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The Effect of Animal and Plant-Based Protein On Enamel Micro-hardness: An in vitro Study

Hayvansal ve Bitkisel Protein Kaynaklı İçeceklerinin Mine Mikro Sertliği Üzerine Etkisi: in vitro Çalışma

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ÖZ

Amaç: Protein açısından zengin içecekler dişleri diş erozyonuna karşı koruma potansiyeline sahiptir. Ancak, protein açısından zengin içeceklerin dişler üzerindeki etkileri ile ilgili araştırmalar sınırlıdır. Bu nedenle, bu çalışma günümüzde sıklıkla tüketilen bitkisel ve hayvansal kaynaklı protein açısından zengin içeceklerin in vitro koşullarda mine mikro sertliği ve yüzey pürüzlülüğü üzerindeki etkisini araştırmayı amaçlamıştır.

Gereç ve Yöntemler: Çekilen daimi arka dişler (15 premolar ve 15 molar) bukkal yüzeyleri açıkta kalacak şekilde akrilik bloklara gömüldü. Dişler her grupta 6 diş olacak şekilde; G1-kakao aromalı protein açısından zengin süt; G2-çikolata aromalı peynir altı suyu proteini tozu; G3-bezelye proteini tozu; G4-pirinç proteini tozu; ve G5-yapay tükürük (kontrol) gruplarına ayrıldı. Her içeceğe ait pH değerleri kaydedildi. Ayrılan dişler bu içeceklerde her gün 5 dakika olmak üzere 30 gün boyunca inkübe edildi. Başlangıç, 4.haftanın sonu ve 3. ayın sonunda dişlerin mikro sertlik ve yüzey pürüzlülüğü ölçümleri yapıldı.

Bulgular: Çalışmada kullanılan hem bitkisel hem de hayvansal tüm protein içeceklerinin mine mikro sertliğini artırdığı tespit edilmiştir. Bezelye proteini tozuyla yapılan içecek mine mikro sertliğinde en yüksek artışa neden olmuştur. Öte yandan, pirinç proteini tozu içeceğinin diş yüzey pürüzlülüğünü diğer içeceklere göre önemli ölçüde azalttığı belirlenmiştir.

Sonuç: Bu çalışmada kullanılan tüm protein içeceklerinin mine mikro sertliğini farklı oranlarda artırdığı bulunmuştur. Proteince zengin bu içeceklerin dişler üzerindeki etkileri içeceğin kimyasal bileşimine ve dişlerle temas süresine göre farklılık gösterdiği tespit edilmiştir.

Anahtar Kelimeler: Mine mikrosertliği, Protein içecekleri, Yüzey Prozitesi, Diş

ABSTRACT

Objectives: Protein-rich beverages have the potential to protect the teeth against dental erosion. However, there is a lack of research on the effects of protein-rich beverages on the teeth. Therefore, this study aimed to investigate the effect of plant and animal-based protein-rich beverages, that are frequently consumed today, on enamel microhardness and surface roughness under in vitro conditions..

Materials and Methods: The extracted permanent posterior teeth (15 premolars and 15 molars) were embedded in acrylic blocks, leaving the buccal surfaces of the teeth exposed. The extracted teeth were then divided randomly into five groups (n=6 per group): G1-cocoa-flavored protein-rich milk; G2-chocolate-flavored whey protein powder; G3-pea protein powder; G4-rice protein powder; and G5-artificial saliva (control). The pH of each beverage was assessed. The teeth were incubated in these beverages every day for 5 minutes for 30 days. Microhardness and surface roughness were measured at the baseline, at the 4th week and the third months.

Results: It was found that all the protein beverages used in the study, both plant and animal-based, increased enamel microhardness. The beverage made with pea protein powder showed the highest increase in enamel microhardness. On the other hand, the rice protein powder beverage has been found to decrease tooth surface roughness significantly higher than the other beverages.

Conclusions: It was found that all protein beverages used in this study increased enamel microhardness at different rates as the effects of these beverages on teeth depend on the chemical composition, contact time with teeth and pH.

