

Evaluation of some properties of fermented milk beverages that affect the demineralization of dental enamel

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Abstract: The aim of this *in vitro* study was to evaluate the erosive capacity of fermented milk beverages, as well as some of their properties that affect the demineralization of dental enamel (pH, buffering capacity, fluoride, calcium and phosphorus contents). Three different batches of 6 commercial brands of fermented milk beverages were analyzed. pH evaluation was accomplished using a potentiometer. The buffering capacity was measured by adding 1 mol L⁻¹ NaOH. Fluoride concentration was assessed by an ion specific electrode after hexamethyldisiloxane-facilitated diffusion, and calcium and phosphorus concentrations were assessed by a colorimetric test using a spectrophotometer. Sixty specimens of bovine enamel were randomly assigned to 6 groups (n = 10). They were exposed to 4 cycles of demineralization in the fermented milk and remineralization in artificial saliva. Enamel mineral loss was determined by surface microhardness (%SMHC) and profilometric tests. The samples' pH ranged from 3.51 to 3.87; the buffering capacity ranged from 470.8 to 804.2 µl of 1 mol L⁻¹ NaOH; the fluoride concentration ranged from 0.027 to 0.958 µgF/g; the calcium concentration ranged from 0.4788 to 0.8175 mgCa/g; and the phosphorus concentration ranged from 0.2662 to 0.5043 mgP/g. The %SMHC ranged from -41.0 to -29.4. The enamel wear ranged from 0.15 µm to 0.18 µm. In this *in vitro* study, the fermented milk beverages did not promote erosion of the dental enamel, but rather only a superficial mineral loss.

Descriptors: Fermentation; Probiotics; Dental caries; Dental enamel; Demineralization.

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Introduction

In the last decades, the supply of fermented milk beverages has been growing in the Brazilian market because their consumption has been stimulated due to the alleged benefits of probiotics to the general health.¹ Probiotics are defined as live microorganisms that, when administered in adequate amounts, are beneficial to the host.^{1,2}

Most fermented milk beverages present an acidic pH, and contain sugar and *Lactobacillus* or *Bifidobacterium* that produce organic acids. These characteristics can facilitate the adherence of *L. salivarius* to the dental surface and enhance the cariogenicity of *S. mutans*.² In the dental literature, a study by Shibata *et al.*³ (1977) was found on the cariogenic potential of fermented milk. The authors observed the effects of two fer-

mented milk beverages, “Yakult” and “Calpis”, on the development of caries in hamsters and mice. The results indicated that the continuous administration of the beverages promoted caries lesions in the animals. In addition, the induction of erosive lesions could be observed in the enamel of molars.³ On the other hand, due to their derivation from milk, fermented milk beverages present calcium and phosphate in their mineral composition, and the simple increment of those minerals in the acidic beverages reduces the erosive effect of those substances.⁴

The influence of ions on the erosive effect of beverages has been the aim of several studies. Calcium and phosphate contents have a protective effect in yogurt, which has a low pH (~4), and yet it has no erosive potential.⁵ Several properties related to soft drinks have an impact on their erosive potential on teeth after long exposure times, and the addition of calcium and phosphate considerably decreased their erosive potential.⁶ Calcium was found to be effective in reducing the erosive potential of most acidic beverages, while the effect of phosphorus and fluoride, in association with calcium, was less clear.⁷

In view of an increasing consumption of these products, the growing launch of children-oriented advertising campaigns (aiming at an age bracket with greater risk for the development of caries), and the lack of studies concerning the effects of the consumption of fermented milk beverages on the conditions of oral health, the aim of the present *in vitro* study was to evaluate the erosive capacity of fermented milk beverages and some of their properties that affect the demineralization of dental enamel (pH, buffering capacity, fluoride, calcium and phosphorus concentrations).

Material and Methods

Sample selection

Three batches of 6 different commercial brands of fermented milk beverages (A - Parmalat® Grape, - Parmalat, Carambeí, PR, Brazil; B - Chamyto® - Nestlé Brasil Ltda., Araras, SP, Brazil; C - Paulista® - Paulista, Poços de Caldas, MG, Brazil; D - Batavito® - Indústria de Alimentos Batávia S.A, Carambeí, PR, Brazil; E - Yakult® - Yakult S/A Indústria e Comércio, Lorena, SP, Brazil; and F - Vigor® - Vigor,

São Paulo, SP, Brazil) were purchased for analysis of their physicochemical properties.

pH analysis

The pH of the products was determined at room temperature using a pH electrode (2A14-KA Analyzer, São Paulo, SP, Brazil) coupled to a potentiometer (290A, Orion Research Inc., Boston, MA, USA).

