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RESEARCH ARTICLE

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Effect of Almond Powder and Banana Milk on the Microhardness of Erosive Lesions of Primary Enamel Caused by Jelly Gum and Jelly Candy: An *In Vitro* Study

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Abstract:

Introduction: Demineralization and remineralization are naturally occurring processes. Since the type of food and its ingredients have a significant effect on the above-mentioned processes, this study aimed to investigate the effect of almond powder and banana milk on primary enamel erosive lesions caused by jelly gum and jelly candy.

Method: This *in vitro* study was conducted on 80 primary molar teeth. After the initial microhardness measurement, half of the teeth were immersed in an edible jelly solution, while the other half were immersed in a jelly gum solution for 5 minutes twice a day for 5 days. At the end of the fifth day, the microhardness of the samples was measured again. The teeth in each group were then randomly divided into four subgroups (n = 10) for immersion in 80% almond suspension, banana milk, artificial saliva, and fluoride gel for 15 minutes. Next, the microhardness of the teeth was measured for the third time, and the results were analyzed using ANOVA, ANCOVA, and post-hoc tests ($\alpha = 0.05$).

Results: The microhardness of the specimens in the jelly group was significantly higher than that of the specimens in the jelly gum group. The microhardness of the specimens in the saliva group was significantly the lowest ($p < 0.05$); while the other remineralizing agents showed no significant difference with each other ($p > 0.05$).

Discussion: This study demonstrated that both jelly and jelly gum significantly reduced enamel microhardness, with jelly gum showing a stronger demineralizing effect. Almond powder and banana milk effectively restored enamel hardness, exhibiting remineralization comparable to fluoride. These findings suggest that natural, calcium-rich foods such as almonds and banana milk, may serve as viable alternatives for managing early erosive lesions in children.

Conclusion: According to the results, jelly candy and jelly gum increase dental erosion, and the demineralizing effect of jelly gum is greater than that of jelly candy. Almond and banana milk cause enamel remineralization, similar to fluoride.

Keywords: Erosion, Demineralization, Remineralization, Jelly candy, Jelly gum, Almond, Banana milk.

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1. INTRODUCTION

Dental erosion is a significant cause of tooth destruction in children and adolescents. In recent decades, following the reduction in the rate of dental caries in children and adolescents, especially in developed countries, there has been a significant increase in other dental conditions, such as dental erosion [1].

Dental erosion is a chemical process that is common in children. During this process, the hard tooth structure undergoes acid dissolution without the involvement of microorganisms [1-3]. Early erosive damage to the tooth structure can lead to severe loss of tooth structure, resulting in subsequent tooth hypersensitivity and aesthetic problems; therefore, its early diagnosis and prevention of progression are of great significance [2]. Intrinsic factors, extrinsic factors, or both can cause dental erosion in both children and adults. Intrinsic factors include vomiting and gastroesophageal reflux, while, extrinsic factors include industrial sources, medications, and diet [4, 5]. Due to the high rate of consumption of acidic foods and drinks, diet is considered a major cause of dental erosion [4]. Since the type of food and its ingredients have a significant impact on the process of dental erosion, it is essential to identify foods with a less erosive nature and those with remineralizing properties.

The main ingredients of jelly candy and jelly gum include gelling agents (such as gelatin or pectin), water, sugar, and edible acids (such as citric acid and maleic acid) [6]. The presence of an acidic environment is necessary for the gelation process of jelly and jelly gum. The acid content of foods can lead to dental erosion.

Due to the complications of dental erosion, it is necessary to restore the damaged tooth structure. There are several methods for enamel remineralization [3]. Using calcium and phosphorus ions is one suggested method for this purpose. Calcium and phosphorus ions are the main constituents of the tooth enamel, and sufficient amounts of these ions can lead to remineralization [7]. Several studies have investigated the efficacy of food products containing calcium and phosphorus for remineralizing tooth enamel, and have demonstrated their optimal effectiveness for this purpose [8, 9].

