Research Article

Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1434306

Demineralization effects of breast milk, formula milk and cow's milk on the primary teeth. A study of SEM-EDX analysis

Anne sütü, formül süt ve inek sütünün süt dişleri üzerindeki demineralizasyon etkileri. Bir SEM-EDX analizi çalışması

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Posted date:09.02.2024 Acceptance date:01.04.2024

Abstract

Purpose: It was aimed to investigate demineralization effects of breast milk, formula milk and cow's milk on deciduous teeth with SEM-EDX analysis.

Materials and methods: Demineralization effects of breast milk (Group 1), 3 different formula milks: Aptamil Pronutra-3 (Group 2), Hipp Organic-3 (Group 3), SmA Optipro-2 (Group 4) and cow's milk (Group 5) on newly extracted caries-free primary incisors were investigated on 5 teeth in each group. Each tooth was split in the sagittal direction from the midline and embedded in 2 separate acrylic blocks with their labial surfaces exposed. Half of the teeth was exposed to the milk material for 15 minutes, 4 times a day for 5 days, while the other half was kept in artificial saliva only. After the exposures, Scanning Electron Microscopy (SEM) images of the labial surfaces were taken and Energy Dispersive X-ray Spectroscopy (EDX) analyzes were performed. For the statistical analysis Independent-Samples T test, Man Whitney U and Kruskal-Wallis test were used.

Results: The most increased enamel porosity was seen in Group 2. A significant decrease was observed in the amount of Ca on tooth surfaces exposed to breast milk and formula milks, and a significant increase in the amount of both Ca and P after exposure to cow's milk (p=0.009). The decrease observed in the amount of Ca after exposure to breast milk was significantly higher than that of formula milks (p=0.001).

Conclusion: Formula milk and breast milk are not innocent in terms of causing demineralization, therefore awareness of parents and dentists on this issue should be increased.

Keywords: Breast milk, formula milk, Cow's milk, demineralization, SEM-EDX analysis.

Ertugrul CC. Demineralization effects of breast milk, formula milk and cow's milk on the primary teeth. A study of SEM-EDX analysis. Pam Med J 2024;17:510-519.

Öz

Amaç: Bu araştırmada anne sütü, formül süt ve inek sütünün süt dişleri üzerindeki demineralizasyon etkilerinin SEM-EDX analizi ile araştırılması amaçlanmıştır.

Gereç ve yöntem: Anne sütü (Grup 1), 3 farklı formül süt: Aptamil Pronutra-3 (Grup 2), Hipp Organik-3 (Grup 3), SmA Optipro-2 (Grup 4) ve inek sütünün (Grup 5) demineralizasyon etkileri her bir grupta 5 yeni çekilmiş çürüksüz süt kesici dişi üzerinde incelenmiştir. Her diş orta hattan sagittal yönde ikiye bölünerek labial yüzeyleri açıkta kalacak şekilde 2 ayrı akrilik bloğa gömülmüştür. Açıkta kalan diş yüzeyinin yarısı 5 gün boyunca günde 4 kez 15 dakika süreyle incelenecek süt materyaline maruz bırakılırken, diğer yarısı ise sadece yapay tükürük içerisinde tutulmuştur. Deney materyallerine maruziyet sonrası diş yüzeylerinin Taramalı Elektron Mikroskobu (SEM) görüntüleri alınmış ve Enerji Dağılımlı X-ışını Spektroskopisi (EDX) analizleri yapılmıştır. Verilerin istatistiksel analizinde Independent-Samples T testi, Man Whitney U ve Kruskal-Wallis testi kullanılmıştır.

Bulgular: En fazla artan mine pürüzlülüğü Grup 2'de görülmüştür. Anne sütü ve formül sütlere maruz kalan diş yüzeylerinde Ca miktarında anlamlı azalma, inek sütüne maruz kalan diş yüzeylerinde ise hem Ca hem de P miktarında anlamlı artış gözlenmiştir (p=0,009). Anne sütüne maruz kaldıktan sonra Ca miktarında gözlenen azalma, formül sütlere göre anlamlı olarak daha yüksek bulunmuştur (p=0,001).

Sonuç: Bu araştırmanın sonuçlarına göre bebeklik döneminde yaygın kullanılan formül sütler ve anne sütü süt dişlerinde demineralizasyona neden olması açısından şüphe uyandırmaktadır, bu nedenle ebeveynlerin ve diş hekimlerinin bu konuda farkındalığı arttırılmalıdır.

Anahtar kelimeler: Anne sütü, formül süt, inek sütü, demineralizasyon, SEM-EDX analizi.

Ertuğrul CÇ. Anne sütü, formül süt ve inek sütünün süt dişleri üzerindeki demineralizasyon etkileri. Bir SEM-EDX analizi çalışması. Pam Tıp Derg 2024;17:510-519.