Keywords: Enamel, Microhardness, Protein Beverages, Surface Rougness, Teeth.

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INTRODUCTION

The use of protein supplements in daily nutrition has increased due to the rise in healthy eating habits, especially among people who exercise regularly. Additionally, there has been a growing demand for plant-based protein supplements among vegetarian consumers (Kårlund et al., 2019). There is limited research available on the effects of animal or plant-based protein powders and protein riched milk on the tooth structure. However, there are some studies that reported that the increase in fast and on-the-go eating as a result of modern life has led to an increase in the frequency of dental erosion (Sato et al., 2021; Rusyan et al., 2022; Mobley et al., 2009). This has become a common problem for developed societies (Addy & Shellis 2006). Dental erosion is the loss of dental hard tissue with a multifactorial etiology due to prolonged contact and repetition of low pH, unsaturated solutions to the dental tissues (Erdoğan & Bolaca 2023). It can occur due to extrinsic and intrinsic factors. Extrinsic factors result from consuming acidic foods, drinks, and drugs, as well as from occupational exposures. These factors can increase the risk of dental erosion, especially when they are consumed regularly or in large quantities (Moynihan & Petersen 2004). Intrinsic factors, on the other hand, come from stomach acid and can be caused by eating disorders, reflux disease, or alcohol abuse (Kanzow et al., 2016). While acidic foods and drinks with low pH play a significant role, other factors such as calcium, phosphate, and fluorine levels, saliva composition, buffer capacity, diet and consumption habits, lifestyle, and oral hygiene practices also contribute to the erosive process. These factors, combined with abrasion from incorrect oral hygiene practices, can lead to dental erosion (Inchingolo et al., 2023).

Various methods have been attempted to prevent dental erosion due to its irreversible nature.

One such method is the addition of calcium and phosphate salts to acidic beverages. However, this approach can result in an undesirable taste due to the high calcium content. An alternative method that has shown positive results is the addition of protein supplements, such as ovalbumin and casein, to acidic beverages (Ferreira et al., 2015). Fluoride is a frequently used substance to enhance the remineralization of enamel. However, because of the risk of toxicity associated with high concentrations of fluoride, alternative ingredients are being considered.

Studies have shown that milk and dairy products, such as cheese and yoghurt, exhibit cariostatic effects on human and animal models because of their phosphoprotein, casein, and calcium content. Whey extract is a dairy product that contains a high concentration of casein phosphopeptide-amorphous calcium phosphate (CPP-ACP). CPP-ACP is defined as a milk product that aids remineralization and prevents dental caries (Nobahar et al., 2020). While studies have shown that milk has a protective effect against dental erosion, it is often consumed with flavored beverages rather than pure milk. It is assumed that these added ingredients may interfere with the protective effect of milk against dental erosion (Lachowski et al., 2014).

Currently, consumers who prioritize their health are changing their food preferences to attain

specific physiological advantages, minimize the risk of certain diseases, and align with foods

that fulfill their body's essential nutritional needs (Kadam et al., 2018). As the demand for protein-enriched food products increases, interest in plant-based alternatives is also growing. The substitution of milk protein with plant protein will mainly occur in prepared foods, such as nutritional drinks (Arranz et al., 2023). There has been a growing demand for plant-based protein supplements among vegetarian consumers. There is limited research available on the effects of animal or plant-based protein powders and protein milk on tooth structure. Therefore, this study aimed to investigate the effects of protein-based beverages, which are commonly consumed currently, on enamel structure *in vitro*.

MATERIALS AND METHODS

Study Design

This study was approved by the ethics committee Marmara University, Institute of Health Sciences Non-Interventional Clinical Research Ethics Committee (Approval Date:19.12.2023, Approval Number: 114).

