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Influence of bottled salad dressings on the development of enamel erosion in the presence or absence of salivary pellicle – An in vitro study

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ABSTRACT

Background: Acidic beverages are believed to elevate the risk of enamel surface erosion. In addition to the intake of soft drinks, the increased consumption of salad dressings has been linked to a higher prevalence of dental erosion. Therefore, the current study aimed to investigate the influence of bottled salad dressings on the development of enamel erosion in the presence or absence of pellicle through in vitro experiment.

Methods: Preliminary pH and calcium analyses of solutions were performed. Highest pH and calcium content was found for sandwich spread i.e., 4.69 and 55.4 mg/100 g grams, respectively. Eighty tooth specimens (measuring 4 × 4 × 3 mm) were prepared from extracted human premolars and randomly assigned to four groups (group 1: orange juice; group 2: eggless plain mayonnaise; group 3: sandwich spread; and group 4: thousand island dressing) with 20 samples in each group. Ten tooth specimens from each group were immersed in 20 ml of the respective solutions for 5 min (control group). The remaining ten tooth specimens from each group were submerged in 5 mL saliva vials for 3 min to facilitate salivary pellicle formation before being immersed in their respective solutions for 5 min (saliva-covered group). Pre and post-experimental assessments of enamel roughness and hardness were conducted using a surface roughness tester and Knoop Hardness indenter, respectively. **Results:** Overall, enamel roughness was notably elevated in the control group, with the eggless plain mayonnaise (0.52 ± 0.38) and thousand island dressing groups (0.57 ± 0.29) showing a significant increase in surface roughness post-test (p = 0.05). Nevertheless, there was no significant difference in the enamel roughness between the groups. On the other hand, regardless of the presence/absence of the salivary pellicle, a marked decrease in enamel hardness was observed among all groups except for group 3 (sandwich spread) with a mean score of 311.5 ± 82.6 (p < 0.05).

Conclusion: A significant increase in surface roughness and reduction in enamel hardness was observed with salad dressings. However, in vitro formed salivary pellicle showed a protective effect against tooth erosion.

1. Introduction

Dental erosion is defined as the chemical loss of mineralized tooth substance caused by exposure to acids not derived from oral bacteria.¹ The potential causative factors of dental erosion can be categorized as intrinsic or extrinsic. Intrinsic tooth erosion occurs as a result of Gastroesophageal Reflux Disease (GERD) and voluntary regurgitation of gastric acids, commonly observed in individuals with anorexia or bulimia.² On the other hand, extrinsic tooth erosion may be attributed to environmental factors,³ lifestyle,⁴ dietary choices,⁵ and the use of

certain medications, such as vitamin C supplements, aspirin, and bronchodilators like beclomethasone dipropionate, fluticasone, salmeterol, and terbutaline sulfate.⁶ Of all, diet is regarded to be the primary extrinsic factor contributing to the aetiology of tooth erosion.⁵ This can be ascribed to the excessive intake of soft drinks, alcoholic beverages, salad dressings, citrus fruits, and fruit juices, with prevalent acidity primarily stemming from citric and maleic acids in these commonly consumed acidic beverages.⁷ Notably, numerous studies have investigated and demonstrated the erosive effects of citric, maleic, phosphoric and other acids.^{8–12} Importantly, the increased prevalence of dental

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erosion has been linked not only to the consumption of soft drinks but also to the intake of vinegar present predominantly in salad dressings, pickles, citrus fruits and acidic berries.¹³

Tooth erosion is significantly influenced by the frequency, duration, and type of acidic exposure.¹⁴ Moreover, the erosive potential of an acidic food product is determined by several factors such as acid strength, pH, temperature, and the concentration of phosphate, calcium, and fluoride ions.¹⁵ In addition, pH and buffering capacity of saliva, as well as the formation of the salivary pellicle, is known to alter the erosive potential of an acid. The adsorbed phosphoproteins and mucins found in salivary pellicle serve to reduce the solubility of the enamel surface, acting as a perm-selective membrane.¹⁶ In recent years, various in vitro studies have been conducted to assess the erosive potential of acidic food on tooth enamel.^{8–11}

However, limited focus has been devoted to explore the erosive potential of bottled salad dressings, with previous studies overlooking crucial influence of the salivary pellicle on tooth erosion.^{17,18} Therefore, to understand the role of salivary pellicle, the present study aimed to investigate the influence of bottled salad dressings on the development of enamel erosion in the presence or absence of the salivary pellicle through an in vitro experiment. The current study proposes a null hypothesis, stating that the presence of the salivary pellicle has no impact on enamel erosion caused by bottled salad dressings.