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Introduction

The main cause of early childhood caries (ECC), which may cause severe pain and loss of function, therefore may affect nutrition and general health status of the infants [1, 2] is repetitive and prolonged breast-feeding or bottle-feeding with formula milk, especially during sleep at night [3, 4].

Breast milk which is the most valuable nutrient in infant nutrition, contains protein, fat, lactose and iron, and besides being an important source of energy for brain development, it increases calcium absorption and is beneficial for microorganisms in the body [5]. However, Birkhed et al. [6] reported that lactose can cause dental caries by increasing the adhesion of caries-causing microorganisms to tooth surfaces and causing demineralization. Bowen and Lawrence [7] emphasized that tooth decays are more common in children who take breast milk compared to children who take formula milk, and that oral hygiene practices should be done more carefully in these children. On the other hand, Erickson and Mazhari [2] stated that it is not possible for breast milk to have an effect on early childhood caries.

Formula milks, another nutritional source in infant nutrition, are complex compounds containing fermentable carbohydrates such as sucrose, lactose and glucose, which play a leading role in the etiology of dental caries [8]. Despite the proteins, fats, vitamins and minerals such as calcium and phosphate in their content, their cariogenic effects have been detected and reported in many studies [9-12]. Generally, it is stated that a wide variety of carbohydrates in the structure of formula milk are effective in the formation of dental caries, but on the other hand, peptides, caseins, ions and vitamins in the structure of formula milk can prevent the cariogenic activity of sucrose [8, 10]. Formula milks are generally produced in a similar structure to breast milk. There are similar amounts of lactose in breast milk (6.7 to 7.8 g/dL) and formula milk (8-8,3 g/dL), but there is more protein content in formula milk (2-2,5 g/dL) than in breast milk (0.9-1.2g/dL) [8, 13].

Another commonly consumed nutrient in early childhood nutrition is cow's milk. It is stated that cow's milk protects from dental caries due to its high calcium and phosphorus content and buffering activity of milk proteins [14, 15]. It shows anticariogenic effect thanks to the proteins in the form of casein particles in the cow's milk structure. These particles are calcium phosphate complexes in highly stable form [16]. In addition, it has been stated that the antibacterial enzymes, vitamin D and fluoride in cow's milk also play a role in the caries preventive effect [17, 18]. However, despite these advantages, cow's milk consumption is not recommended in the first year of life [19].

Demineralisation is the process of removing minerals such as calcium and phosphate from the hydroxyapatite crystals of the tooth enamel and is the initial stage for the formation of dental caries. Studies about the demineralisation effect of breast milk, formula milk and cow's milk are few and the results are controversial [20]. In this study, it was aimed to investigate and compare the possible demineralization effects of indispensable infant nutrients such as breast milk, formula milk and cow's milk on deciduous tooth surfaces with SEM-EDX analysis.

Materials and methods

Permission was obtained from Pamukkale University Non-Interventional Clinical Research Ethics Committee for the study.

Preparation of the tooth specimens

In order to be used in in-vitro experiments, a total of 25 newly extracted, caries-free healthy human primary teeth for 5 groups that were planned to be included in the study, with 5 teeth in each were collected. The collected teeth were kept in 0.1% thymol solution at room temperature until the time of the experiment. Before the experiments, the crowns of the teeth were separated from enamel-cement junction with the help of a diamond separe under water cooling. Then, the crown part of the teeth was divided into two from the midline in the sagittal direction, and each half of the teeth was embedded in 2 separate acrylic blocks with their labial surfaces up. Finally, these labial surfaces, which will be exposed to the test material, were polished using green and red color Shofu Super-Snap polishing discs (Shofu Inc., Kyoto, Japan) respectively.

Milk materials used in the experiments

Breast milk samples (Group 1) taken from a volunteer mother who was breastfeeding a 9-12 month-old baby were stored in separate storage bags in a deep freezer at -16°C until the experiment day, and before the each experiment, only the package to be used was thawed and brought to 37°C. Three different formula milks with similar contents of sugar (8-8.3 g/100 mL), calcium (75-76.2 mg/100 mL) and phosphorus (43-52 mg/100 mL) which are commonly found in the market and suitable for 9-12 months old babies: Aptamil Pronutra 3 (Group 2), Hipp Organic 3 (Group 3) and SMA Optipro 2 (Group 4) were freshly prepared in accordance with the manufacturer's instructions before the experiments and used at 37°C. Finally, a brand of Ultra High Temperature (UHT) cow's milk (Pınar Whole Milk) (Group 5), which is easily accessible in the market, was used at 37°C by opening a new package in each experiment.

Endogenous pH measurement of milk materials

Before the experiments, endogenous pH of 5 different test materials was measured with a digital pH meter (WTW InoLab pH 7110, Germany) at room temperature by placing the measuring tip of the device directly into the material. The pH meter accurate to 0.1 was first calibrated according to manufacturer's instructions using buffer standarts of pH 7 and pH 4. As much of 10 mL of each milk material was placed in a beaker, the pH meter was immersed into the milk and the value was recorded.