The teeth used in this study were collected within a 2-month period. The extracted permanent posterior teeth (15 premolars and 15 molars) were embedded in acrylic blocks, leaving the buccal surfaces of the teeth exposed. The extracted teeth were then divided randomly into five groups (n=6 per group): G1-cocoa-flavored protein-rich milk; G2-chocolate-flavored whey protein powder; G3-pea protein powder; G4-rice protein powder; and G5-artificial saliva (control). The formula of the artificial saliva formula was adjusted to contain 0.62g KCl, 0.17g $CaCl_2$, 1.1gK_2HPO^4, 0.3g KH_2PO_4, 0.87g NaCl, and a pH of 7 in 1000ml. The teeth were incubated in these beverages every day for 5 minutes for 30 days. Microhardness and surface roughness were measured at the baseline, at the 4^{th} week and the third month. The pH of each beverage was also assessed.

Specimen Preparation

The collected permanent premolar and molar teeth were cleaned using pumicewater slurry with a polishing brush at a low-speed handpiece to remove debris or calculus before the study. The crown was separated from the root with the help of a diamond disc. To assess the microhardness of the samples, the teeth were embedded in an auto-polymerizing acrylic resin, leaving a portion of the buccal enamel visible. The sample number was inscribed at the base of each sample. Sample surfaces were polished using 400,600, 1200-grit abrasive paper.

The samples were randomly divided into 5 groups with 3 premolars and 3 molars in each group.The prepared samples were kept in distilled water at room temperature until the experiment was performed.

The Beverages

In this study, the effects of beverages containing animal and plant-based proteins on tooth enamel hardness and roughness were investigated. The source of animal-based proteins were the protein enriched milk (SEK, Türkiye) and whey protein (Fellas, Türkiye). The source of plant-based proteins were rice protein powder (Saf, Türkiye) and pea protein powder (Saf, Türkiye).

The beverages to be tested were prepared at a protein concentration of 8.8%. The beverages were re-prepared every week and kept in the refrigerator during this period. The pH values of the beverages used in the experiment were measured with a pH meter (Mettler Toledo,Switzerland)

Experimental Procedure

Tooth samples were kept in the prepared beverages for 5 minutes daily, then washed with distilled water and dried. Tooth samples were stored in artificial saliva at 37°C until the next immersion period. The control group was kept in artificial saliva throughout the experiment and the artificial saliva solution was renewed daily for all samples. This experiment was continued for 1 month with measurements of microhardness and surfaceroughness. After 1 month, to obtain an additional 3 months of data, the tooth samples were kept in the prepared beverages for 7.5 hours and the microhardness and surface roughness measurements were repeated. Based on the estimate that these beverages were consumed for 5minutes per day, it was assumed that 7.5 hours of immersion would simulate 3 months of use of these beverages (Shiozawa et al., 2015).

Surface Microhardness Test

Microhardness measurements were made with the Vickers microhardness machine (Falcon 400, Innovatest) at Istanbul Kent University. After drying the samples, microhardness was measured at the center of the polished area in each sample using a Vickers hardness tester (Alrahlah et al., 2023). For this purpose, a 50 g load was applied to three points at the center of each sample for 15 seconds by the diamond indenter of the device. Each load application created a diamond-shaped indentation on the samples. The average of the three measurements was taken and used as the Vickers hardness value.

Surface Roughness Test

Surface roughness measurements were made with the portable surface roughness tester (SJ-410, Mitutoyo) at Istanbul Kent University. The Ra parameter was measured at a traversing speed of 0.5 mm/s and a cut-off length of 0.08 λc. The surface of the samples was measured

three times, and the resulting roughness value was determined by averaging the measurements.

Statistical Analysis

Statistical analyses were performed using the GraphPad Prism 6.0 package program (GraphPad Software, San Diego, CA, USA). Results were presented as mean and standard deviation (SD).

The normality of the distribution of all data was determined. The data showed a normal distribution, thus parametric tests were used. One-Way Analysis of Variance (One-Way ANOVA) was used to compare the means of more than two groups and identify differences. The post hoc Tukey test also analyzed the difference in variable subgroups. The cut-off point for significance was applied to interpretations, where $p < 0.05$ was regarded as significant.