Milk is a dairy product containing calcium and phosphate. Consuming milk offers numerous health benefits for both children and adults, and can help increase their calcium intake. Consumption of flavored milk has become popular as a way to increase milk consumption and calcium intake among children. Although sweeteners are added to flavored milk (*e.g.*, banana milk), it is considered a rich source of proteins, calcium, phosphorus, and vitamins [10]. Milk, as a rich source of calcium and phosphate, can exert remineralizing effects. Also, it creates a protective film in combination with saliva and enhances the deposition of minerals and organic substances on the enamel surface [11].

Nuts such as almonds contain high amounts of micronutrients, including minerals (calcium, magnesium and potassium). Almonds have the highest amount of calcium among nuts. Consuming almonds protects against demineralization, prevents hypertension and insulin resistance, and lowers the risk of cardiovascular diseases by providing high amounts of micronutrients and low amounts of sodium [12].

Dental treatment of young children is challenging and cost-prohibitive; on the other hand, many chemicals used to prevent caries are not prescribed for young children or should be used with utmost caution, necessitating the use of affordable and readily available substances with minimal complications [13].

An *in vitro* study evaluated the erosive nature of fruit drinks enriched with calcium ions on primary teeth. The microhardness values of the samples in the non-enriched drink group decreased during the experiment, suggesting erosion. Calcium enrichment of fruit drinks appears to decrease their erosive potential on primary teeth [14].

Another *in vitro* study evaluated the effects of acidic drinks (soda, soy-based apple juice, and strawberry juice) on the microhardness of primary enamel. They demonstrated that soy drink resulted in a significant reduction in surface microhardness. This reduction was greater for the strawberry juice compared to the soy drink, and soda significantly decreased the surface micro-hardness. All the tested beverages adversely affected the microhardness of primary enamel, but soda was the most harmful for the enamel [15].

Considering the complications of erosive lesions, the need for treatment of erosive lesions caused by the consumption of jelly gum, and the fact that the role of calcium and phosphorus contents of almonds and banana milk has not been previously studied in treatment of such lesions, this study aimed to investigate the effect of almond powder and banana milk on erosive lesions resulting from exposure to jelly gum and jelly candy.

2. MATERIALS AND METHODS

This *in vitro* study was ethically approved by the ethics committee of Shahed University (IR.SHAHED.REC. 1400.131). The samples included 80 extracted sound primary maxillary and mandibular molars. Primary molar teeth with sound buccal or lingual surfaces with no caries, hypoplasia, restoration, crack, or fracture were included in the study. Absence of caries was confirmed by using the World Health Organization criteria. Also, the teeth had no abrasion, crack, or hypocalcification in clinical examination. The teeth were collected from dental clinics in Tehran city and had been extracted due to physiological root exfoliation. During the collection period, the teeth were cleaned and stored in a glass container containing saline at room temperature. The sample size was calculated according to the following formula:

$$n = (Z\alpha/2+Z\beta)2 * (p1(1-p1)+p2(1-p2)) / (p1-p2)2$$

The parameters used in the above formula were as follows: α (type I error) = 0.05, β (type II error) = 0.20 (power = 80%), expected difference in microhardness (Δ) = 20 units, and standard deviation (σ) = 15 units. Based on these values, the calculated sample size was 10 samples per subgroup, which resulted in a total of 80 primary molar teeth (8 subgroups × 10 samples).

The surface of the teeth was mechanically cleaned from plaque and debris by using a prophylaxis paste containing fluoride-free pumice, a low-speed handpiece with a rotation range of 500 to 1500 rpm, and a prophy brush.

The teeth were mounted in acrylic resin; next, a square-shaped sticker measuring 5 x 5 mm was adhered to the middle third of the buccal surface of the teeth, and all the remaining surfaces were covered with transparent auto-polymerizing acrylic resin such that after polymerization of the acrylic resin, the exposed surface of all teeth was the same [16].

The exposed surface of the samples was polished with abrasive paper under water coolant. Next, the initial microhardness of the smooth surface was measured using a Vickers hardness tester (Zwick Roell, Ulm, Germany) with a 50 g load applied for 10 seconds [2, 17].

