0.0083, indicating no statistically significant differences in duration among these groups.

Table 1. Results of the Kruskal-Wallis Test for the Time of Initial Formation of White Spot Lesions on Tooth Enamel after Treatment in Each Group

Source of Variation	Chi-Square	Degrees of Freedom (df)	p
Time	25,770	3	0,000*

* significant if $p < 0,001$

Table 2. Results of the Mann-Whitney U Test for the Time of Initial Formation of White Spot Lesions on Tooth Enamel after Treatment in Each Group

Group	p
CA – CN	0,510
CA – KBC	0,188
CA – SL	0,000*
CN – KBC	0,038
CN – SL	0,000*
KBC – SL	0,000*

* significant if $p < 0,0083$



(A)



(B)

Figure 1. Photographic documentation of white spot lesions, comparing the surface condition of the tooth samples before (A) and after (B) treatment.

The results of the Mann-Whitney post hoc test showed that the lemon juice group differed significantly from the apple cider vinegar, pineapple vinegar, and kombucha groups. The pH measurements of the acidic beverages in this study showed that lemon juice had the lowest pH (1.9), followed by kombucha (2.27), pineapple vinegar (2.36), and apple cider vinegar (2.45). Beverages with low pH have high erosive potential due to their acidogenic effects. The lemon juice group showed the fastest formation of white spot lesions compared to the other groups because it had the highest acidity level, causing the environment to fall well below the critical pH of enamel (pH 5.5). Acidic conditions cause the hydroxyapatite crystals that make up enamel to dissolve, releasing calcium and phosphate ions from the enamel structure. Continuous mineral loss increases the porosity of the enamel surface, which clinically appears as white spot lesions (Abouneel et al., 2016).

According to Klimek-Szczykutowicz et al., lemon juice contains citric acid as its primary acidic component. The erosive potential of lemon juice is influenced not only by its very low pH but also by the chemical properties of citric acid, which can accelerate the dissolution of tooth enamel minerals. Citric acid, the dominant acid in lemon juice, acts through two simultaneous demineralization mechanisms on tooth enamel. The first mechanism is direct acid attack, in which H^+ ions protonate the carbonate and phosphate groups in the crystal lattice of biological hydroxyapatite, thereby weakening the coordination of calcium ions within the crystal structure. The second mechanism is chelation, in which citrate anions ($R-COO^-$) actively bind to calcium ions and release them from the hydroxyapatite surface, thereby directly accelerating the rate of enamel mineral dissolution. Patten et al. explain that, with citric acid, both mechanisms occur simultaneously at the acid–enamel interface (Nernst layer), making it more erosive than other organic acids in a similar pH range. Undissociated citric acid is also able to diffuse into the enamel structure and dissociate within the enamel's water content, so that demineralization occurs not only on the surface but also in the subsurface layers—a characteristic feature of white spot lesions (Patten et al., 2022; Buzatu et al., 2024).

A study conducted by Buzatu et al. assessed the effects of acidic beverages—namely lemon juice, apple cider vinegar, Sprite, and white wine—on tooth structure using SEM to analyze the enamel surface and Diagenometer to analyze tooth surface density. The results of this study showed that lemon juice and apple cider vinegar had a significantly greater effect on the process of tooth demineralization—as indicated by the presence of white spot lesions—compared to other acidic beverages. These findings are

consistent with a study by Lile et al., which reported that exposure to lemon juice caused more severe changes in enamel morphology compared to several other acidic beverages. Scanning Electron Microscopy (SEM) observations revealed increased surface porosity, partial loss of enamel prism structure, and the formation of deeper, more irregular erosion patterns in the group exposed to lemon juice. The combination of the lowest acidity level and citric acid content—the main component of lemon juice—explains why the SL group had the shortest time to white spot lesion formation (average 3.00 minutes) compared to all CA, CN, and KBC groups. (Buzatu et al., 2026)

The results of the Mann–Whitney post hoc test showed no statistically significant differences among the apple cider vinegar, pineapple vinegar, and kombucha groups in the onset time of white spot lesion formation. These results indicate that the three solutions have relatively similar effects in causing enamel demineralization. Based on the measurement results, the pH values were 2.45 for apple cider vinegar, 2.36 for pineapple vinegar, and 2.27 for kombucha; all solutions had very high acidity levels and were well below the critical pH of enamel, which is approximately 5.5. Under these conditions, the solutions can trigger enamel demineralization.⁵ The pH values of the three solutions are relatively close, resulting in a relatively similar onset time for white spot lesion formation; therefore, the analysis did not reveal any significant differences.

The pH value of kombucha measured in this study was 2.27. Cohen et al. reported that the pH of kombucha generally ranges from 2.5 to 3.5; however, under certain fermentation conditions, the pH can drop to 1.95–2.38 due to increased production of organic acids. (Cohen et al., 2023) Therefore, the pH of 2.27 obtained in this study is still scientifically acceptable as a

characteristic of kombucha with a high level of fermentation. The results show that the kombucha group had a lower pH than apple cider vinegar and pineapple vinegar, but took longer to form white spot lesions. These results indicate that pH is not the sole factor determining the rate of enamel demineralization.

According to Carvalho and Lussi, the potential for erosion and demineralization of a beverage is influenced not only by pH, but also by the type of acid, buffer capacity, mineral content, and the acid's ability to chelate calcium ions. Click or tap here to enter text. Furthermore, Abou Neel et al. explain that the process of enamel demineralization is influenced by the balance of calcium and phosphate ions in the environment surrounding the enamel, so that the chemical composition of a solution can affect the rate of hydroxyapatite dissolution. Kombucha is a fermented beverage containing various organic acids, such as acetic acid, gluconic acid, and glucuronic acid, as well as bioactive compounds derived from tea. The presence of these components is thought to influence the interaction between the solution and the enamel surface, so that the formation of white spot lesions does not occur as rapidly as would be predicted based on pH alone. This finding is consistent with the study by Kolumban et al., which showed that acidic solutions with low pH can result in varying degrees of demineralization due to differences in their chemical characteristics. Therefore, although kombucha has a lower pH than apple cider vinegar and pineapple vinegar, its chemical characteristics and acid composition are thought to cause white spot lesions to form over a longer period of time (Cohen et al., 2023)

4. CONCLUSION

Based on the research results, there were significant differences in the onset time of white spot lesions on tooth enamel following immersion in apple cider vinegar, pineapple vinegar, kombucha, and lemon juice. There were no significant differences among the apple cider vinegar, pineapple vinegar, and kombucha groups, likely due to their relatively similar pH values. Kombucha has a lower pH than apple cider vinegar and pineapple vinegar, yet it took longer for white spot lesions to form because lesion formation is influenced not only by pH but also by each beverage's chemical characteristics and acid composition. Lemon juice is the beverage with the greatest potential to accelerate the formation of white spot lesions compared to apple cider vinegar, pineapple vinegar, and kombucha. To minimize the effects of acidic drinks, it is recommended to consume them using a straw and rinse with water to remove acid residue that sticks on tooth surface.

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