2. Materials and methods

Ethical approval for this study was obtained from the Institutional Ethics Committee of Osmania Medical College, Hyderabad (IEC/OMC/2022/M.No.(8)/Acad-81). In addition, permission to operate the necessary equipment was granted by the National Mineral Development Corporation (NMDC), Hyderabad. It is important to mention that this study adhered to the CRIS (Checklist for Reporting In vitro Studies) guidelines.¹⁹

2.1. Sample size calculation

Based on the previous literature,¹⁷ with an expected proportion of 0.50 and a precision of 5% at a confidence interval of 95% and power of 80%, a minimum sample size of 80 was required. Anatomically and morphologically intact human premolar teeth extracted for orthodontic purpose, with no surface defects (dental fluorosis, enamel hypoplasia, fractures, attrition, abrasion) and dental caries were utilized for the study.

2.2. Test solutions

Furthermore, the study included salad dressings with different consistencies, namely eggless plain mayonnaise, sandwich spread and thousand island dressing. Additionally, commercially available orange juice was selected for analysis (Table 1). To ensure uniformity in the manufacturing process, salad dressings from the same company were considered for the evaluation of pH and calcium content.

2.3. Preliminary pH and calcium analysis

Adopting Association of Official Analytical Chemists (AOAC) guidelines,²⁰ pH and calcium analysis of salad dressings and orange juice was done using pH electrode (Mettler Toledo pH and conductivity meter, AG S No.: B231163144) and Atomic Absorption Spectrophotometry (AZ GFAAS, Model: Zeenit 700P, SNo. 150 Z7P1712), respectively. The obtained results revealed the following pH levels and calcium concentrations for the respective solutions—Eggless plain mayonnaise: pH = 4.69, calcium concentration = 21.6 mg/100 g; sandwich spread: pH = 4.50, calcium concentration = 55.4 mg/100 g; thousand island dressing: pH = 3.64, calcium concentration = 18 mg/100 g; and Orange Juice: pH = 4.25, calcium concentration = 14.9 mg/100 mL.

Table 1

Ingredients of bottled salad dressings and orange juice.

Product	Ingredients according to manufacture
Eggless plain mayonnaise	Water, Refined Soyabean Oil, Synthetic Vinegar [Water, Acetic Acid (INS260)], Sugar, Emulsifiers and Stabilizers (INS1442, INS1450, INS415), Iodised Salt, Spices and Condiments, Acidity Regulator (INS330), Preservatives (INS211, INS202), Nature-Identical Flavouring Substances, Antioxidant (INS319) and Sequestrant (INS385).
Sandwich spread	Refined Soyabean Oil, Water, Synthetic Vinegar [Water, Acetic Acid (INS260)], Sugar, Cheese (4.0%), Milk Solids, Emulsifiers and Stabilizers (INS1442, INS1450, INS415), Red Chillies (2.25%), Iodised Salt, Tomato Paste, Spices and Condiments, Acidity Regulator (INS330), Preservatives (INS211, INS202), Nature-Identical Flavouring Substances and Sequestrant (INS385). Allergen Information: Contains Milk.
Thousand island dressing	Water, Refined Soyabean Oil, Synthetic Vinegar [Water, Acetic Acid (INS260)], Sugar, Cucumber (10.0%), Milk Solids, Tomato Paste (5.0%), Emulsifiers and Stabilizers (INS1442, INS1450, INS415), Iodised Salt, Spices and Condiments, Acidity Regulators (INS260, INS330), Preservatives (INS211, INS202), Antioxidant (INS319) and Sequestrant (INS385). Allergen Information: Contains Milk.
Orange juice	Water, Sugar, Orange Juice Concentrate (6.5%), Orange Cells (2.4%), Acidity Regulator Citric Acid, Flavours (Nature-Identical and Natural), Iodised Salt, Antioxidant (Ascorbic Acid), Lemon Juice Concentrate, Pepper Black Powder and Black Salt. Reconstituted 38% Orange Juice.