Preparation of artificial saliva

The artificial human saliva to be used in the research was prepared by a scientist from Department of Biochemistry of the Faculty of Science according to the recipe and method specified in the literature [21] excluding only sorbitol [22]. It contained Methyl-p-hydroxybenzoate 2.00g, carboxymethyl cellulose 10.0 g, KCl 0.625 g, $MgCl_2.6H_2O$ 0.059 g, $CaCl_2.2H_2O$ 0.166 g, K_2HPO_4 0.804 g, KH_2PO_4 0.326 g in 1000 mL of deionized water. The protein and mucin content of the natural saliva was simulated by the first two components, which increased the viscosity of the saliva, while the other ingredients provided the inorganic components at levels comparable with that of natural saliva. The pH of the artificial saliva was adjusted to 6.75 using KOH.

Immersion procedures

- During the experiment, the prepared acrylic blocks were kept into artificial saliva in a 5 mL beaker for each group in an oven (Nüve Oven KD400, İstanbul, Türkiye) at 37°C.
- Half of the divided tooth samples were exposed to the test material (experiment) and the other half was kept in artificial saliva throughout the experiment (control).
- Half teeth in the experimental groups were removed from the artificial saliva 4 times a day and kept in the tested milk material in the oven at 37°C for 15 minutes.
- At the end of the immersion period, the teeth were washed with distilled water and placed in artificial saliva again and kept in the oven until the next experiment.
- The experiments were repeated for 5 days and for each material, a total of 20 applications of 15 minutes were made on the tooth surface.

SEM-EDX analyzes

After the last experiments, the teeth were removed from the artificial saliva and posterior dehydration was performed in an ascending ethanol series (25, 50, 75, 95 and 100%). Following the specimens were mounted on stubs, sputter-coated with a rate of 80% gold and 20% palladium and got ready for scanning electron microscopy imaging.

A total of 10 half-tooth specimens in each group (5 experiments, 5 controls) observed at 20 kV accelerated voltage at 500, 1000, 1500 and 3000 times magnification with an SEM (Zeiss Supra 40 VP, Carl Zeiss SMT Inc., Oberkochen, Germany). SEM images of each experimental and control tooth sample were analyzed qualitatively. Erosion signs such

as loss of integrity, irregular enamel surface, roughness, crater formation, porosity, pitted or cracked surfaces and sporadic rod ends were investigated in the images. In addition, the changes in the mineral level of the enamel surfaces caused by the milk materials were evaluated using energy dispersive X-ray spectroscopy (EDX) (Zeiss Supra 40 VP, Carl Zeiss SMT Inc., Oberkochen, Germany) on a total of 50 tooth samples that 10 half tooth surfaces in each group. As a result of the surface element analysis of each tooth surface, Ca and P values were recorded quantitatively (wt%).

Statistical analyzes

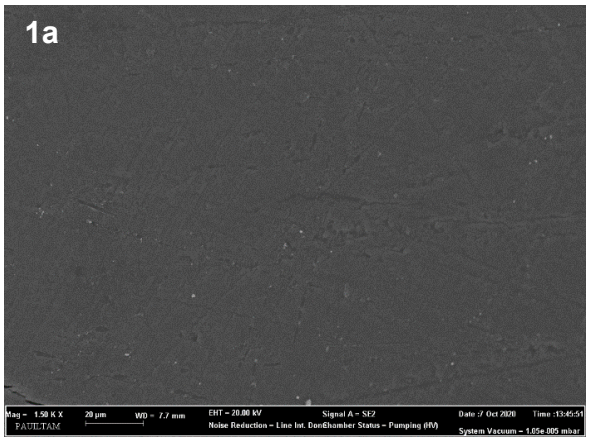
Data were analyzed with the SPSS package program (SPSS v23.0, SPSS Inc., Chicago, IL, USA). For the analysis of the differences intra-groups, the data which meet the prerequisites of parametric tests were analyzed with Independent-Samples T test, and the values which did not meet the prerequisites of parametric tests with the Man Whitney U test. For the analysis of the differences between groups Kruskal-Wallis test was used.