RESULTS

Microhardness Results

The Vickers microhardness (VHN) values were presented at Fig. 1, Fig. 2. and Fig. 3. The microhardness of the tooth enamel kept in artificial saliva (control group) did not show a statistically significant change at the fourth week and the third month compared to the baseline (Fig. 1).

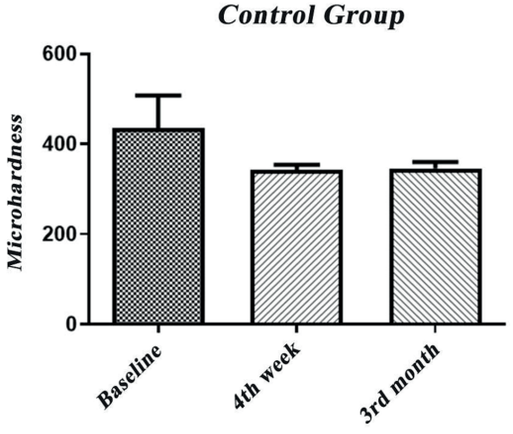

Figure 1: Microhardness of Control Group

When the teeth were kept in animal protein-based beverages, cocoa-flavored high protein milk significantly increased microhardness after 4 weeks and 3 months compared to the baseline. Whey protein beverage did not cause any significant change in microhardness after 4 weeks and 3 months compared to the baseline (Fig. 2).

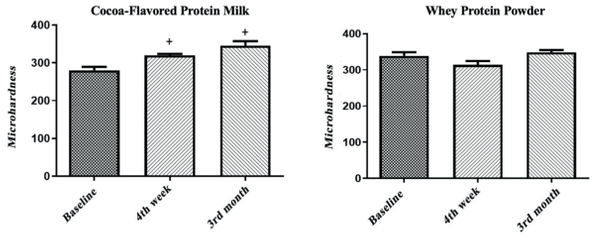

Figure 2: Effect of Animal-Based Proteins on Microhardness

When the teeth were kept in plant protein-based beverages, pea protein beverages significantly increased the microhardness at the end of week 4 and month 3 compared to the baseline. Keeping the teeth in rice protein beverage did not significant change the microhardness at the end of week 4 but exhibited a significant increase at the end of 3 months period (Fig. 3).

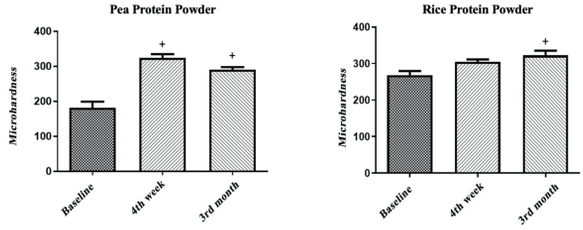

Figure 3: Effect of Plant – Based Proteins on Microhardness

Surface Roughness Results

The surface roughness values were presented at Fig. 4, Fig. 5 and Fig. 6.

The surface roughness values of the tooth enamel kept in artificial saliva (control group) did not significantly change at ehe end of week 4 and month 3 compared to the baseline (Fig. 4). When the teeth were kept in animal protein-based beverages, cocoa flovred high-protein milk did not change the surface roughness of the tooth enamel significantly at the end of week 4, but decreased the surface roughness at the end of third month compared to the baseline. Keeping teeth in a whey protein beverage significantly decreased the surface roughness at the end of week 4 and month 3 compared to the baseline (Fig. 5).