2.4. Preparation of tooth specimens

The labial surfaces of the collected teeth were examined with the naked eye for any observable macroscopic surface defects. The premolar teeth were vertically sectioned, thereby separating the labial and lingual surfaces.²¹ Tooth specimens measuring (4 × 4 × 3) mm were then prepared using a water-cooled ultra-thin 0.3 mm silicon carbide (SiC) disc and a micromotor. Any subsequent dentin required to achieve the specified dimensions of the specimen was carefully retained. Subsequently, the specimens underwent a cleaning process using an ultrasonic device (Ultrasonic scalar) for 5 min and were examined for any visible cracks or microscopic irregularities using scanning electron microscopy (SEM). Eighty tooth specimens were then stored at a low temperature (4 °C) until the commencement of the experiment.

2.5. Collection of stimulated saliva

Stimulated saliva was collected from a single healthy female volunteer aged 24 years, with a negative medical history of systemic diseases like diabetes, hypertension, antibiotic therapy within the last 3 months, adverse habits like smoking and alcohol consumption, and currently not pregnant or lactating. Additionally, the volunteer displayed no evidence of untreated dental caries, periodontal disease, or other oral ailments. Paraffin-stimulated whole saliva samples were specifically collected between 9:00 a.m. and 12:00 p.m. The volunteer refrained from using fluoride toothpaste and consuming food or drinks for 2 h prior to saliva collection. The volunteer was then asked to chew on a piece of paraffin for 10 min and was instructed to spit saliva into graduated test tubes without swallowing for every minute. The initial 2-min salivary collection was excluded from the analysis. Subsequently, stimulated saliva (200 mL) was collected over a period of two days and a salivary pool was obtained. Saliva from the collected salivary pool was transferred into 40 vials, each containing 5 mL. The vials were kept in ice throughout the whole saliva collection period to ensure that the properties remained unaltered.²²

2.6. Distribution of tooth specimens

Due to strict adherence to the inclusion criteria, all the samples exhibited identical characteristics, necessitating the use of the lottery method for allocation. Eighty tooth specimens were distributed among four groups (Group 1: orange juice, Group 2: eggless plain mayonnaise, Group 3: sandwich spread, and Group 4: thousand island dressing), with each group comprising twenty samples (Fig. 1). Within each group, the twenty specimens were further categorized into two subgroups: a saliva-covered (with salivary pellicle) and a control group (without salivary pellicle), with ten specimens in each.

2.7. Erosion experiment

2.7.1. Control group (without salivary pellicle)

Ten specimens from each group (Group 1: orange juice, Group 2: eggless plain mayonnaise, Group 3: sandwich spread, and Group 4: thousand island dressing) were directly submerged in 20 mL of their corresponding solutions for 5 min. Subsequently, the specimens were rinsed with distilled water for 10 s to ensure the complete removal of any remnants of the solutions.

2.7.2. Saliva-covered group (with salivary pellicle)

Ten specimens from each group were immersed in 5 mL pooled saliva vials for 3 min to facilitate the formation of a salivary pellicle. The specimens were delicately handled, and a representative sample from each group (a total of 4 specimens) was transported for SEM analysis to confirm the presence of the salivary pellicle (Figs. 2 and 3). Simultaneously, the remaining nine specimens from each group were submerged in 20 mL of their respective solutions for a period of 5 min. Following this, the specimens were rinsed with distilled water for 10 s to guarantee the removal of remnants.

2.8. Measurement of enamel erosion

The surface morphology, including pre- and post-experiment surface roughness (in μm) and enamel hardness (in KHN) of all specimens, were measured using a surface roughness tester (Mitutoyo SJ-410) and a Knoop hardness tester (Mitutoyo HV-100), respectively. The enamel roughness was quantified as “Rq” [Root Mean Square (RMS)], representing the average roughness of a surface. The enamel hardness of 270–350 KHN was set as a standard range.²³

2.9. Statistical analysis

Upon completion of the experiment, the data were statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 25.0 for Windows (IBM, New York). The Shapiro-Wilk test was conducted to assess the normality of the data. As the data exhibited non-normal distribution, non-parametric tests were selected for data analysis. The pre and post-experimental roughness (Rq) and hardness (KHN) within each group were analyzed using the Wilcoxon Signed Rank test.