After measuring the initial surface microhardness, the teeth were randomly assigned to two groups (n=40) for immersion in jelly (group 1) and jelly gum (group 2). In group 1, the teeth were immersed in a jelly solution for 5 minutes twice a day for 5 days. It was prepared by dissolving 5 g of jelly (Farmand, Karaj, Iran) in 2 mL of artificial saliva. In Group 2, the teeth were immersed in a jelly gum solution for 5 minutes, twice a day, for 5 days. It was prepared by dissolving 5 g of jelly gum (Farmand, Karaj, Iran) in 2 mL of artificial saliva. The secondary microhardness of the samples was then measured. The samples were subsequently washed with distilled water for 20 seconds. Additionally, they were stored in an artificial saliva solution composed of NaCl, $CaCl_2$, NaH_2PO_4, and NaN_3 during the study period. The artificial saliva was replaced daily. At the end of 5 days, the microhardness of the samples was measured again [1]. Next, each group was randomly divided into four groups (n = 10), as shown in Table **1**.

Table 1. Study groups.

Groups	Number
Jelly as a demineralizer and almond powder as the remineralizing agent	10
Jelly as a demineralizer and banana milk as the remineralizing agent	10
Jelly gum as a demineralizer and almond powder as the remineralizing agent	10
Jelly gum as a demineralizer and banana milk as the remineralizing agent	10
Jelly as a demineralizer and artificial saliva (negative control)	10
Jelly as a demineralizer and fluoride solution (positive control)	10
Jelly gum as a demineralizer and artificial saliva (negative control)	10
Jelly gum as a demineralizer and fluoride solution (positive control)	10

The teeth in each subgroup were exposed to either 80% almond suspension (5 g of almond kernel powder dissolved in 6 mL of artificial saliva), banana milk (Mihan, Eslamshahr, Iran), artificial saliva (pH=6.5-7,) or fluoride gel (1.23% sodium fluoride; Cina, Karaj, Tehran) for 15 minutes. Subsequently, the microhardness of the teeth was measured again for the third time.

The results were expressed as mean and standard deviation. The changes in microhardness of the teeth were statistically analyzed. ANCOVA was used to adjust for the effect of initial microhardness. One-way ANOVA was applied to compare the groups, followed by the Stat Plus Advanced post-hoc tests for pairwise comparisons. For pairwise group comparisons following one-way ANOVA, we applied Tukey’s HSD post-hoc test (StatPlus Advanced software) at a significance level of 0.05. All statistical analyses were performed using SPSS version 24 (SPSS Inc., IL, USA) at a 0.05 level of significance.

3. RESULTS

In this study, 80 samples were examined in 8 subgroups. First, the initial microhardness of the samples was measured before their immersion in the demineralizing solutions. The results indicated a significant difference in microhardness between the groups at baseline. In other words, the groups were not homogeneous in terms of initial microhardness. Thus, to compare the effects of jelly and jelly gum (as demineralizing agents) on microhardness, the difference between the groups at baseline was adjusted by ANCOVA.

To use ANCOVA, the homogeneity of the variance was first assessed by Levene's test, which showed that the assumption of the homogeneity of the variances was met (F=0.634, $p > 0.05$).

ANCOVA showed a significant difference in micro-hardness between the two groups of jelly and jelly gum after adjusting for their initial microhardness values, as shown in Fig. (**1**), the microhardness of the jelly group was significantly higher than that of the jelly gum group (p=0.001) (Fig. **1**).

The results indicated a significant difference in microhardness of the four subgroups irrespective of the group (jelly gum/jelly) ($p < 0.05$, Table **2**). Therefore, to compare the effects of different remineralizing agents, the difference among the groups after demineralization had to be adjusted. Thus, to compare the effects of remineralizing agents on microhardness, the difference among the subgroups after demineralization was adjusted by ANCOVA, and then the groups were compared (Table **2**). The Levene's test showed that the assumption of the homogeneity of the variances was met (F=2.34, $p > 0.05$).

