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Original Article

The effects of fermented milk intake on the enamel surface

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ABSTRACT

Objectives: The aim of this study was to evaluate the extent of the potential erosion of enamel induced by three different types of commercial fermented milk using the pH cycle model. **Methods:** Specimens were treated and soaked up in three types of fermented milk and in mineral water for 10 min, four times a day for 8 days, and all of the specimens were immersed in artificial saliva outside of treatment times. The microhardness of the surface was measured by a microhardness tester, and a scanning electron microscope (SEM) was used to identify the enamel surface morphology. **Results:** The differences in the surface microhardness (ΔVHN) of enamel were different among the groups ($p<0.05$). The four groups were in descending order of ΔVHN: the liquid type group, condensed-drink type group, condensed-stirred type group, and control group. The liquid type group had a higher ΔVHN than the other two fermented milk groups ($p<0.05$). Based on SEM observation, the most severe surface damage was due to the liquid type of fermented milk. **Conclusions:** Customers' careful discretion is advised when purchasing these types of fermented milk. This information is anticipated to be of much value in the prevention of dental erosion.

Key Words: Dental erosion, Fermented milk

Introduction

Lactic acid bacteria refer to all bacteria that produce lactic acid as a metabolite. Lactic acid bacteria exhibits beneficial effects on the human body, such as intestinal regulation, immunity reinforcement, improvement in hepatic cirrhosis, anticancer activity, cholesterol reduction, and skin enhancement [1]. Such effects were first acknowledged when Elie Metchinikoff attributed the longevity of Bulgarian people to fermented milk intake and proposed the respective theory in the early 20th century, after the discovery of lactic acid bacteria in the 19th century by Louis Pasteur. Numerous effects of fermented milk with lactic acid bacteria were subsequently identified, and with the known nutritional values of milk, the global consumption of fermented milk has increased [2].

However, despite these beneficial effects, the low pH of fermented milk may cause dental erosion [3], a condition induced by the chemical reaction mediated by acid, not caused by bacteria, to lead to the loss of dental hard tissue [4]. The condition is caused by biological factors, such as saliva, acquired pellicle, tooth structure, soft tissue, and tongue position; behavioral factors, such as eating and drinking habits, a decrease in saliva secretion, and excessive habits to maintain oral hygiene; and chemical factors, such as the intake of acidic foods, including fermented milk [5]. Pereira et al. [6] investigated the factors influencing dental erosion in preschool children and showed that the intake of acidic foods led to a 2.74-fold higher rate of dental erosion. Korkmaz & Kaptan [7] also reported dental erosion in 21.8% of students aged 7-14 years in association with the intake of sodas, fruit juices, and fruit yogurts.

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Among the overseas studies regarding dental erosion in relation to fermented milk intake, Lodi et al. [8] conducted an *in situ* study to show that fermented milk caused demineralization of bovine tooth enamel by lowering the pH of the biofilm, and Pimentel Lopes De Oliveira et al. [9] showed a decrease in the enamel surface microhardness after tooth exposure to fermented milk. Studies conducted in South Korea also reported a decrease in the enamel surface microhardness of deciduous teeth using liquid-type fermented milk [10] and bovine teeth [11].

In South Korea, fermented milk is divided into liquid and condensed types depending on the solid-not-fat content, and the condensed type is further categorized into the stirred type and drink type [2]. Based on this, a previous study selected one representative product for each type of fermented milk and evaluated the risk of dental erosion [12]. The results showed that the highest risk was from liquid-type fermented milk, while the two type of condensed-type fermented milk shared similar risks. However, as the study did not take into account the influence of saliva and simply compared the risks in accordance with the time of immersion, a follow-up study seemed necessary to investigate the influence of saliva, whose role in the oral cavity includes not only self-purification, buffering, and antimicrobial activity, but also enamel demineralization and remineralization [13].

Thus, this study aimed to determine the effects of the intake of commercially available liquid type, condensed-stirred type, and condensed-drink type fermented milk on the enamel surface and the related risk of dental erosion. For this, the enamel surface microhardness was measured using the process of the pH cycle with artificial saliva, and scanning electron microscopy (SEM) was performed.

Methods

1. Materials

1) Samples

A representative product of each of the liquid, condensed-stirred, and condensed-drink types of fermented milk was selected based on the mean pH that approximated to the pH of each product type reported in previous studies, including that of Ko et al. [3] <Table 1>. Mineral water was used as the control, and all of the samples were kept in refrigeration for subsequent use.