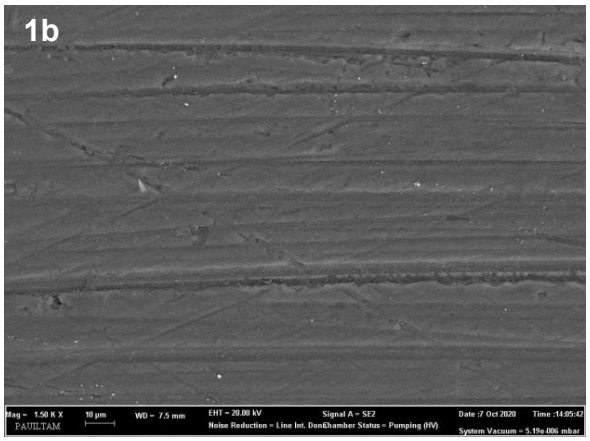
Results

The endogenous pH values of the tested milk materials are shown in Table 1. SEM images of the groups are shown in Figure 1. Qualitative evaluation of SEM images of experimental tooth surfaces in each group are listed in Table 2. A statistically significant decrease was observed in the amount of Ca on tooth surfaces exposed to breast milk and all the formula milks, on the other hand a statistically significant increase was observed in the amount of Ca of the teeth exposed to cow's milk. The amount of phosphorus increased in the Group 1, 3 and 5 after exposure to the tested milk material, and decreased in the Group 2 and 4. The mean SEM-EDX findings of Ca and P minerals of the teeth in each group and the differences within each group are given in Table 3. The decrease observed in the amount of Ca on the tooth surfaces exposured to breast milk was statistically significantly higher than that of formula milks (p=0.001). The differences observed in terms of the mean Ca decrease between the groups are given in Table 4.

Table 1. Endogenous pH values of the milk materials at room temperature

Groups	Milk materials	Endogenous pH value
Group 1	Breast milk	7.22
Group 2	Formula milk (Aptamil Pronutra 3)	7.16
Group 3	Formula milk (Hipp Organic 3)	7.20
Group 4	Formula milk (SMA Optipro 2)	7.19
Group 5	Cow's milk	7.16


1a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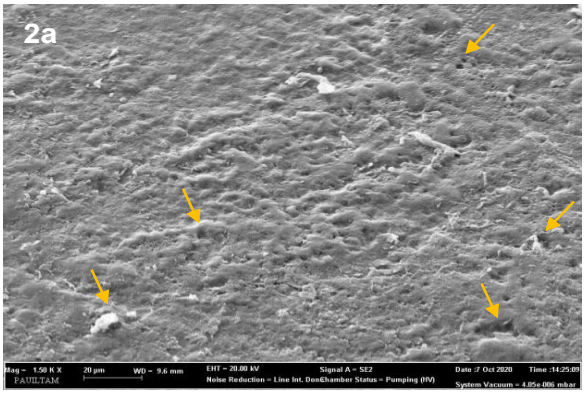
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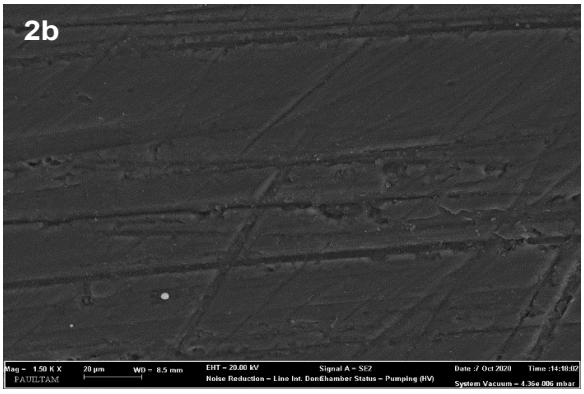
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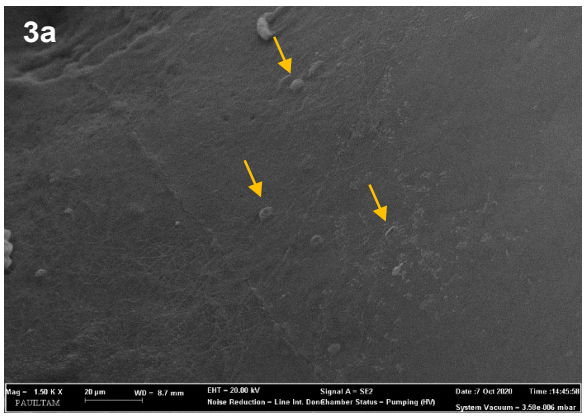
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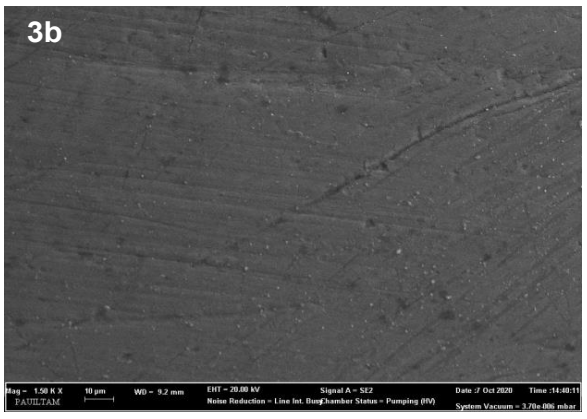
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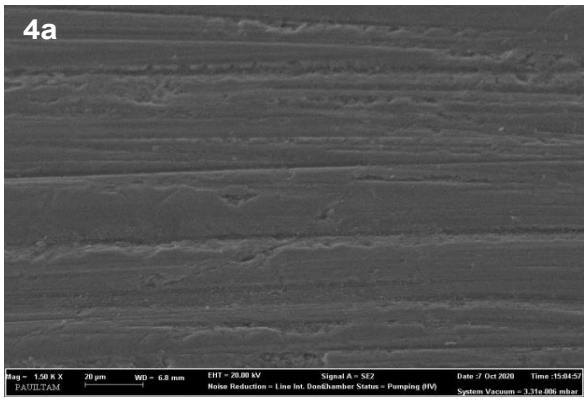
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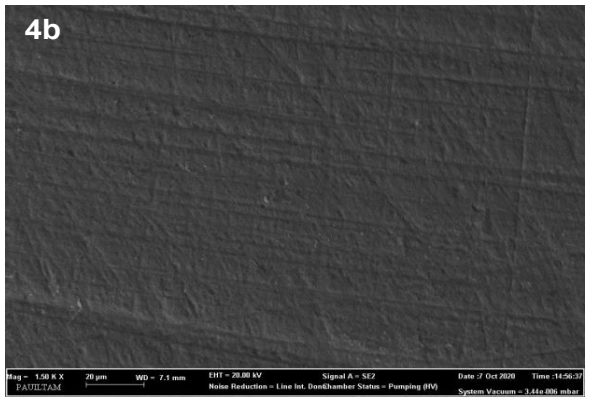
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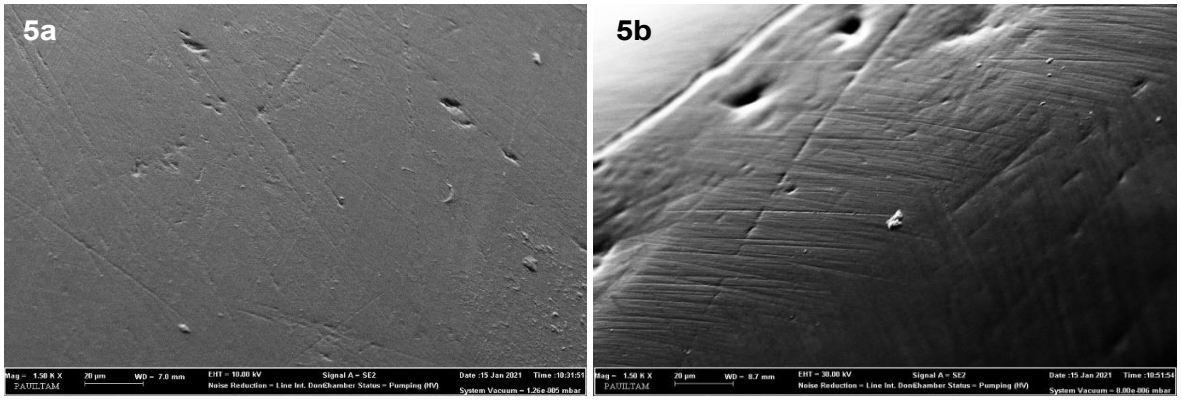