ANCOVA showed a significant difference in microhardness among the four subgroups irrespective of their group (jelly gum/jelly) after adjusting for their microhardness after demineralization (p=0.000). Pairwise comparisons of the subgroups (Table **3**) revealed that the microhardness of the saliva subgroup was the lowest, and the microhardness of the saliva subgroup was significantly lower than the microhardness of other groups ($p < 0.05$). However, no significant difference was found in the microhardness of other groups in pairwise comparisons ($p > 0.05$). Figures **2** and **3** show the effect of remineralizing agents on the microhardness of teeth demineralized by jelly gum and jelly, respectively; As shown in both figures, almond powder and banana milk significantly improved enamel microhardness compared to saliva in teeth demineralized by jelly gum and jelly. The values were comparable to fluoride, with no statistically significant differences between these three agents ($p > 0.05$). Saliva consistently showed the lowest microhardness recovery, confirming its limited remineralizing potential under these conditions (Figs. 2 and 3).

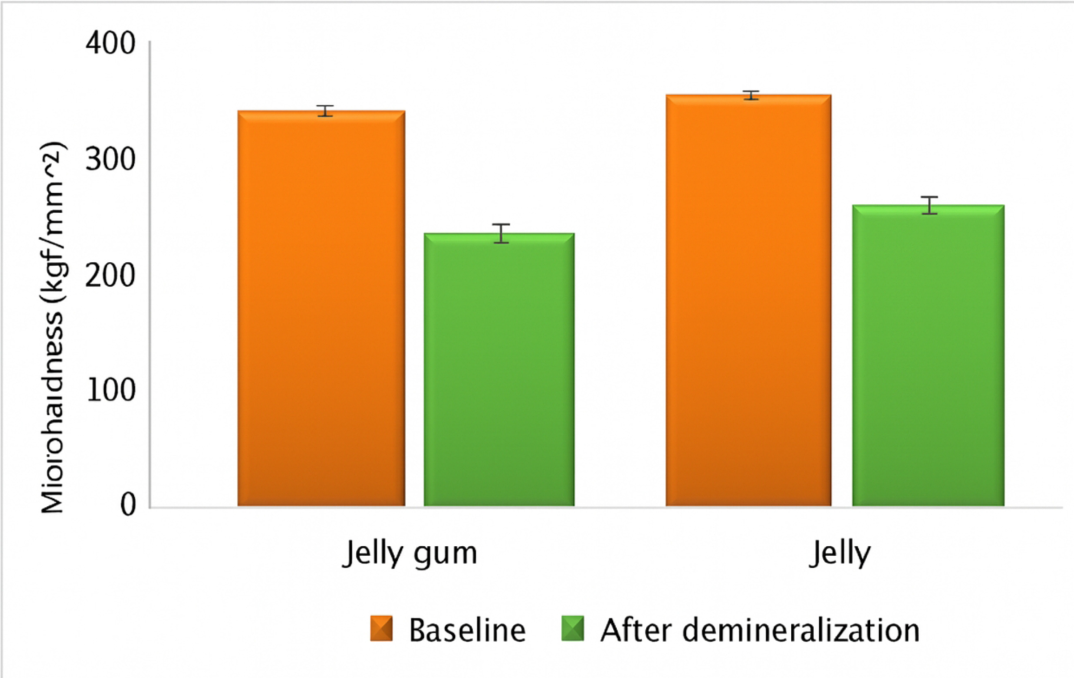

Fig. (1). Comparison of the demineralizing effects of jelly gum and jelly candy on primary teeth.
Y-axis: microhardness (kg/mm^2). X-axis: treatment group (jelly vs. jelly gum). Error bars represent standard deviation (SD).

Table 2. Comparison of microhardness (kg/mm^2) of the four subgroups after demineralization irrespective of the group (jelly gum/jelly candy) (n=10).

Subgroup	Mean	Standard Deviation	Minimum	Maximum	*p*-value
Almond	250.80	36.91	183.67	315.67	0.022
Banana milk	248.48	31.55	198.33	320.33	
Saliva	221.32	37.66	173.33	291.33	
Fluoride	245.93	25.07	194.00	304.67	

Table 3. Pairwise comparisons of the subgroups in terms of microhardness.