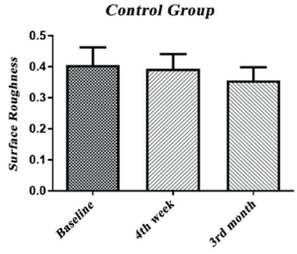

Figure 4: Surface Roughness of Control Group

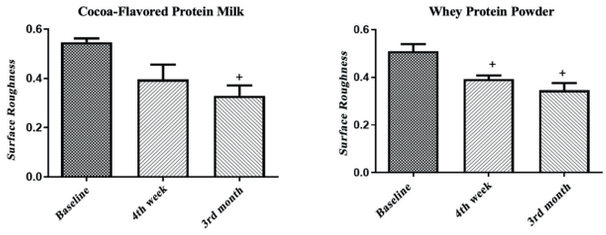

Figure 5: Effect of Animal-Based Proteins on Surface Roughness

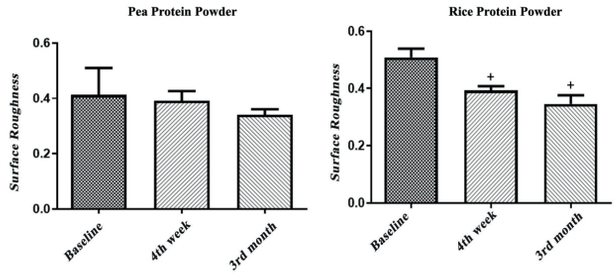

Figure 6: Effect of Plant-Based Proteins on Surface Roughness

When the teeth were kept in plant protein-based beverages, the surface roughness values of the tooth enamel kept in a pea protein beverage group did not change significantly at the end of week 4 and month 3 compared to the baseline. Keeping the teeth in the rice protein beverages, significantly decreased the surface roughness values of tooth enamel at the end of week 4 and month 3 compared to the baseline.

DISCUSSION

Poor eating habits are one of the main factors in the formation of erosive tooth wear (Dugmore & Rock 2004), but it has been reported that healthy eating habits can have positive effects on teeth (Huew et al., 2011). Since demineralization and remineralization cycles are continuous in the mouth, Ca and P ions lost during demineralization can be returned to the tooth structure by the remineralization process. In this study, it was aimed to show the effect of beverages with different types of protein content as a demineralizing or remineralizing agent after immersion in these beverages. An in vitro study was conducted using extracted teeth to provide more controlled conditions.

Nowadays, dieters, vegetarians and athletes have started to prefer high protein drinks as meal supplements. Studies on the effects of these protein-rich drinks, which can be of plant or animal origin, on oral and dental health are limited. Therefore, the aim of this study was to evaluate the erosive potential of the selected animal and plant

protein-based beverages by measuring the tooth enamel microhardness and surface roughness.

In this study, an in vitro experimental model was used considering the effects of patient follow-up and standardization stages. In vitro experimental models are frequently used methods in the study of demineralization and remineralization procedures for enamel. Mudumba et al. stated that long-term exposure to acid attacks was used for 10-60 minutes and short-term exposure for 1-4 minutes in in vitro study models. Bashir and Lagerlöf stated that saliva saturated with calcium and phosphate returned to its previous saturation 5 minutes after exposure to citric acid. Many similar dental erosion studies have also followed 5-minutes immersion cycles (Shiozawa et al., 2015). In this study, dental samples were immersed in protein drinks once a day for 5 minutes to simulate daily protein drink consumption of consumers and then the samples were stored in artificial saliva at 37°C to simulate the oral environment. The samples from the control group were kept in artificial saliva solution without any treatment. After the four-week experimental period, samples were immersed in beverages for 7.5 hours at a time to simulate 3 months of use, based on the work of Guler et al., (2005).

Studies have shown that casein and ovalbumin can adsorb onto the enamel surface, reduce hydroxyapatite dissolution, and these proteins can effectively reduce the erosive potential of acidic solutions and commercially available soft drinks (Arends et al., 1986; Hemingway et al. 2011; Reynolds & Black 1987; Ferreira et al., 2015).