Figure 1. SEM images (1500x and 20.00 kV) of the groups

1a: Image of half tooth surface exposed to breast milk (experiment), **1b:** The other half of the tooth exposed to breast milk which is kept in only artificial saliva (control)
2a: Image of half tooth surface exposed to formula milk Aptamil Pronutra 3 (experiment), **2b:** The other half of the tooth exposed to formula milk Aptamil Pronutra 3 which is kept in only artificial saliva (control)
3a: Image of half tooth surface exposed to formula milk Hipp Organic 3 (experiment), **3b:** The other half of the tooth exposed to formula milk Hipp Organic 3 which is kept in only artificial saliva (control)
4a: Image of half tooth surface exposed to formula milk SmA Optipro 2 (experiment), **4b:** The other half of the tooth exposed to formula milk SmA Optipro 2 which is kept in only artificial saliva (control)
5a: Image of half tooth surface exposed to cow's milk (experiment), **5b:** The other half of the tooth exposed to cow's milk which is kept in only artificial saliva (control)

Table 2. Qualitative evaluation of SEM images of experimental tooth surfaces in each group

Group 1 **Breast milk**	Microstructure and integrity of the enamel is generally preserved. Little porosity is seen. No crater formation or sporadic rod ends.
Group 2 **Formula milk** **(Aptamil Pronutra 3)**	Is the group that the enamel integrity shows the most deterioration. Irregular and porous enamel surface. Crater formation, roughness and etched prism patterns are seen.
Group 3 **Formula milk** **(Hipp Organic 3)**	Increased enamel porosity. No crater formation or etched prism pattern but few sporadic rod ends available.
Group 4 **Formula milk** **(SMA Optipro 2)**	Microstructure and integrity of the enamel is generally preserved. No porosity, crater formation or sporadic rod ends.
Group 5 **Cow's milk**	Smooth enamel surface. No evidence of erosion. Is the group that experimental and control half of tooth sample gives the most similar appearance.