2) Bovine tooth

A permanent incisor was obtained from the frozen bovine upper jaw. For disinfection and sterilization, the tooth was immersed in 0.1% thymol until specimen preparation.

Table 1. Materials used in the experiment

Classification	Group	Brand name	Manufacture
Control	Mineral water	Jeju SamDaSoo	Jeju Special Self-Govering Province Development Corp
Fermented milk	Liquid	Enyo Applecarrot	Maeil
	Condense - stirred	Super100 Premium Blueberry	Korea Yakult
	Condense - drink	Wiepeonhan Gut	Maeil

2. Methods

1) pH and titratable acidity

Using 20 mL of the samples, the pH and titratable acidity were measured. For titratable acidity, the required volumes of 1 M NaOH to reach pH 5.5 and pH 7.0 were measured. For each measurement, a pH meter (3-star; Thermo Orion, Beverly, CA, USA) was used, while each sample was stirred at 200 rpm, and the mean of triplicate measurements was estimated.

2) Specimen preparation

From the surface of a sound bovine tooth without dental caries or cracks, a 3 mm diameter cylindrical enamel sample was obtained. After embedding the specimen in an acrylic rod using acrylic resin, the specimens were polished in sequence using #60, #240, #600, and ɤ alumina oxide sand paper (Carbimet, Buehler, Illinois, USA). The required number of specimens for each experimental group was determined based on the values reported in a previous study regarding dental erosion and fermented milk intake [11] using the G*power 3.1.3 program. The testing power was 100% for 12 specimens in each group. Hence, a total of 48 specimens were selected for use across the four groups in this study.

3) Surface microhardness measurement

The surface microhardness was measured as the Vickers hardness number (VHN) for 0.5 mm towards the interior from the surface at the top, bottom, left, and right of the enamel. A microhardness tester was used (Fm-7; Future-tech Corp., Kanagawa, Japan), while the enamel surface was positioned perpendicular to the tester, and pressure was applied at 200 g weight for 10 s. The diameter was measured under 400× magnification. The specimens with 280–310 VHN were selected for subsequent analyses.

4) Cycle treatment of experimental materials and artificial saliva

For the cycle treatment of experimental materials and artificial saliva, the methods described in the study by Yoon et al. [14] were used, whereby the specimens were immersed in 20 mL of experimental samples for 10 min with 2 h intervals four times a day over a period of 8 days, while all of the specimens were immersed in artificial saliva when not being treated [14] <Table 2>. The artificial saliva was prepared by mixing the reagents $CaCl_2 \cdot 2H_2O$ (0.0213%), gastric mucin (0.22%), NaCl (0.038%), KH_2PO_4 (0.0738%), and KCl (0.1114%) to distilled water, and adjusting the pH to 7.0 using NaOH. The immersion in artificial saliva was performed in an incubator set at 36.5℃ to reproduce the oral cavity temperature, and stirring was performed to prevent precipitation.

Table 2. Time table of cycle for 8 days

Time	Treatment
12:30 - 12:40	Treatment solution (experiment material)
12:40 - 14:30	Remineralization (artificial saliva)
14:30 - 14:40	Treatment solution (experiment material)
14:40 - 16:30	Remineralization (artificial saliva)
16:30 - 16:40	Treatment solution (experiment material)
16:40 - 18:30	Remineralization (artificial saliva)
18:30 - 18:40	Treatment solution (experiment material)
18:40 - 08:30	Remineralization (artificial saliva)
08:30 - 12:30	Measurement (surface microhardness)

5) Post-treatment evaluation

(1) Surface microhardness measurement

During the process of cycle treatment, the surface microhardness was measured each day to examine the changes over 8 days. The surface microhardness was measured at a point 0.1 mm away from the previously measured point and toward the center from the four initial measurement points. The measured values were analyzed based on the difference between the initial and final (day 8) values.

(2) Enamel surface morphology

After the final measurement of the surface microhardness, a representative specimen was selected for each group for the morphological observation. The selected specimen was dried at 60℃ for 2 days, and fixed to the copper plate to be platinum-coated in a vacuum state. Using a SEM (FE-SEM; S-4700; Hitachi, Tokyo, Japan) under 15 kV, the enamel surface morphology was studied at 50,000× and 100,000× magnification.