Group (I)	Group (J)	Mean Difference (I-J)	Standard Error	*p*-value*	95% CI for the Difference	
					Lower Limit	Higher Limit
Almond	Saliva	62.385*	6.651	0.000	44.360	80.409
	Banana milk	13.044	6.332	0.257	-4.117	30.206
	Fluoride	6.017	6.339	1.000	-11.163	23.197
Saliva	Banana milk	-49.340*	6.604	.000	-67.236	-31.444
	Fluoride	-56.368*	6.556	.000	-74.134	-38.602
Banana milk	Fluoride	-7.028	6.333	1.000	-24.190	10.135

Note: CI: Confidence interval; * Stat Plus Advanced.

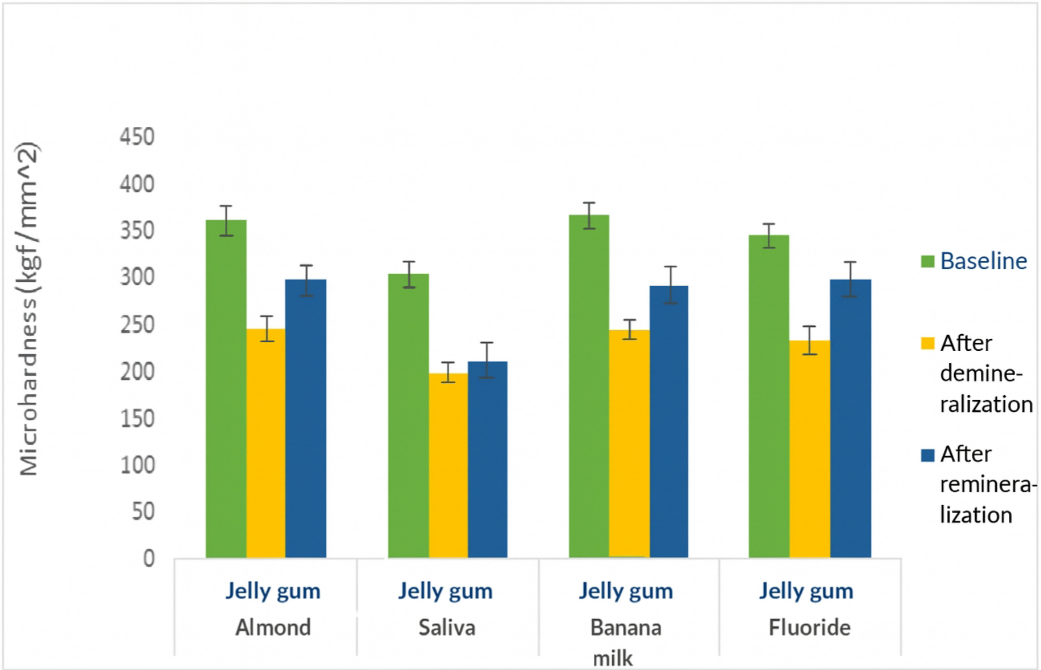

Fig. (2). Effect of remineralizing agents on the microhardness of teeth demineralized by jelly gum.
X-axis: treatment groups (almond suspension, banana milk, fluoride gel, artificial saliva). Y-axis: Vickers microhardness (kg/mm^2). Error bars represent standard deviation (SD).