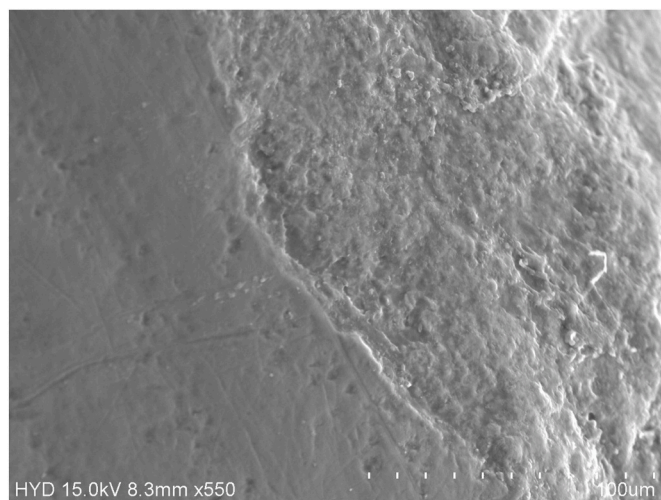


Fig. 2. SEM photograph (700 ×) showing tooth specimen without salivary pellicle (bar equals to 50 micrometers).

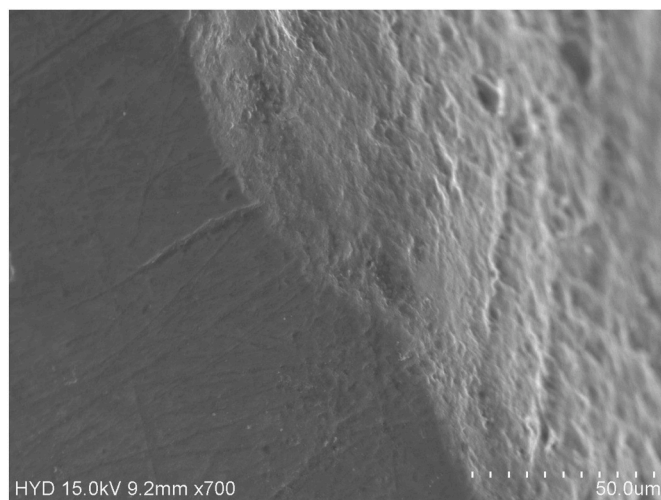


Fig. 3. SEM photograph (700 ×) showing tooth specimen with salivary pellicle (bar equals to 50 μm).

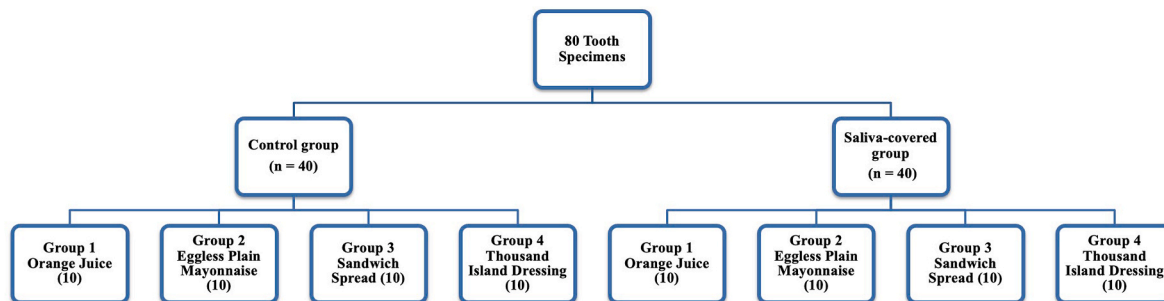


Fig. 1. Distributions of tooth specimens.

3. Results

For both control and saliva-covered groups, no significant differences were observed in the mean enamel roughness and hardness scores between the groups (Group 1: orange juice, Group 2: eggless plain mayonnaise, Group 3: sandwich spread, and Group 4: thousand island dressing) either pre-test or post-test (Tables 2 and 3). However, within the control group, a significant increase in mean enamel roughness scores was noted for eggless plain mayonnaise (0.52 ± 0.38) and thousand island dressing (0.57 ± 0.29), with a p-value of 0.05. On the other hand, the post-test comparison of thousand island dressing revealed that the increase in enamel roughness was both significant and higher in the control group (0.57 ± 0.29 , $p = 0.02$) (Table 4).