Table 3. SEM-EDX findings of Ca and P minerals of the teeth

Groups	Milk materials	Minerals wt%	Control mean±SD	Experiment mean±SD	*p** value
Group 1	Breast milk	Ca	34.48±0.11	26.99±0.14	0.009
		P	12.91±0.17	14.13±0.17	0.000
Group 2	Formula milk (Aptamil Pronutra 3)	Ca	33.39±0.08	28.90±0.19	0.009
		P	12.99±0.23	10.71±0.20	0.009
Group 3	Formula milk (Hipp Organic 3)	Ca	38.74±0.15	34.52±0.15	0.009
		P	11.98±0.29	14.91±0.15	0.009
Group 4	Formula milk (SMA Optipro 2)	Ca	29.68±0.19	24.95±0.33	0.009
		P	11.23±0.23	10.46±0.14	0.000
Group 5	Cow's milk	Ca	24.67±0.33	30.89±0.22	0.009
		P	9.82±0.40	12.56±0.33	0.009

SEM-EDX: Scanning Electron Microscopy- Energy Dispersive X-ray Spectroscopy
Ca: Calcium, P: Phosphorus, $p \leq 0.05$ values indicate a statistically significant difference
*p** value: differences of the Ca and *P* values between control and experimental groups in each group

Table 4. Intergroup differences in the decrease in calcium values

Groups	Milk materials	Mean decrease in calcium values (wt%)	Mean rank*	*p* value
Group 1	Breast milk	7.49	18.00[a]	**0.001**
Group 2	Formula milk (Aptamil Pronutra 3)	4.49	8.60[bc]	
Group 3	Formula milk (Hipp Organic 3)	4.22	3.40[b]	
Group 4	Formula milk (SMA Optipro 2)	4.73	12.00[cd]	

***** Same superscript lowercase letters show statistical similarity, different lowercases show statistical difference
$p \leq 0.05$ values indicate a statistically significant difference

Discussion

Nutrition type and nutrient intake are important factors in the etiology of early childhood caries. The cariogenic potential of consuming sucrose-containing liquids with a bottle is well known, but studies comparing breast milk, cow's milk, and formula milk are few and the results are controversial [5, 20, 23]. As a result of this in-vitro study investigating the possible demineralization effects of breast milk, commercially available formula milks and cow's milk on primary teeth, among all the tested milk materials with similar pH values, the material that caused the most deterioration quantitatively in the microstructure of the deciduous tooth enamel was the formula milk named Aptamil Pronutra 3, followed by the formula milk named Hipp Organic 3 that caused some porosity on the enamel surface. In the SEM images, the image in which the integrity of the enamel structure was best preserved was observed in the tooth sample exposed to cow's milk. In a similar study including the results of SEM analysis of the tooth surfaces after exposure to formula milk, breast milk, saliva and saline materials, similarly the roughest surface was observed in the formula milk group [5].

According to SEM-EDX results of the present study, the material causing the most Ca loss from deciduous tooth enamel was breast milk, followed by formula milk, but after exposure to cow's milk, the amount of Ca and P in deciduous tooth enamel increased. This result is consistent with a meta-analysis in the literature reporting that the cariogenic potential of breast milk is higher than that of cow's milk [20]. It also overlaps with the opinion that cow's milk is protective against dental caries and has more mineral content than breast milk [14, 15], however it is inconsistent with the finding that formula milk causes more inorganic substance loss than breast milk [5].

Liquids containing lactose can cause sudden decreases in oral pH and thus create an environment prone to caries. However, milk is a complex structure consisting of calcium, phosphorus, protein, vitamins and fats as well as lactose [24]. It has been reported that breast milk has lower mineral content, higher lactose level (7 versus 3%) and lower protein content (1.2 g/100 mL versus 3.3 g/100 mL) compared to cow's milk, but these content differences are not indicative of their cariogenic potential [25]. In this study, not only Ca and P levels decrease in tooth samples exposed to breast milk, but also more microstructural deterioration was observed compared to those exposed with cow's milk. Therefore, the data suggest that the content differences between breast milk and cow's milk directly affect their demineralizing potential.

Formula milk, which is another baby bottle feeding material, can be a cause of tooth decay due to various carbohydrates in it [8]. It has been stated that formula milk taken with a bottle during sleep at night, remains stagnant in the mouth due to low saliva flow and suckle-sleep-suckle cycle, and this causes the enzymatic deterioration of the casein which is the protective protein in its structure [26]. In this research, as a result of SEM analysis, Aptamil Pronutra 3 and Hipp Organic 3 material caused prominent roughness on the surface of the primary teeth, and as a result of the EDX analysis, both materials caused a significant loss of Ca and also a significant decrease in the amount of P on the surfaces of the deciduous teeth exposed to Aptamil Pronutra 3. In a study examining the decrease in plaque pH caused by infant formula milk, it was reported that the formula milk named Aptamil Pronutra 2 was one of the formula milks that caused the most decrease in plaque pH [23]. Researchers stated that this may be due to the lower fat content of this material compared to others [23].