Buffering capacity

Evaluation of the buffering capacity (BC) was accomplished using 5 ml of each product, which were stored in a plastic vial (J10 - Injeplast, São Paulo, SP, Brazil) to determine the initial pH. One mol L⁻¹ NaOH (Dinâmica Reagentes Analíticos, São Paulo, SP, Brazil) was added in 25 µl increments, until the pH reached 7.0.⁸

Fluoride analysis

Fluoride concentrations were determined after overnight hexamethyldisiloxane (HMDS)-facilitated diffusion according to the method described by Taves⁹ (1968) and modified by Whitford¹⁰ (1996), using a fluoride ion-specific electrode (model 9409, Thermo Electron Corporation, Beverly, MA, USA) and a miniature calomel reference electrode (Accumet #13-620-79 Fischer Scientific, Pittsburgh, PA, USA), coupled to a potentiometer (290A, Orion Research Inc., Boston, MA, EUA).

Calcium analysis

Calcium concentration was analyzed by a colorimetric test (Spectrophotometer - Hitachi Lts, Tokyo, Japan) using the cresolphthalein method (Bioclin® Kit, Belo Horizonte, MG, Brazil).

Phosphorus analysis

Phosphorus concentration was analyzed by the colorimetric test (Spectrophotometer - Hitachi Lts, Tokyo, Japan) according to the method described by Fiske, Subbarow¹¹ (1925).

Enamel block preparation and de-remineralization cycles

Enamel blocks measuring 4 x 4 x 2 mm were obtained from bovine incisor teeth previously stored in

2% formaldehyde solution (pH 7.0) for one month. They had their surface serially polished (Politriz APL-4, Arotec, Cotia, SP, Brazil) for surface microhardness determination (five indentations in different regions of the blocks, 25 g, 5 s, HMV-2000; Shimadzu Corporation, Tokyo, Japan).

Sixty blocks with a mean surface microhardness between 358.6 and 379.8 Knoop Hardness Number (KHN) were divided into 6 groups corresponding to each fermented milk beverage tested. In the first 24 hours, the blocks were immersed in artificial saliva. After that, they were subjected to four cycles of demineralization (15 ml of the fermented milk for 5 min) and remineralization (15 ml of artificial saliva for 60 min). After each treatment, the specimens were rinsed under deionized water.¹²

In order to maintain reference surfaces for lesion depth determination by profilometry, two layers of nail varnish were applied on half of the surface of each block.

Enamel block analysis

Enamel mineral loss was determined by surface microhardness (SMH)¹² and profilometric tests.

The nail varnish over the reference surfaces was carefully cleaned with acetone-soaked (Ace-Rio, Rioquímica, São José do Rio Preto, SP, Brazil) cotton wool. The blocks were dried, and the enamel wear (R) was determined in relation to the reference surface by profilometry, using a rugosimeter (T1000 Tester; Hommelwerke, VS-Schwenningen, Germany). On each specimen, 5 readings were performed by scanning from the reference to the exposed surface.

Statistical analysis

ANOVA and Tukey's tests were carried out for statistical analysis. The correlations between the percentage of superficial microhardness change (%SMHC) and the variables such as pH, buffering capacity, and the fluoride, calcium and phosphate contents in the fermented milk beverages were analyzed using Spearman's correlation coefficient. The significance level was set at 5%.

Results

The samples' pH ranged from 3.51 to 3.87 (Table 1).

The buffering capacity ranged from 470.8 to 804.2 μL of 1 mol L^{-1} NaOH (Table 1).

The fluoride concentration ranged from 0.027 to 0.958 $\mu\text{g F/g}$ and the phosphorus concentration ranged from 0.2662 to 0.5043 mg P/g (Table 1).

A direct relationship could be observed ($r = 0.9308$) with a significance of 0.0084 between pH and buffering capacity.

The calcium concentration ranged from 0.4788 to 0.8175 mg Ca/g (Table 2).

The %SMHC ranged from -41.0 to -29.4. There was significant difference between brands A, B and C (Table 3). Brands E and F also showed a statistically significant difference in relation to brands A and B.

The enamel wear (R) ranged from 0.15 μm to 0.18 μm , but the profilometric test was not capable of showing the presence of significant enamel wear in relation to the reference surface (Table 3).

The %SMHC (%SMHCxF: $r = 0.339$; $p = 0.510$; %SMHCxCa: $r = 0.901$; $p = 0.014$; %SMHCxP:

Table 1 - Mean and standard deviation of the pH, buffering capacity, fluoride concentration and phosphorus concentration of the three batches of the different commercial brands of fermented milk beverages.