Furthermore, in the control group, a significant reduction in mean enamel hardness was observed between the pre-test and post-test for all groups (Group 1: orange juice, Group 2: eggless plain mayonnaise, Group 3: sandwich spread, and Group 4: thousand island dressing), with $p < 0.05$. Meanwhile, within the saliva-covered group, a notable decrease in mean enamel hardness scores was evident across all groups (Group 1: orange juice, Group 2: eggless plain mayonnaise, and Group 4: thousand island dressing), with a p-value less than 0.05, except for sandwich spread (Group 3) ($p = 0.11$), as indicated in Table 5.

4. Discussion

The current study was designed to investigate the influence of bottled salad dressings on the development of enamel erosion, considering the presence or absence of the salivary pellicle in an in vitro experiment. The findings revealed a substantial rise in enamel surface roughness and a reduction in enamel hardness upon exposure to salad dressings. However, the salivary pellicle showed a protective effect, leading to the rejection of the null hypothesis.

Given that premolars are frequently extracted for orthodontic purposes, they were utilized for this study. Unlike previous in vitro studies where bovine teeth were utilized,^{8–11} human teeth were selected for this investigation. This choice was motivated by the fact that bovine enamel crystallites have a larger diameter compared to human enamel (with a ratio of 1.6:1) and distinct variations in protein contents.²⁴ Additionally, the use of human tooth specimens, as opposed to bovine enamel, enhances the relevance and applicability of the study's findings to humans.

This study incorporated eggless plain mayonnaise, sandwich spread, and thousand island dressing to cover a wide variety of acidic salad dressings with varying consistencies commonly available in the market. Mayonnaise, a blend of oil, egg yolk, vinegar, lemon juice, and seasonings, serves multiple culinary purposes. Conversely, sandwich spread, a spreadable condiment used on bread, offers distinct characteristics.

Table 2

Comparison of pre-test and post-test mean enamel roughness scores between groups based on salivary pellicle.

Groups	Mean ± SD Enamel roughness in µm			
	CONTROL		SALIVA-COVERED	
	Pre-test	Post-test	Pre-test	Post-test
Group 1 (Orange juice)	0.33 ± 0.21	0.37 ± 0.24	0.52 ± 0.31	0.50 ± 0.37
Group 2 (Eggless plain Mayonnaise)	0.40 ± 0.21	0.52 ± 0.38	0.43 ± 0.25	0.40 ± 0.26
Group 3 (Sandwich spread)	0.44 ± 0.27	0.47 ± 0.25	0.36 ± 0.21	0.31 ± 0.21
Group 4 (Thousand island dressing)	0.43 ± 0.18	0.57 ± 0.29	0.41 ± 0.30	0.34 ± 0.25
p-value	0.66	0.34	0.63	0.61

Control subgroup – without salivary pellicle.

Saliva-covered subgroup – with salivary pellicle.

* $p \leq 0.05$ - Statistically significant.

Table 3

Comparison of pre-test and post-test mean enamel hardness scores between groups based on salivary pellicle.

Groups	Mean ± SD Enamel roughness in µm			
	CONTROL		SALIVA-COVERED	
	Pre-test	Post-test	Pre-test	Post-test
Group 1 (Orange juice)	386.9 ± 44.1	339.2 ± 49.4	353.5 ± 20.1	292.0 ± 29.0
Group 2 (Eggless plain Mayonnaise)	346.1 ± 45.4	307.5 ± 46.5	319.2 ± 57.4	279.1 ± 54.2
Group 3 (Sandwich spread)	343.9 ± 54.1	296.9 ± 58.2	325.3 ± 56.4	311.5 ± 82.6
Group 4 (Thousand island dressing)	347.5 ± 52.0	274.4 ± 62.9	315.1 ± 47.6	269.8 ± 39.9
p-value	0.15	0.15	0.26	0.29

KHN – Knoop Hardness Number.

Control subgroup – without salivary pellicle.

Saliva-covered subgroup – with salivary pellicle.

* $p \leq 0.05$ - Statistically significant.