Energy Dispersive X-ray Spectroscopy used in our research is an analytical technique used in conjunction with SEM to perform elemental analysis or examine the chemical characterization of a sample [27]. The principle of operation in this system is the emission of energy in the form of X-ray photons when electrons from external sources collide with atoms of the material. Thus, the X-ray characteristic of each element is formed. The characterization capabilities of this system are mainly due to the unique atomic structure of each element, which allows for the formation of peaks in the X-ray spectrum [28]. In this method, the relative number of X-rays detected by the energy spectrum is obtained and the quantitative determination of the elements can be made using a computer-based program [27, 28].

The milk materials examined in our research are indispensable for infant nutrition. For this reason, it is not possible to give up their consumption in order to prevent dental caries. However, it is not impossible to be protected from the cariogenic effects of these indispensable nutrients. Thanks to such in-vitro studies, it is revealed that nutritional materials such as formula milk and breast milk are not innocent in terms of causing dental caries and the awareness of parents and dentists on this issue should be increased. Dental caries can be prevented with a few simple precautions and lifestyle changes in infants who continue breast-feeding or bottle-feeding with formula milk. In the current guidelines of the AAPD, it is stated that brushing teeth with smear amount of fluoride toothpaste prevents dental caries in children aged 0-3 [29]. Due to the decrease in the flow rate of saliva and thus the protective effect of saliva from dental caries during sleep at night, especially after 1 year of age cessation of feeding during sleep at night is also effective in protecting from early childhood caries [30]. Present results were showing that cow's milk can play a protective role against dental caries, but wrong dietary habits such as adding refined

sugar, honey or various sweeteners into cow's milk drunk in early childhood may negatively change the cariogenic potential of cow's milk.

As a result of this in-vitro study, formula milks can cause deterioration in the microstructure of the enamel of the primary teeth. Breast milk and the examined formula milks cause a significant decrease in Ca amounts in primary tooth enamel, while exposure to cow's milk causes a significant increase in Ca and P amounts.

Conflict of interest: No conflict of interest was declared by the authors.

References

1. Ripa LW. Nursing caries: a comprehensive review. Pediatr Dent 1988;10:268-282.
2. Erickson PR, Mazhari E. Investigation of the role of human breast milk in caries development. Pediatr Dent 1999;21:86-90.
3. Mazhari F, Talebi M, Zoghi, M. Prevalence of early childhood caries and its risk factors in 6-60 months old children in Quchan. Dent Res J 2008;4:96-101.
4. Schroth RJ, Halchuk S, Star L. Prevalence and risk factors of caregiver reported severe early childhood caries in Manitoba First Nations children: results from the RHS Phase 2 (2008-2010). Int J Circumpolar Health 2013;72:21167. https://doi.org/10.3402/ijch.v72i0.21167
5. Lee MR, Lee CJ, Park JH. The Roughness & micro-hardness on the deciduous teeth according to formula milk or human milk. Int J Clin Prev Dent 2011;7:179-188.
6. Birkhed D, Ohlsson A, Svensson C, Edwardsson S, Imfeld T. Milk and lactose-acid production in human dental plaque. J Dent Res 1981;60:1245.
7. Bowen WH, Lawrence RA. Comparison of the cariogenicity of cola, honey, cow milk, human milk, and sucrose. Pediatrics 2005;116:921-926. https://doi.org/10.1542/peds.2004-2462
8. Martin CR, Ling PR, Blackburn GL. Review of infant feeding: key features of breast milk and infant formula. Nutrients 2016;8:279. https://doi.org/10.3390/nu8050279
9. Moynihan PJ, Wright WG, Walton AG. A comparison of the relative acidogenic potential of infant milk and soya infant formula: a plaque pH study. Int J Paediatr Dent 1996;6:177-181. https://doi.org/10.1111/j.1365-263X.1996.tb00238.x
10. Grenby TH, Andrews AT, Mistry M, Williams RJH. Dental caries-protective agents in milk and milk products: investigations in vitro. J Dent 2001;29:83-92. https://doi.org/10.1016/S0300-5712(00)00061-0
11. Danchaivijitr A, Nakornchai S, Thaweeboon B, Leelataweewud P, Phonghanyudh A, Kiatprajak C. The effect of different milk formulas on dental plaque pH. Int J Paediatr Dent 2006;16:192-198. https://doi.org/10.1111/j.1365-263X.2006.00722.x
12. Chaudhary SD, Chaudhary M, Singh A, Kunte S. An assessment of the cariogenicity of commonly used infant milk formulae using microbiological and biochemical methods. Int J Dent 2011;320798. https://doi.org/10.1155/2011/320798
13. Ballard O, Morrow AL. Human milk composition: nutrients and bioactive factors. Pediatr Clin North Am 2013;60:49-74. http://dx.doi.org/10.1016/j.pcl.2012.10.002
14. Rugg Gunn AJ, Roberts GJ, Wright WG. Effect of human milk on plaque pH in situ and enamel dissolution in vitro compared with bovine milk, lactose, and sucrose. Caries Res 1985;19:327-334. https://doi.org/10.1159/000260863
15. Bowen WH, Pearson SK. Effect of milk on cariogenesis. Caries Res 1993;27:461-466. https://doi.org/10.1159/000261581
16. Rollema HS. Casein association and micell formation. In Advanced Dairy Chemistry: Proteins (ed. Fox PF). London: Elsevier Applied Science, 1992;1:111-140.
17. Reynolds EC. The prevention of sub-surface demineralization of bovine enamel and change in plaque composition by casein in an intra-oral model. J Dent Res 1987;66:1120-1127. https://doi.org/10.1177/00220345870660060601
18. Peres RCR, Coppi LC, Volpato MC, Groppo FC, Cury JA, Rosalen PL. Cariogenic potential of cows', human and infant formula milks and effect of fluoride supplementation. Br J Nutr 2009;101:376-382. https://doi.org/10.1017/S0007114508020734
19. American Academy of Pediatrics. Breastfeeding and the use of human milk. Work Group on Breastfeeding. Pediatrics 1997;100:1035-1039. https://doi.org/10.1542/peds.100.6.1035
20. Aarthi J, Muthu MS, Sujatha S. Cariogenic potential of milk and infant formulas: a systematic review. Eur Arch Paediatr Dent 2013;14:289-300. https://doi.org/10.1007/s40368-013-0088-6
21. McKnight Hanes C, Whitford GM. Fluoride release from three glass ionomer materials and the effects of varnishing with or without finishing. Caries Res 1992;26:345-350. https://doi.org/10.1159/000261466
22. Amaechi BT, Higham SM, Edgar WM. Factors influencing the development of dental erosion in vitro: enamel type, temperature and exposure time. J Oral Rehabil 1999;26:624-630. https://doi.org/10.1046/j.1365-2842.1999.00433.x