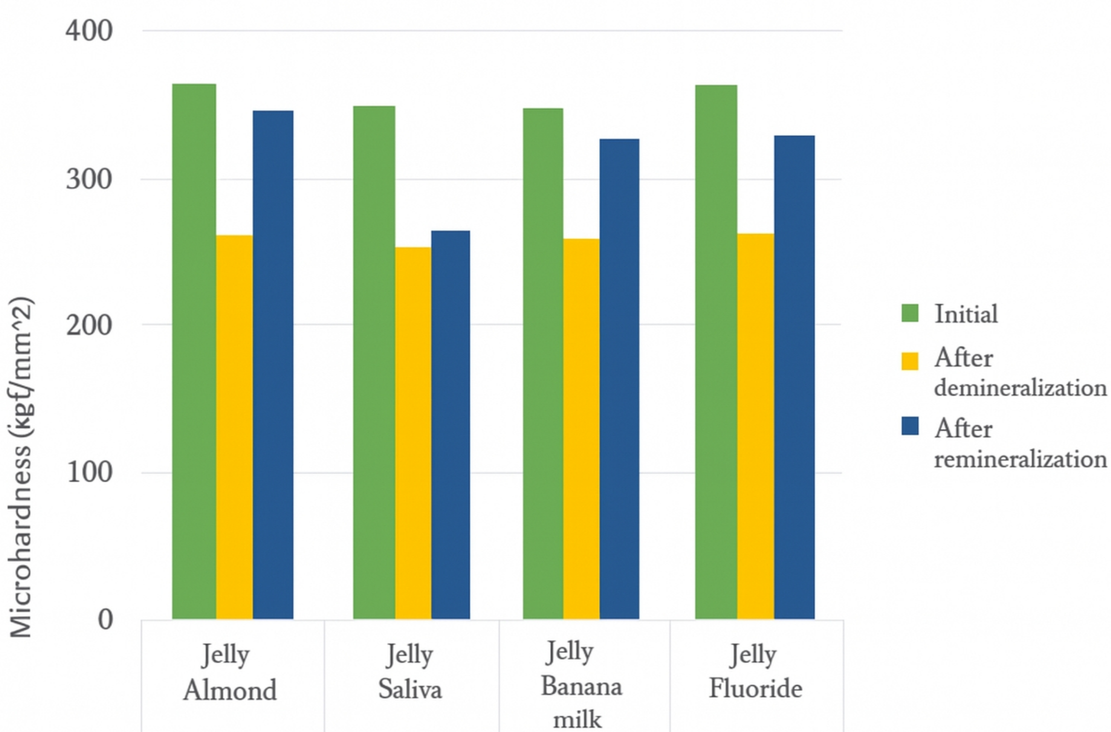

Fig. (3). Effect of remineralizing agents on the microhardness of teeth demineralized by jelly.
X-axis: treatment groups (almond suspension, banana milk, fluoride gel, artificial saliva). Y-axis: Vickers microhardness (kg/mm^2). Error bars represent standard deviation (SD).

4. DISCUSSION

The present results showed that the demineralizing effect of jelly gum was greater than that of jelly because the microhardness of the specimens in the jelly group was significantly higher than that of the specimens in the jelly gum group. The greater demineralizing effect of jelly gum compared to jelly could be attributed to the higher concentration of jelly gum and its adhesion to the tooth surface.

Jelly and jelly gum both contain citric acid, phosphoric acid, ascorbic acid, malic acid, tartaric acid, oxalic acid, and carbonic acid, which cause dental erosion and damage the tooth structure.

Mohammadi *et al*. [2] demonstrated that chocolate and candy decreased the microhardness of enamel in primary and permanent teeth, with candy having a greater adverse effect due to its lower pH compared to chocolate [2]. Gonçalves *et al*. [18] used scanning electron microscopy and observed that all acidic drinks caused gradual demineralization and chemical dissolution of both organic components and mineral matrix of the tooth structure.

The enamel demineralization process depends on the pH and the contents of calcium, phosphate, and fluoride. These factors also determine the degree of mineral saturation of the tooth structure [3]. Therefore, a sub-saturation state leads to the dissolution of hydroxyapatite and the release of calcium and phosphate ions from the enamel surface, while a supersaturated state of these ions results in the re-deposition of hydroxyapatite or remineralization, creating an intact enamel surface. Thus, an increase in surface microhardness value reveals an increase in remineralization; whereas, a decrease in microhardness indicates a reduction in remineralization (and increased demineralization).

The present results indicated that the remineralizing properties of almond and banana milk were almost the same, and comparable to those of fluoride. Dedhia *et al*. [14] demonstrated the preventive effect of calcium enrichment on dental erosion, showing that the enrichment of fruit drinks with calcium reduced their erosive potential on primary teeth. Lachawski *et al*. [11] claimed that milk or a combination of chocolate or coffee with milk would have a protective effect on the enamel surface against erosive substances.