Commercial Brand	pH	Buffering Capacity (μL)	Fluoride concentration ($\mu\text{gF/g}$)	Phosphorus concentration (mgP/g)
A	3.83 \pm 0.023	470.8 \pm 19.09	0.027 \pm 0.005	0.3286 \pm 0.015
B	3.65 \pm 0.006	620.8 \pm 19.09	0.246 \pm 0.023	0.5040 \pm 0.001
C	3.52 \pm 0.006	804.2 \pm 19.09	0.196 \pm 0.021	0.4939 \pm 0.003
D	3.87 \pm 0.015	570.8 \pm 7.22	0.029 \pm 0.001	0.5043 \pm 0.005
E	3.51 \pm 0.026	779.2 \pm 52.04	0.148 \pm 0.051	0.5029 \pm 0.003
F	3.54 \pm 0.127	733.3 \pm 40.18	0.958 \pm 0.189	0.2662 \pm 0.001

Table 2 - Mean and standard deviation of the calcium concentration and variation coefficient in the three batches of the different commercial brands of fermented milk beverages.

Commercial Brand	Calcium concentration (mg Ca/g) on the label	Calcium concentration (mg Ca/g) in the test	Variation Coefficient (%)
A	0.8000	0.4788 ± 0.0607	- 40.15
B	0.7375	0.8054 ± 0.0197	9.21
C	0.7385	0.7295 ± 0.0278	- 1.22
D	0.8000	0.8175 ± 0.0165	2.18
E	0.8625	0.7211 ± 0.0330	- 16.39
F	0.8250	0.7426 ± 0.0641	- 9.99

$r = 0.385$; $p = 0.450$; %SMHCxpH: $r = -0.275$; $p = 0.598$; %SMHCxBC: $r = 0.329$; $p = 0.524$) and enamel wear (RxF: $r = -0.620$; $p = 0.189$; RxCa: $r = -0.882$; $p = 0.020$; RxP: $r = -0.154$; $p = 0.771$; RxpH: $r = 0.516$; $p = 0.295$; RxBC: $r = -0.664$; $p = 0.151$) presented correlation only related to the calcium ion.

Discussion

Several studies have shown the benefits to the general health of the consumption of probiotics, and some have investigated the relationship of this consumption with oral health.¹⁻⁴

The low pH of fermented milk beverages can be associated with the onset of caries lesions and erosions in the oral cavity, as demonstrated by Shibata *et al.*³ (1977), who evaluated the effect of the continuous administration of the fermented milk beverages “Yakult” and “Calpis” in mice and hamsters. In that study, caries and erosion lesions were observed in the enamel of molars of the animals.

As observed in the present study, all of the fermented milk beverages analyzed presented a low pH (Table 1), which may contribute to the dissolution of the dental structure according to reports found in the related literature.^{6,8,13} However, this demineralization effect cannot be attributed to foods and beverages taking into account only the pH. Other factors should also be considered, including the physical and chemical properties that affect the adhesion of microorganisms to the dental surface, the stimulation of salivary flow, the buffering capacity of beverages and the presence of fluoride, calcium and phosphate.¹⁴⁻¹⁶

In the present study, the buffering capacity,

Table 3 - Mean and standard deviation of the %SMHC and enamel wear of the different commercial brands of fermented milk beverages.

Commercial Brand	%SMHC	Enamel wear (µm)
A	- 41.0 ± 3.72 ^a	0.17 ± 0.06 ^A
B	- 29.4 ± 4.38 ^b	0.17 ± 0.04 ^B
C	- 32.2 ± 5.65 ^c	0.17 ± 0.04 ^B
D	- 30.2 ± 2.53 ^{c,b}	0.17 ± 0.03 ^B
E	- 33.3 ± 2.90 ^c	0.18 ± 0.03 ^{B,A}
F	- 29.7 ± 4.16 ^c	0.15 ± 0.03 ^B

and the fluoride, calcium and phosphorus concentrations were also analyzed. The buffering capacity of a substance is related to the resistance to pH changes. It was observed that the commercial Brand C presented the largest buffering capacity, requiring 804.2 µl of 1 mol L⁻¹ NaOH to reach neutral pH. A direct relationship was observed ($r = 0.9308$) with a significance of 0.0084 between pH and buffering capacity. This result was corroborated by Edwards *et al.*⁸ (1999), making it possible to infer that, the smaller the pH of the beverage, the larger its buffering capacity, thus allowing the acid to stay longer in the oral cavity.