Table 4

Comparison of pre-test and post-test mean enamel roughness scores within the group based on salivary pellicle.

Groups	SUBGROUPS	Enamel Roughness in µm		
		Mean ± SD		p-value
		Pre-test	Post-test	
Group 1 (Orange juice)	CONTROL	0.33 ± 0.21	0.37 ± 0.24	0.28
	SALIVA-COVERED	0.52 ± 0.31	0.50 ± 0.37	0.95
p-value		0.14	0.57	–
Group 2 (Eggless plain mayonnaise)	CONTROL	0.40 ± 0.21	0.52 ± 0.38	0.05*
	SALIVA-COVERED	0.43 ± 0.25	0.40 ± 0.26	0.50
p-value		0.63	0.57	–
Group 3 (Sandwich spread)	CONTROL	0.44 ± 0.27	0.47 ± 0.25	0.07
	SALIVA-COVERED	0.36 ± 0.21	0.31 ± 0.21	0.20
p-value		0.57	0.16	–
Group 4 (Thousand island dressing)	CONTROL	0.43 ± 0.18	0.57 ± 0.29	0.05*
	SALIVA-COVERED	0.41 ± 0.30	0.34 ± 0.25	0.87
p-value		0.39	0.02*	–

Control subgroup – without salivary pellicle.

Saliva-covered subgroup – with salivary pellicle.

* $p \leq 0.05$ - Statistically significant.

Thousand island dressing, typically a salad sauce made with a base of oil and vinegar or mayonnaise, serves as another variant. Each salad dressing features unique utility, characteristics, and chemical composition. The viscosity of eggless plain mayonnaise and sandwich spread was found to be higher, exhibiting a more spoonable texture compared to thousand island dressing. This may be attributed to varying levels of emulsifiers and thickening agents in their composition. In this study, commercially available salad dressings were utilized instead of home-made dressings due to the varied proportions of ingredients and the need for standardization across salad dressing samples. In addition, as orange juice is considered a standard control for assessing erosive effects on enamel,²⁵ it was employed as a reference in the present study for comparative purposes.

Despite the availability of other quantitative methods,²⁶ this study utilized surface profilometry and the Knoop hardness indenter as they effectively quantify the loss of dental hard tissue, providing relevant information on both enamel surface hardness and roughness. Surface

Table 5

Comparison of pre-test and post-test mean enamel hardness scores within the group based on salivary pellicle.

Groups	Subgroups	Enamel hardness in KHN		p-value
		Mean \pm SD		
		Pre-test	Post-test	
Group 1 (Orange juice)	CONTROL	386.9 \pm 44.1	339.2 \pm 49.4	0.02*
	SALIVA-COVERED	353.5 \pm 20.1	292.0 \pm 29.0	0.005*
p-value		0.04*	0.10	–
Group 2 (Eggless plain mayonnaise)	CONTROL	346.1 \pm 45.4	307.5 \pm 46.5	0.05*
	SALIVA-COVERED	319.2 \pm 57.4	279.1 \pm 54.2	0.005*
p-value		0.48	0.35	–
Group 3 (Sandwich spread)	CONTROL	343.9 \pm 54.1	296.9 \pm 58.2	0.01*
	SALIVA-COVERED	325.3 \pm 56.4	311.5 \pm 82.6	0.11
p-value		0.31	0.48	–
Group 4 (Thousand island dressing)	CONTROL	347.5 \pm 52.0	274.4 \pm 62.9	0.005*
	SALIVA-COVERED	315.1 \pm 47.6	269.8 \pm 39.9	0.009*
p-value		0.14	0.52	–

KHN – Knoop Hardness Number.

Control subgroup – without salivary pellicle.

Saliva-covered subgroup – with salivary pellicle.

* $p \leq 0.05$ - Statistically significant.

roughness can be measured using three parameters: **R_a**, indicating the arithmetic average roughness of a surface; **R_q**, denoting the RMS average roughness of a surface; and **R_z**, representing the ten-point mean surface roughness, calculated as the difference between the five tallest peaks and five deepest valleys within the surface. In this study, the “**R_q**” value was considered as the parameter to measure enamel roughness, as it is sensitive to minute changes in the surface roughness.