6) Data analysis

To analyze the between-group differences in the pH and titratable acidity of experimental samples, nonparametric methods, specifically the Kruskal-Wallis test and Mann-Whitney U test, were used. The data of the surface microhardness satisfied the normality and distribution homogeneity; thus, the analysis used the paired t-test and one way analysis of variance as the parametric methods. Tukey test was used as a post-hoc test. Statistical analyses were performed using the Statistical Packages for Social Science 21.0 (IBM Corp., Armonk, NY, USA).

Results

1. pH and titratable acidity

The fermented milk samples used in this study varied in pH. The mean pH for the three different fermented milk types was 3.92±0.32, which was lower than that of the control. Among the three types, the pH of the liquid type was the lowest. A significant between-group difference in pH was found for the three types ($p<0.05$).

At a pH of 5.5, the titratable acidity did not differ between the liquid and condensed-drink types ($p>0.05$), but the other types showed variations. At a pH of 7.0, the three fermented milk types varied significantly ($p<0.05$). Among the three types, the condensed-stirred type had the highest titratable acidity to necessitate a greater amount of NaOH to increase pH <Table 3>.

Table 3. The pH and titratable acidity of experiment materials

Unit : Mean±SD

Group	pH	p^*	Titratable acidity (mL)*			
			pH 5.5	p^*	pH 7.0	p^*
Control	7.66 ± 0.01[d]	0.015	-	0.034	-	0.026
Liquid type	3.47 ± 0.02[a]		1.02 ± 0.02[a]		1.23 ± 0.02[a]	
Condense - stirred type	4.09 ± 0.01[b]		1.18 ± 0.02[b]		2.02 ± 0.06[c]	
Condense - drink type	4.19 ± 0.01[c]		0.95 ± 0.04[a]		1.70 ± 0.04[b]	

*by Kruskal-Wallis test

[a,b,c,d] The same letter indicates no significant difference by Mann-Whitney test

2. Enamel surface microhardness

The enamel surface microhardness of each specimen after the 8 days treatment was reduced in all of the groups except the control group ($p<0.05$). The changes in enamel surface microhardness (ΔVHN) after 8 days indicated significant between-group variations ($p<0.001$) <Fig. 1>. Among the three fermented milk types, the liquid type showed the largest ΔVHN, while the other two types did not significantly differ in ΔVHN ($p>0.05$) <Table 4>.

Table 4. Differences in surface microhardness after treatment for 8 days

Unit : Mean±SD

Group	N	Time			Difference	p^{**}
		Before (0 day)	After (8 days)	p^*		
Control	12	291.78 ± 7.87	290.76 ± 9.17	0.523	-1.02 ± 5.35[a]	0.001
Liquid type	12	291.89 ± 7.80	199.15 ± 12.37	< 0.001	-92.75 ± 11.23[c]	
Condense - stirred type	12	292.02 ± 7.92	280.13 ± 8.27	< 0.001	-11.89 ± 5.21[b]	
Condense - drink type	12	291.60 ± 7.77	274.00 ± 8.01	< 0.001	-17.60 ± 7.13[b]	

*by paired t-test, **by one-way ANOVA

[a,b,c] The same letter indicates no significant difference by Tukey test at α = 0.05

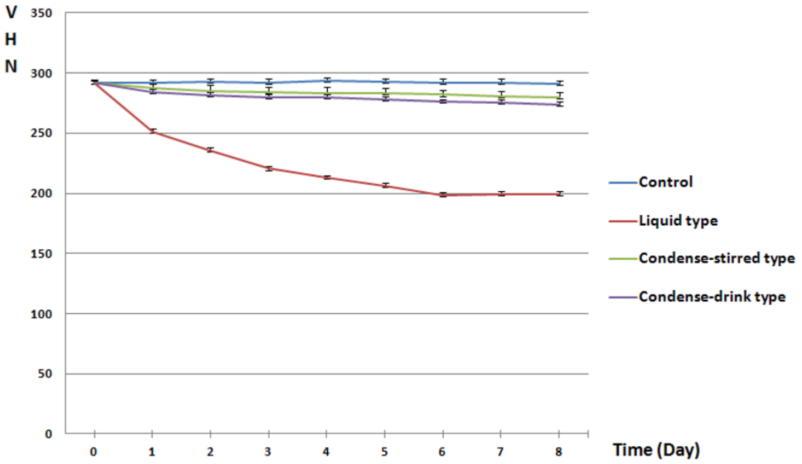

Fig. 1. Changes in enamel surface hardness (VHN) over exposure time to the experiment materials