Evaluation of the fluoride concentration is justified due to its important role in the prevention of the formation of caries lesions, a condition that takes place in the presence of an acidic environment, mainly through a topical action.¹⁷ Although the presence of acid is an important factor in the development of both caries and erosion, these two conditions involve quite different processes. In the

caries process, fluoride can exert several functions, including the reduction of enamel demineralization and the stimulation of enamel remineralization.¹³ Furthermore, fluoride can accumulate on the dental plaque and then become available for long periods in the oral cavity.¹⁸ The addition of fluoride to the solution does not alter the pH, although it assumes a protective function, reducing dental erosion.¹⁹ However, it should be borne in mind that, once the erosion takes place, the presence of fluoride is not capable of promoting remineralization, differently from what occurs during the development of a caries lesion.¹³

Although none of the products indicated the presence of fluoride on their labels, it could be observed in this study that all beverages presented fluoride in their composition. Considering that the optimum fluoride dose to prevent dental fluorosis is 0.05-0.07 mg F/kg/day, most products (commercial brands A, B, C, D and E) showed a fluoride concentration which was lower than that reference, except for commercial brand F, which presented an average concentration of 0.958 µg F/g. Several studies have been conducted to investigate the concentration of fluoride in foods and beverages marketed in Brazil.²⁰⁻²² In a recent study, the presence of fluoride was detected in all types of milk analyzed, and in a brand of ready-to-drink chocolate milk, a concentration of 1.6 µg F/mL was found.²¹ It can be suggested, based on the relevant studies found in the related literature, that the presence of fluoride adds a protective effect to the solution, but it is important to point out that high fluoride concentrations in children's food and beverages can create a risk for dental fluorosis when a concomitant ingestion of other fluoride sources is considered.^{13,19}

Analysis of the calcium and phosphorus contents was accomplished due to their importance in the reduction of the cariogenic and erosive potential when present in the final form of foods and beverages.^{16,23} The addition of calcium to acidic solutions is preferable to that of phosphate because, when the same concentration of both elements was compared, calcium was more efficient in the prevention of erosion.¹⁹ Furthermore, the addition of calcium did not alter the flavor, differently from phosphate

that can produce a metallic taste in the foods.⁶ The addition of calcium and phosphate to acidic solutions changed the onset of the acid attack to human enamel, and these elements proved more efficient in protecting the enamel when their concentrations were increased.²⁴ *In situ* studies have confirmed the protecting effect of calcium and its advantage in reducing the dental erosion provoked by acidic beverages.²³ All of the fermented milk beverages analyzed in this study presented calcium in their composition, but differences could be observed among the values presented on the labels and those obtained with the test. Most of the beverages (brands B, C, D, E and F) presented a small difference between both values, except for brand A, which presented a variation coefficient (%) of -40.15% (Table 2). Although it has not been informed on the label, all of the fermented milk beverages analyzed presented phosphorus in their composition (Table 1). This information is important to the consumer, since the addition of calcium and phosphorus to acidic beverages reduces their erosive potential.⁶

Moreover, in the present study, no significant correlation was observed between the %SMHC or enamel wear and the evaluated ion concentrations, except for the calcium ion. Larsen, Nyvad²⁵ (1999) stated that the potential of a soft drink to erode dental enamel depends not only on the pH, but also on its buffering capacity. This fact may be influenced by the concentrations of different ions, such as phosphate, fluoride and calcium.¹² The related literature is contradictory regarding the erosive potential of beverages containing these ions. The effect of fluoride on dental erosion has been studied under various conditions with conflicting results.^{19,26,27} It is possible that increasing the fluoride concentration in acidic drinks could contribute to reduce their erosive potential. However, a review paper showed that fluoride admixtures to acidic solutions in a concentration excluding toxicological side effects seem unable to arrest erosive lesions.²⁸ Other studies have shown that beverage modification by addition of calcium is efficient in preventing erosion.^{29,30} Our results corroborate those of previous studies, showing a correlation between both the percentage of superficial microhardness change and enamel wear with

the calcium concentration in the products.

Conclusions

Some characteristics which may promote demineralization of the dental enamel, such as low pH and high buffering capacity, were observed in the products analyzed. On the other hand, the presence of fluoride, calcium and phosphorus ions was ob-

served, which may reduce the dissolution of dental structure produced by acidic substances. In this *in vitro* study, the fermented milk beverages did not promote erosion of the dental enamel, but rather only a superficial mineral loss. *In situ* and clinical studies are recommended to evaluate the erosive potential of fermented milk beverages.

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