



Article

Milk Matters? Comparing Dairy and Plant-Based Milks for Post-Erosive Enamel Mineral and Hardness Recovery: An In Vitro Study

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Abstract

Background/Objectives: The effects of commercial plant-based beverages on eroded enamel remain uncertain. This study compared post-erosive enamel elemental composition, SEM image-derived surface roughness, and microhardness after exposure to one bovine, one almond, and one coconut milk product. **Methods:** Fifty bovine incisors were randomly allocated to five groups ($n = 10$): a never-eroded water control; a cola-eroded water control; and three cola-eroded groups treated with the respective milk products. Each tooth was immersed individually in a separate container containing 250 mL of the assigned medium. Each daily cycle comprised 3 h of cola exposure followed by 3 h of milk or water exposure, repeated for four consecutive days, with interim storage at 37 °C. Endpoint calcium, phosphorus, and Ca/P ratio were assessed by energy-dispersive X-ray spectroscopy, microhardness by Vickers indentation, SEM image-derived Ra by standardized grayscale analysis, and morphology by scanning electron microscopy. Tooth-level outcomes were compared using one-way ANOVA followed by Tukey HSD tests ($\alpha = 0.05$). **Results:** Significant group differences were identified for elemental composition, image-derived Ra, and microhardness ($p < 0.001$). All milk groups had higher calcium, phosphorus, and microhardness and lower image-derived Ra than the eroded water control. The bovine product had the highest treatment-group calcium and microhardness and the lowest image-derived Ra, followed by almond and coconut. Phosphorus did not differ significantly between the bovine and almond groups. The bovine-group Ca/P ratio did not differ significantly from the never-eroded control; this does not establish equivalence or mineral-phase restoration. **Conclusions:** The tested bovine product showed more favorable endpoint calcium, image-derived Ra, and microhardness than the tested plant-based products under this accelerated in vitro protocol. The findings support product-specific differences in mineral/property recovery, not demonstrated enamel regeneration or clinical efficacy.



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Keywords: dental erosion; enamel mineral recovery; plant-based beverages; almond milk; coconut milk; bovine milk; microhardness; surface roughness; scanning electron microscopy; energy-dispersive X-ray spectroscopy

1. Introduction

Soft drinks are one of the largest categories in the beverage industry, and their sales have grown substantially over recent decades. Children, teenagers, and young adults con-

sume them most frequently [1]. They are non-alcoholic and may be classified as carbonated or non-carbonated [2]. Carbonated beverages are the third most consumed non-alcoholic drinks worldwide and generally contain carbon dioxide, sweeteners, caffeine, phosphoric and citric acids, high-fructose corn syrup, and various flavorings and colorants [3]. Common examples include Pepsi, Coca-Cola, Sprite and Red Bull [4], with Coca-Cola holding a leading market position owing to its relatively low price [5]. Because of their low pH and high sugar content, frequent consumption has been associated with a range of adverse health effects including diabetes, obesity, nutritional deficiency, dental caries and other systemic conditions [6], and rising consumption has been linked to a higher incidence of enamel erosion [7].

Dental erosion is an irreversible chemical process characterized by acid dissolution of hard dental tissue without the involvement of acids of bacterial origin [8]. It is classified by aetiology as intrinsic or extrinsic [9]. The intrinsic form results from exposure to endogenous acids and is seen in conditions such as gastro-oesophageal reflux, gastric ulcers, hiatus hernia and bulimia, whereas the extrinsic form arises from dietary acids, acidic drinks or low-pH medications such as vitamin C and aspirin [10]. Continued acid exposure causes demineralization, the dissolution of the mineral content of the tooth, which weakens its structure [11]. Residual softened enamel may undergo partial rehardening through mineral uptake, but enamel tissue lost through erosive wear cannot be regenerated by this process [8]. Remineralization of non-cavitated carious lesions involves mineral deposition within a retained crystal framework [12] and should be distinguished from recovery after surface erosion. Saliva is central to this process, supplying minerals and buffering acids to neutralize the oral environment [11]; however, saliva alone struggles to compensate for continuous acid exposure, resulting in tooth wear [2].

Remineralization can also be promoted extrinsically. Fluoride-based products have been the cornerstone of enamel remineralization for decades [12]: their effects on mineral uptake and acid resistance depend on the formulation and lesion model [13]. It can be delivered through toothpastes, varnishes, mouth rinses and gels [12]. Natural food products such as grape seed extract, aloe vera, ginger and honey have also been investigated [14]. Dairy products, including milk, cheese, and yogurt, are considered non-cariogenic and may support mineral recovery through their high calcium and phosphate content, buffering capacity, and protective milk proteins, particularly casein [15]. Casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) is a two-phase system that deposits onto tooth surfaces and raises calcium levels in both enamel and the plaque biofilm [12]. Bovine milk itself provides a rich source of lipids, proteins, amino acids, vitamins and minerals [16].