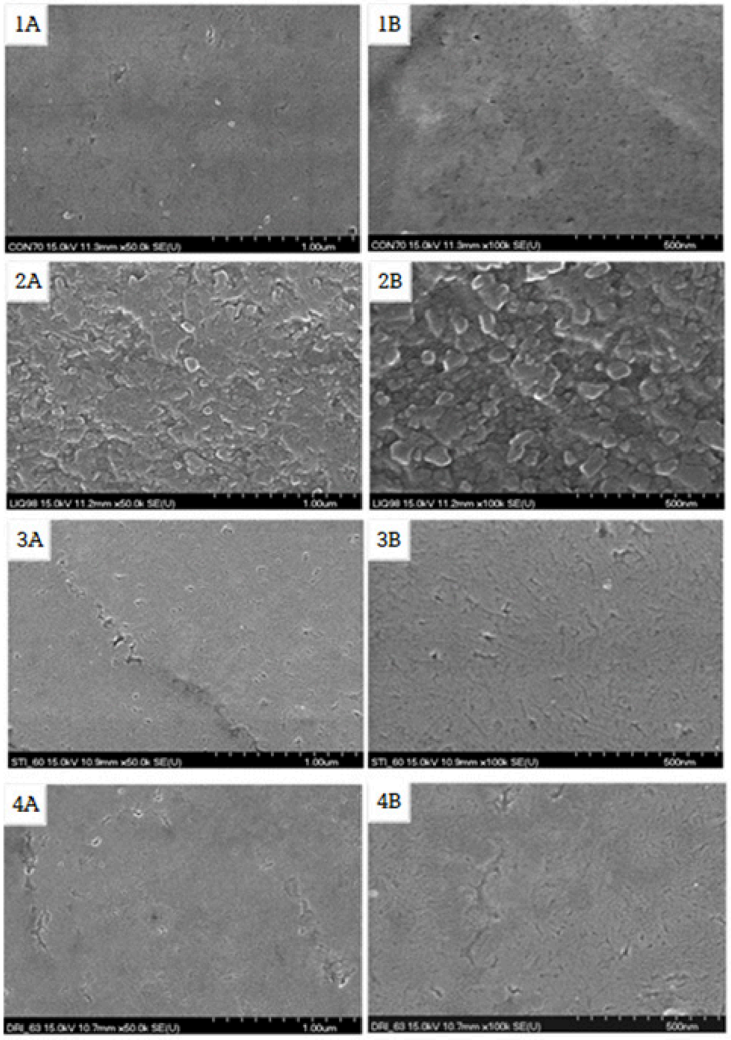

Fig. 2. SEM image of enamel surface after treatment on enamel (1: Control, 2: Liquid type, 3: Condense-stirred, 4: Condense-drink, A: × 50,000, B: × 100,000)

3. Enamel surface morphology

Compared to the smooth surface of the control group, the liquid-type group had rough surfaces with irregular crystals that suggested considerable damage to the enamel surface, while the other two groups had subtle damage observed under 100,000× magnification as surface cracks <Fig. 2>.

Discussion

Dental erosion is regarded as an important cause of dental structural loss in children, adolescents, and adults [15]. This study selected fermented milk as a functional food product consumed across all age groups and evaluated the risk of dental erosion by reproducing the conditions of the oral cavity.

The mean pH of the fermented milk types used in this study was 3.92±0.32 with a high level of acidity that predicted a high risk of dental erosion. Grenby et al. [16] claimed that titratable acidity, along with pH, was a critical indicator of the risk of dental erosion. In this study, the titratable acidity measured alongside pH indicated that among the three fermented milk types, the condensed-stirred type required the greatest amount of NaOH to reach pH 5.5 and pH 7.0, showing the highest titratable acidity. This may be due to the viscosity of this type being higher than those of the other two types, making the dilution by NaOH more difficult than in liquid states. Kargul et al. [17] claimed that for fruit yogurt, the fruit type was a determinant of the titratable acidity, and based on this, further studies should investigate the effects of blueberry in the condensed-stirred fermented milk used in this study and the general influence of fruit types on titratable acidity.