According to the results obtained in this study, commercially available plant and animal proteins did not have a negative effect on teeth microhardness and roughness. In addition to the fact that the beverages used in the study did not have a negative effect on contact with teeth, they also had positive effects on microhardness and porosity. The results of this study show that consumption of whey protein beverage did not lead to a statistically significant change in microhardness at the end of 3 months compared to the baseline level. In contrast, Rezvani et al., (2015) in their study comparing the effect of casein phosphopeptide – amorphous calcium phosphate (CPP-ACP) and whey extract (as a natural CPP-ACP) on enamel microhardness, found promising findings in terms of the effectiveness of whey extract on enamel microhardness. The reason for this difference is probably attributable to the methodological differences between the two studies and the varying durations of contact with the material. In this study, there was a significant increase in enamel microhardness of teeth kept in cocoa-flavored protein milk at 4 weeks and 3 months compared to the baseline level. A similar result was observed in a study conducted by Khan et al., (2022) where an increase in enamel microhardness was noted after continuous immersion in plain milk and flavored milk.

In the literature, there were no study that evaluates the effect of pea and rice protein beverages on dental erosion, therefore, this study will form the basis for future studies on the effects of plant-based proteins on teeth.

According to the plant-based protein beverages results, at the end of the week 4 and month 3, pea and rice protein beverages increased the enamel microhardness compared to baseline. Both of these plant-based proteins provided protection against enamel surface softening.

In order to explain these differences, it was assumed that the nature, aminoacid content and molecular weight of the both animal and plant based proteins could affect their adsorption to enamel.

According to previous studies, the effect of treatment solutions on surface roughness varies depending on the type of material (Münchow et al., 2014; Al-Samadani, 2013). Ferreira et al,. (2015) reported that casein did not prevent dental erosion caused by orange juice, whereas commercially available calcium-modified fruit juice reduced erosion of both enamel and dentin. In this study while the immersion of teeth in high-protein milk reduced the surface roughness at the end of the month 3, whey protein beverage reduced both at the end of the week 4 and month 3. According to this result, whey protein showed an earlier effect on surface roughness than high-protein milk. When the teeth immersed in plant based-protein beverages, only rice protein based beverage decreased the surface roughness, pea protein did not cause any difference at the surface rougness. This result shows that rice proteins positively affect surface roughness compared to pea proteins.

CONCLUSION

The consumption of protein-rich beverages has a beneficial impact on dental health and may contribute to protective effects by supporting proper oral hygiene practices. The results of this study provide a basis for subsequent *in vivo* studies.

Conflicts of Interest

The author declares that they have no conflicts of interest.

REFERENCES

1. Addy M, Shellis RP. Interaction between attrition, abrasion and erosion in tooth wear. Dental Erosion 2006; 20:17-31.
2. Al-Samadani KH. Effect of energy drinks on the surface texture of nanofilled composite resin. J. Contemp. Dent. Pract. 2013;14(5):830-835.
3. Alrahlah A , Khan R, Al-Odayni A B, Saeed W S, Bautista L S, Alnofaiy I A, & De Vera M A
4. T. Advancing Dimethacrylate Dental Composites by Synergy of Pre-Polymerized TEGDMA
5. Co-Filler: A Physio-Mechanical Evaluation. Biomimetics, 2023; 8(8), 577.
6. Arends J, Schuthof J, Christoffersen J. Inhibition of enamel demineralization by albumin in vitro.Caries Res.1986;20(4):337-340.