23. Nozari A, Ghaderi F, Madadelahi S. pH-value decrement of saliva-plaque solution after mixing with four customary used infant milk formulas: an in vitro study. J Pediatr Dent 2014;2:92-95. https://doi.org/10.4103/2321-6646.145583
24. Peres RC, Coppi LC, Franco EM, Volpato MC, Groppo FC, Rosalen PL. Cariogenicity of different types of milk: an experimental study using animal model. Braz Dent J 2002;13:27-32.
25. Seow WK. Biological mechanisms of early childhood caries. Community Dent Oral Epidemiol 1998;26:8-27. https://doi.org/10.1111/j.1600-0528.1998.tb02090.x
26. Erickson PR, McClintock KL, Green N, LaFleur J. Estimation of the caries-related risk associated with infant formulas. Pediatr Dent 1998;20:395-403.
27. Mejia Olea O, Contreras Bulnes R, Zamudio Ortega RM, Morales Luckie RM, Olea Cardoso O, López Castañares R. Scanning electron microscopy and energy dispersive spectroscopy microanalysis applied to human dental specimens under laser irradiation for caries prevention. Formatex 2014:70-77.
28. Asaka Y, Miyazaki M, Aboshi H, et al. EDX fluorescence analysis and SEM observations of resin composites. J Oral Sci 2004;46:143-148. https://doi.org/10.2334/josnusd.46.143
29. American Academy of Pediatric Dentistry.Fluoride therapy. The Reference Manual of Pediatric Dentistry. Chicago, Ill: American Academy of Pediatric Dentistry 2021:302-305.
30. Branger B, Camelot F, Droz D, et al. Breastfeeding and early childhood caries. Review of the literature, recommendations, and prevention. Arch Pediatr 2019;26:497-503. https://doi.org/10.1016/j.arcped.2019.10.004

Acknowledgments

Lots of thanks to Asst. Prof. Dr. Berna Kavakcioglu Yardimci from Pamukkale University, Faculty of Science, Department of Biochemistry for the preparation of artificial saliva used in the experiments.

Funding: This research is supported by Pamukkale University Scientific Research Projects Department (project number: 2020BSP008).

Ethics committee approval: Permission was obtained from Pamukkale University Non-Interventional Clinical Research Ethics Committee for the study (permission date: March 03, 2020, number:5).

Authors' contributions to the article

C.C.E. constructed the main idea and hypothesis of the study. C.C.E. developed the theory and arranged/edited the material and method section. C.C.E. has done the evaluation of the data in the Results section. Discussion section of the article was written by C.C.E. and C.C.E. reviewed, corrected and approved the final version.