Due to the better access of the enamel's superficial layer to environmental ions, the chances of remineralization would be higher, and the risk of demineralization would be lower in the presence of higher amounts of calcium, phosphate, and magnesium ions on the enamel surface, and their optimal saturation state. Consequently, large amounts of acid-resistant hydroxyapatite crystals form on the surface, resulting in an increase in enamel microhardness [19]. The formation of a strong superficial layer on the enamel surface has a protective effect against the continuation of demineralization of the underlying minerals, and limits the release of calcium and phosphate ions from the tooth structure. It also prevents the penetration of hydrogen and acids into the subsurface areas and inhibits the progression of erosive lesions and cavitation as such.

The findings of the present study indicated that almond and banana milk were capable of successfully remineralizing the primary enamel exposed to jelly candy and jelly gum. A previous study on foods containing calcium and phosphate indicated their optimal remineralizing effect. Rezvani *et al*. [20] demonstrated that the sesame extract gel, a rich source of calcium and phosphate, increased enamel microhardness even more than commercially available fluoride products. They reported a significant increase in enamel microhardness of human permanent premolars following the consumption of foods containing calcium and phosphate. Ahmadvand *et al*. [21] showed the optimal efficacy of eggshell, as a source of calcium, magnesium, phosphorus, strontium, and fluoride, for remineralization of caries-like enamel lesions.

The balance between the remineralization and demineralization cycles depends on the concentration of calcium and phosphate ions, and is somewhat affected by the salivary level of alkaline phosphatase [5]. It is essential to maintain a saturated state of calcium and phosphate in the saliva to create a balance between the remineralization and demineralization cycles. In the present study, both almond powder and banana milk caused remineralization of the tooth structure, similar to a fluoride solution, due to the presence of calcium and phosphorus in their composition.

Although this was an *in vitro* study, our findings are supported by clinical studies reporting similar protective effects of calcium-rich dietary agents on enamel.Dedhia *et al*. [14] demonstrated that calcium-fortified fruit beverages significantly reduced the erosive potential on primary teeth. Likewise, Lachowski *et al*. [11] showed that milk and milk-based drinks could protect the enamel surface against erosive substances in vivo. These reports provide clinical correlation to our results, confirming that the remineralizing effect of almond powder and banana milk can be attributed to their high calcium and phosphate content.

The present results showed that jelly candy and jelly gum increased tooth erosion and demineralization due to their high sugar and acidic content, while almond and banana milk accelerated the enamel remineralization process due to the presence of phosphorus and calcium in their composition; therefore, they may be included as remineralizing substances in children's diets.

5. STUDY LIMITATIONS

This study had certain limitations. This was an *in vitro* study and may not fully represent intraoral conditions such as saliva flow and biofilm. Variations in baseline hardness may also have influenced results. Future *in vivo* studies are needed to validate these findings.

CONCLUSION

Both jelly candy and jelly gum caused enamel erosion, with jelly gum showing greater demineralizing potential. Almond powder and banana milk demonstrated remineralizing effects similar to fluoride, suggesting their potential as natural dietary adjuncts to protect children’s teeth.

AUTHORS' CONTRIBUTIONS

The authors confirm their contribution to the paper as follows: R.H., M.M., M.B.R.: Study conception and design and analysis and interpretation of results; F.K., M.G., M.H.N.: Data collection; M.G., M.H.N.: Draft manuscript. All authors reviewed the results and approved the final version of the manuscript.

LIST OF ABBREVIATIONS

ANCOVA	=	Analysis of Covariance
ANOVA	=	Analysis of Variance
HSD	=	Honestly Significant Difference

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Ethics Committee of Shahed University, Iran (IR.SHAHED.REC.1400.131).

HUMAN AND ANIMAL RIGHTS

All procedures performed in studies involving human participants were in accordance with the ethical standards of institutional and/or research committee and with the 1975 Declaration of Helsinki, as revised in 2013.

CONSENT FOR PUBLICATION

Written informed consent was obtained from parents/guardians for the use of extracted teeth.

AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of the article will be available from the corresponding author [R.H] upon reasonable request.

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None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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