Because a substantial proportion of the population is lactose intolerant, allergic to cow's milk protein, or follows a vegan diet, plant-based beverages are increasingly used as alternatives to dairy milk [17]. Plant-based beverages can be prepared from grains, legumes, nuts, and other plant sources [15]. Coconut-based beverages are derived from coconut and may contain substantial lipid fractions, whereas almond beverages are produced from almonds dispersed in water and are typically formulated as commercial milk alternatives [18–21]. Whether these alternatives confer comparable benefits to eroded enamel has received limited attention. A previous investigation compared bovine and plant-based milks on cola-eroded human premolars using scanning electron microscopy and X-ray microanalysis [17].

The present study therefore aimed to evaluate and compare the effects of bovine, almond, and coconut milk on the elemental composition, SEM image-derived surface roughness, microhardness, and surface morphology of bovine enamel after Coca-Cola erosion. Bovine incisors were used because they are an acceptable substitute for human teeth in *in vitro* demineralization and remineralization research [22]. The null hypothesis

was that no difference would be found among the three tested milk products for elemental composition, image-derived Ra, or microhardness.

2. Materials and Methods

2.1. Ethical Approval and Sample Size

This study was reviewed and approved by the Research and Ethics Committee of the Faculty of Dentistry, The British University in Egypt (Project No. FD BUE REC 25-059). Fifty healthy lower bovine incisors were included, with 10 specimens per group. A sensitivity calculation in G*Power version 3.1 [23] for a fixed-effects omnibus one-way ANOVA with five groups and $\alpha = 0.05$ indicates that $N = 50$ provides approximately 77.3% power to detect Cohen's $f = 0.50$. Each tooth was exposed independently in its own container and constituted one experimental unit. Bovine teeth were selected for their large, relatively flat labial surfaces and their reported structural and compositional similarity to human teeth [22].

2.2. Specimen Preparation

Bovine teeth were cleaned to remove residual soft tissue and debris, disinfected using 0.1% Thymol (Muby Chemicals, Mumbai, India), and stored in distilled water at 4 °C for 24 h until use. Each crown was separated from the root using a dental bur in a high-speed handpiece under continuous water coolant and then mounted in self-cured acrylic resin (Acrostone, Cairo, Egypt) with the labial enamel surface exposed. No grinding or polishing of the enamel surface was performed, preserving the native surface for microscopy and microhardness assessment. Consequently, natural variation in surface curvature and baseline mineralization was retained. Each enamel specimen was prepared with dimensions of approximately 8 × 8 mm (± 1 mm), resulting in an exposed enamel surface area of approximately 64 mm² per specimen. Each enamel specimen was immersed individually in a separate container, with one specimen per container and 250 mL of immersion solution per specimen. Accordingly, each experimental group comprised 10 independently exposed specimens in 10 separate exposure containers. The same individual-container arrangement was maintained during both the erosive challenge and subsequent treatment immersion. Baseline and immediate post-erosion measurements were not obtained for individual specimens; the design therefore compares independently randomized groups at the final endpoint. Following each treatment exposure, the enamel specimens were rinsed and stored individually in distilled water in a laboratory incubator maintained at 37 °C until the following day, when the next experimental cycle was initiated. This overnight storage period was maintained consistently throughout the four-day experimental cycling protocol.

2.3. Erosive Challenge and Post-Exposure Treatment Cycling

Specimens were randomly allocated to five groups of ten using a computer-generated sequence of random numbers before any exposure procedure. Each tooth was immersed individually in a separate container containing 250 mL of the assigned medium; therefore, each group comprised 10 independently exposed specimens and 10 independent exposure containers. Teeth in the negative control group were maintained individually in 250 mL of distilled water. All experimental solutions were pre-equilibrated to 37 °C before exposure of the enamel specimens. Each of the 40 specimens assigned to the erosion groups was placed individually in 250 mL of Coca-Cola (Coca-Cola HBC, Cairo, Egypt) for 3 h. The pH of the cola and the milk products was measured before specimen insertion using a Jenway 3510 Standard Digital pH Meter (Cole-Parmer, Vernon Hills, IL, USA) and was 2.5 for the cola, 6.39, 6.93 and 6.80 for the three milk products used in the study. The immersion media were static during exposure to provide a simpler, reproducible protocol, allowing the

chemical characteristics of each immersion medium to remain the principal experimental variable rather than introducing a further hydrodynamic effect with agitation that could accelerate enamel dissolution. Following the erosive challenge, specimens were rinsed under running water for 5 min to remove residual acid.