The surface microhardness measured to determine the risk of dental erosion after the process of the pH cycle treatment with artificial saliva showed that while the control group did not change significantly, the three fermented milk samples exhibited a decrease in the surface microhardness as the demineralization effect of the fermented milk was greater than the remineralization effect of the artificial saliva, thus predicting a higher risk of dental erosion upon long-term intake. The liquid type, in particular, led to the greatest loss of minerals on the first day of the pH cycle treatment, while the surface microhardness steadily decreased until day 6 of treatment, ceasing to change afterward. The liquid type also exhibited damage to the surface morphology with a considerable reduction in surface microhardness in comparison to the other two types. This is thought to be due to the lower pH of the liquid type than those of the other two rather than the level of lactic acid bacteria, as all three fermented milk types contained over 0.1 billion LAB/mL. Lussi et al. [4] reported that pH was the most significant determinant of the risk of dental erosion in beverages. Rios et al. [18] also reported that the addition of a base to increase the pH of sodas led to the reduced risk of dental erosion. Based on this, the pH of fermented milk should be increased. Thus, further studies should investigate the suitable pH that prevents dental erosion but preserves the taste.

As the lactic acid bacteria strains varied across the three fermented milk products used in this study, the follow-up study should be a complement in the microbiological approach. In addition, in comparing the two condensed types, the condensed-drink type showed a greater level of reduction in surface microhardness. This may be because, despite similar pH levels, the two types differed in physical appearance. The drink-type could be immediately absorbed by the tooth, while the stirred-type with a higher viscosity likely formed a film on the tooth surface for slower absorption. In this study, the washing conditions were kept identical for all specimens after the immersion in samples; I.e., 30 s washing with distilled water in flow and subsequent immersion in artificial saliva. However, foods with high viscosity could be retained in the oral cavity for a long time to slow down the buffering effect of saliva, and based on this, such retention time in the oral cavity should be taken into account in setting the time of washing for different types of fermented milk, which may then cause a greater reduction in surface microhardness for the condensed-stirred type with its higher viscosity. While this may be useful for comparisons, the incomplete reproduction of the oral cavity posed a limitation.

Another limitation in this study was the reduction in surface microhardness of the bovine tooth prior to their use in the experiments. First, although the measurement of surface microhardness is considered highly suitable for locating the lesions within 50 μm of tooth structure [19], the measurement has to be taken on a flat and smooth surface, and the consequent polishing might have removed the outermost layer of the surface; thus, the impact of erosion could have been greater in observation than in reality. In addition, the bovine tooth was stored in 0.1% thymol for disinfection and sterilization until use, and according to Jeong et al. [20], storing the extracted bovine tooth in 0.1% thymol for 25 days alone reduced 29.6% of the enamel surface microhardness. In this study, likewise, the storage process may have caused a loss of the surface microhardness of the bovine tooth.

As teeth in the oral cavity are maintained in a moist state by saliva, the tooth specimens in this study were also immersed in artificial saliva in an incubator set at 36.5℃ to reproduce conditions similar to those in the oral cavity at all times outside of treatment. Although the use of human saliva would have allowed the reproduction of more similar oral cavity conditions, the use of saliva from different individuals could have affected the results as a variable. In addition, the artificial saliva selected in this study was the one mentioned by Jeong et al. [21] to have the most similar remineralization effects to human saliva. The artificial saliva in an identical condition was used at all times in the present study. However, while human saliva is constantly renewed in the oral cavity, the artificial saliva in this study was replaced only twice a day; thus, the minerals in stagnated artificial saliva might have been engaged in continuous ion exchange with the tooth after demineralization, displaying less minerals than that contained in human saliva.

Several overseas studies also reported that the use of artificial saliva throughout the cycle treatment did not facilitate dental erosion for fermented milk. The cycle treatment in those studies differed from the one in this study: Lodi et al. [22] applied the process of 5 min immersion in fermented milk and 15 min immersion in artificial saliva with four repetitions; Wongkhantee et al. [23] applied the process of 5 s immersion in fermented milk and 5 s immersion in artificial saliva with ten repetitions. The results may thus be dependent on the relatively short time of immersion via 50 s and 20 min exposure to fermented milk. In the case of fermented milk, the fact that it is a health functional food poses means that it is made for regular intake rather than single consumption. Thus, this study applied the process of an 8 days cycle treatment to determine the effects of a long-term intake of fermented milk on the tooth surface.