7. Arranz E, Segat A, Velayos G, Flynn C, Brodkorb A, Giblin L. Dairy and plant based protein beverages: In vitro digestion behaviour and effect on intestinal barrier biomarkers. Food Res. Int. 2023;169:112815.
8. Connie M, Marshall TA, Milgrom P, Coldwell SE. The contribution of dietary factors to dental caries and disparities in caries. Acad. Pediatr. 2009;9(6):410-414.
9. Dugmore CR, Rock WP. The prevalence of tooth erosion in 12-year-old children. Br.Dent. J. 2004;196(5):279-282.
10. Ferreira SS, Tais S, Hara AT, Aoki IV, Sobral MAP. Supplementation of an orange juice with dietary proteins to prevent enamel and dentin erosion.Br. Dent. J. 2015;26(3):263-267.
11. Guler AU, Yilmaz F, Kulunk T, Guler E, Kurt S. Effects of different drinks on stainability of resin composite provisional restorative materials. J. Prosthet. Dent. 2005;94 (2):118-124.
12. Hemingway CA, White AJ, Shellis RP, Addy M, Parker DM, Barbour ME. Enamel erosion in dietary acids: inhibition by food proteins in vitro.Caries Res. 2011;44 (6):525-530.
13. Huew R, Waterhouse PJ, Moynihan PJ, Kometa S, Maguire A.Dental erosion and its association with diet in Libyan schoolchildren. Eur. Arch. Paediatr. Dent. 2011;12(5):234-240.
14. Inchingolo AM, Malcangi G, Ferrante L, Vecchio GD, Viapiano F, Mancini A, Inchingolo F, Inchingolo AD, Venere DD, Dipalma G. Damage from carbonated soft drinks on enamel: a systematic review. Nutrients 2023;15 (7):1785.
15. Kadam B, Ambadkar R, Rathod K, Landge S. Health benefits of whey: A brief review. Int. J. Livest. Res.2018;8(5):31-49.
16. Kanzow P, Wegehaupt FJ, Attin T, Wiegand A. Etiology and pathogenesis of dental erosion. Quintessence Int. 2016;47(4):275-278.
17. Karlund A, Gomez-Gallego C, Turpeinen AM, Palo-Oja Q, El-Nezami H, Kolehmainen M. Protein supplements and their relation with nutrition, microbiota composition and health: is more protein always better for sportspeople? Nutrients 2019;11(4):829.
18. Khan HB, Gulam S, Mohd Sarmin NI, Zahid MF, Hashim NA. The effect of different milk products on enamel hardness: an in vitro study. Compend. Oral Sci.2022;9(1):67-79.
19. Lachowski KM, Ferreira D, Oliveira TA, Pita Sobral MA. Effect of the mixture of coffee or chocolate to milk in the progression of des-remineralization of tooth enamel-An in vitro study. Pesqui. Bras. Odontopediatria Clin. Integr. 2014;14(3):183-190.
20. Moynihan P, Petersen PE. Diet, nutrition and the prevention of dental diseases. Public Health Nutr. 2004;7(1a):201-226.
21. Mudumba VL, Muppa R, Srinivas N, Kumar DM. Evaluation and Comparison of Changes in Microhardness of Primary and Permanent Enamel on Exposure to Acidic Center-filled Chewing Gum: An in vitro Study. Int J Clin Pediatr Dent. 2014 Jan;7(1):24-9. doi: 10.5005/jp-journals-10005-1228. Epub 2014 Apr 26. PMID: 25206233; PMCID: PMC4144056.
22. Münchow EA, Ferreira ACA, Machado RMM, Ramos TS, Rodrigues-Junior SA, Zanchi CH. Effect of acidic solutions on the surface degradation of a micro-hybrid composite resin. Br. Dent. J. 2014;25(4):321-326.
23. Reynolds EC, Black CL. Reduction of chocolate's cariogenicity by supplementation with sodium caseinate. Caries Res.1987;21(5):445-451.
24. Rezvani MB, Karimi M, Rasoolzade RA, Haghgoo R. Comparing the effects of whey extract and casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) on enamel microhardness. J. Dent. 2015;16(1):49.
25. Rusyan E, Grabowska E, Strużycka I. The association between erosive tooth wear and diet, hygiene habits and health awareness in adolescents aged 15 in Poland. Eur. Arch. Paediatr. Dent. 2022;1-9.
26. Tsutomu S, Fukuzawa Y, Kawakami S, Suzuki M, Tanaka Y, Terayama H, Sakabe K. The onset of dental erosion caused by food and drinks and the preventive effect of alkaline ionized water. Nutrients 2021;13(10):3440.
27. Shiozawa M,Takahashi H, Asakawa Y, Iwasaki N. Color stability of adhesive resin cements after immersion in coffee. Clin. Oral Invest. 2015;19:309-317.
28. Yıldırım E, Bolaca A. Evaluation of traumatic dental injuries in patients attending the pediatric dentistry clinic: a retrospective study.Pamukkale Med. J. 2023;16(4):570-578.