Meanwhile, stimulated saliva obtained from a single healthy volunteer was utilized, representing a form of physiological saliva stimulation. Hannig et al.^{16,27} concluded that the protective effect of in situ formed salivary pellicles within 3 min did not exhibit significant differences compared to salivary pellicles formed in 2 h, 6 h, 12 h, and 24 h. The rapid adsorption of salivary proteins and the subsequent formation of a basal pellicle layer, typically 10–20 nm in thickness, function as a resistant barrier to acids, thereby contributing to the anti-erosive potential. Therefore, tooth specimens were immersed in stimulated saliva for a duration of 3 min to facilitate pellicle formation. In the current study, the erosive experiment on tooth specimens was performed for 5 min per sample, simulating the intra-oral contact time of a salad dressing with natural teeth.

Moreover, sandwich spread showed a comparatively lower erosive effect on enamel hardness compared to other salad dressings and orange juice ($p = 0.01$). This observation aligned with the results of the preliminary analysis, suggesting a high pH (4.69) and increased calcium concentration (55.4 mg/100 g) in Group 3. Likewise, Hartz et al.¹⁷ found that calcium-rich dressings resulted in lower enamel wear than orange juice, with a median of 2.4 μ m. A similar study by Zoller et al.¹⁸ concluded that increasing the calcium concentration (by adding at least 20 % plain yoghurt) in commercially available salad dressings led to a significant reduction in tooth erosion ($p < 0.05$). Thus, a high pH level and increased calcium content were identified as factors that could potentially counteract the initial demineralization caused by acids. It is worth noting that other factors, such as viscosity, type of acid, and the presence or absence of vinegar as an ingredient in salad dressing, can also impact the erosive property of these dressings.

The consumption of salad dressings among young adults posits a great deal of attention as immature permanent teeth contain a porous

enamel surface and lack complete mineralization, rendering them more susceptible to acid dissolution until conditioned by salivary ions. Conversely, older individuals with impaired salivary function should be mindful of the increased risk of salad dressing induced dental erosion. In addition, public policies should aim to include preventive advice for their safe consumption. Nevertheless, future in vivo studies are warranted to yield accurate clinical evidence.

Besides, the study is subject to certain limitations, with the primary constraint being its in vitro design and the relatively small sample. Additionally, other factors like mineral analysis of tooth specimens and biological aspects of saliva, including pH, temperature, clearance, buffering capacity, etc., were not taken into account due to logistical constraints.

Despite its limitations, this study holds significance as experiments were carried out under standardized conditions. The preliminary analysis of pH and calcium content adds to the strength of the study. Moreover, the investigation of the anti-erosive impact of the salivary pellicles on tooth specimens with enamel exposed to various salad dressings contribute valuable evidence to the limited scientific understanding of this subject. While it is acknowledged that this in vitro study may not perfectly replicate the actual intra-oral environment, it successfully demonstrates the protective effect of acquired salivary pellicles against tooth erosion induced by salad dressings.

5. Conclusions

This study revealed a notable increase in enamel surface roughness and a reduction in enamel hardness with the use of salad dressings when compared to orange juice. However, the presence of salivary pellicle showcased a protective effect. Therefore, it can be inferred that salad dressings have the potential to be erosive, and their consumption may contribute to dental erosion.

Additionally, this study can aid public health professionals in providing dietary advice and preventive care for individuals at risk of developing dental erosion. It also opens up the possibility of considering modifications to the ingredients in salad dressing to mitigate their erosive impact on dental health.

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Authors' contributions

Dr. Billa Aishwarya Lakshmi, Dr. Jagadeeswara Rao Sukhabogi and Dr. Dolar Doshi contributed to conception, design, data acquisition, analysis and interpretation, drafted the manuscript. Dr. Billa Aishwarya Lakshmi and Dr. Dolar Doshi critically revised and gave final approval for the manuscript. Dr. Billa Aishwarya Lakshmi, Mr. Prashant Sharma and Mr. TVS Subrahmanyam contributed to design, data analysis and interpretation and gave final approval for the manuscript. Dr. Billa Aishwarya Lakshmi and Dr. Jumjala Sasikala contributed to data interpretation and drafted manuscript. “All authors have read the manuscript and gave their final approval and agree to be accountable for all aspects of the work. Each author believes that the manuscript represents honest work”.

Declaration of competing interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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