Liquid type fermented milk in South Korea was first produced in 1971, while the condensed-stirred type was first produced in 1981 and the condensed-drink type was first produced in 1990 [2]. The product has so far been abundantly used as a functional food for intestinal health. Such health-beneficial foods cannot be prohibited solely for the purpose of preventing dental erosion; thus, developing and imparting education regarding methods to minimize dental erosion are of significant importance. Tahmassebi & Duggal reported that the use of straws while drinking fruit juice most effectively minimized enamel demineralization [24]. Thus, consumers of fermented milk should use a straw to drink the liquid and condensed-drink types, taking care not to expose the teeth to the fermented milk. In the case of the condensed-stirred type, a spoon should be used to minimize contact with teeth, while the food should be rapidly ingested to allow sufficient time for remineralization. Moreover, as the addition of calcium, phosphorous, or fluorides to an acidic drink was found to have a preventive effect on dental erosion [25,26], using an additive to prevent dental erosion may be considered in the production of fermented milk.

The introduction of fermented milk to consumers was presumed to have increased the incidence of dental erosion, especially among children [27]. Caglar et al. [28] reported that dental erosion was observed in 36% of children with a fruit yogurt intake of 2.68±2.07 times a week. In the case of children, the enamel structure, especially that of newly formed teeth, is still immature, making them highly susceptible to acidic conditions [29]. Thus, based on the findings in this study, it is hoped that children, parents, and educational personnel would be informed of the risk of dental erosion in fermented milk and the individual effects of different fermented milk types on the tooth surface, so as to prevent high-risk dental erosion cases. Furthermore, continuous studies should be conducted regarding the ways to prevent dental erosion.

Conclusions

This study investigated the effects of the liquid, condensed-stirred, and condensed-drink types of fermented milk on the enamel surface. To this end, pH cycle treatment using artificial saliva was used and subsequently, the surface microhardness was measured and the surface morphology was observed via SEM. The findings in this study were as follows:

1. The pH of fermented milk was the lowest for the liquid type, and the titratable acidity was the highest for the condensed-stirred type in both pH 5.5 and pH 7.0.

2. The difference in surface microhardness (ΔVHN) before and after the 8 days pH cycle treatment using artificial saliva and the experimental materials was the highest for the liquid-type (-92.75±11.23), followed by the condensed-drink type (-17.60±7.13), condensed-stirred type (-11.89±5.21), and control (-1.02±5.35), with significant between-group differences ($p<0.05$). All three types of fermented milk exhibited a large ΔVHN than that of the control. The liquid type showed the largest ΔVHN compared to the other two types ($p<0.05$), which did not show a significant ΔVHN ($p>0.05$).

3. The enamel surface microhardness observed via SEM confirmed that the liquid type caused the most severe surface damage, while the other two types caused more surface cracks than did the control, indicating subtle damage to the enamel surface.

Based on the findings, the intake of fermented milk was shown to influence the enamel surface based on the physical properties and pH. It is thus hoped that high-risk individuals will be informed of the risk of dental erosion in fermented milk to enable them to consider their intake of fermented milk. Moreover, further studies should be conducted on more methods to prevent dental erosion.

Conflicts of Interest

The authors declared no conflict of interest.

Authorship

Conceptualization: KH Kim, CH Choi; Data collection: KH Kim; Formal analysis: CH Choi; Writing - original draft: KH Kim, CH Choi; Writing - review & editing: KH Kim, CH Choi

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유산균 발효유 섭취가 법랑질 표면에 미치는 영향

초록

연구목적: 본 연구는 액상발효유, 스터드 타입 및 드링크 타입 농후발효유가 정상법랑질 표면에 미치는 영향을 알아보고자 하였다. **연구방법:** 3종의 유산균 발효유와 대조군인 제주삼다수에 우치시편을 8일 동안 매일 10분씩 4회 처리하고 그 외의 시간에는 인공타액에 침지하는 pH 순환처리를 시행한 후 표면미세경도 측정과 주사전자현미경을 이용하여 표면 형태를 관찰하였다. **연구결과:** 3종의 유산균 발효유군 모두 대조군에 비해 표면경도차(ΔVHN)가 크게 나타났다. 이 중 액상발효유군은 표면경도차(ΔVHN)가 가장 크게 나타났으며, 주사전자현미경에 의한 법랑질 표면 형태 관찰에서도 가장 심한 표면 손상이 확인되었다. **결론:** 유산균 발효유 종류에 따른 물리적 성상과 pH가 치아표면에 영향을 미치는 것으로 나타났다. 따라서 유산균 발효유의 치아부식증 발생 가능성을 고위험군에게 알림으로써 섭취 시 이를 고려하도록 하며, 치아부식증을 줄일 수 있는 예방적 연구가 계속적으로 필요하리라 생각된다.

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