After the erosive challenge, each specimen remained in an individual container and was immersed for a further 3 h in 250 mL of the medium assigned to its group: distilled water for the positive control; full-cream bovine milk (Juhayna, Cairo, Egypt); Alpro Almond Original (Alpro, Ghent, Belgium); or Alpro Coconut Original (Alpro, Ghent, Belgium). The treatment media were static during immersion. Specimens were then rinsed and stored in distilled water in BINDER GmbH incubator Model B 28 (Tuttlingen, Germany) at 37 °C until the following day. The cycle was repeated on four consecutive days using a laboratory cycling model informed by previous *in vitro* work [24]. A fresh container of Coca-Cola was opened each day to minimize changes associated with storage of previously opened product [25]. The consecutive 3 h acid and 3 h treatment exposures constitute a severe, accelerated laboratory challenge and do not reproduce physiological drinking patterns. Distilled water provided a standardized control and storage medium without the calcium, phosphate, proteins, buffering, and clearance functions of saliva, which simplifies the product comparison but reduces biological relevance. Table 1 summarizes the nutritional information, components and active ingredients of the milk products used in the study, including the pH and relevant mineral/nutritional composition.

2.4. Surface Morphology and Elemental Analysis

Surface morphology was examined by scanning electron microscopy (Quattro S Field Emission Gun Environmental SEM, Thermo Fisher Scientific, Waltham, MA, USA) using low-vacuum mode (50 Pa), an accelerating voltage of 20 kV, and a working distance of 13.0 mm at the Nanotechnology Research Center, The British University in Egypt. Elemental composition was determined by energy-dispersive X-ray spectroscopy at two points per specimen to quantify calcium, phosphorus and oxygen, and the Ca/P ratio was calculated [26,27]. Measurement points were selected from the mid-buccal enamel surface, avoiding cracks, debris, specimen margins and preparation artifacts. All elemental values are reported as atomic percentages.

2.5. SEM Image-Derived Surface Roughness Analysis

Surface texture was quantitatively compared using an image-derived arithmetic mean roughness parameter (Ra) obtained from standardized scanning electron micrographs. Three non-overlapping $\times 1300$ SEM images of the mid-buccal enamel surface were acquired per specimen, exported as 1280×960 pixel TIFF files, median-filtered, and sampled using three-line profiles within a central region of interest. Images were anonymized, and the assessor was blinded to allocation. Acquisition settings and brightness/contrast were held constant across specimens. Because conventional SEM grayscale intensity reflects electron-signal intensity influenced by surface geometry, composition, and detector response rather than independently calibrated vertical height, the resulting Ra values were interpreted as a comparative SEM image-derived roughness index rather than absolute profilometric surface height. Accordingly, Ra is reported in arbitrary units (a.u.) and was used to compare relative surface-texture differences among specimens acquired and processed under identical conditions [26,28].

Table 1. A summary of the nutritional information of the milk products used in the study.

Component/Milk Product	Juhayna Bovine Full Cream Milk	Alpro Almond Milk	Alpro Coconut Milk
Nutritional Information (Typical Values per 100 mL Serve)			
Energy	0 kJ/57 kcal	56 kJ/13 kcal	84 kJ/20 kcal
Fat	3 g	1.1 g	0.8 g
Saturates	-	0.1 g	0.8 g
Mono-unsaturates	-	0.7 g	0 g
Polyunsaturates	-	0.3 g	0 g
Carbohydrates	5 g	0 g	2.7 g
Sugars	-	0 g	1.9 g
Fiber	-	0.3 g	0.1 g
Protein	3 g	0.4 g	0.1 g
Salt	-	0.14 g	0.12 g
Vitamins	Vitamin A 0.512 mg Vitamin B2 0.416 mg Vitamin D 13.13 mcg Vitamin E 1.812 mg	Vitamin A 0.4 mg Vitamin D 0.75 µg Vitamin E 1.8 mg Vitamin B2 0.21 mg Vitamin B12 0.38 µg	Vitamin B12 0.38 µg
Calcium	115 mg [11%]	120 mg [15%]	120 mg [15%]
Other ingredients	100% Natural Milk UHT * full cream natural cow's milk. 3% fat, 8.25% SNF *. Free from milk powder, preservatives & any other additives. * UHT: Ultra-High Temperature * SNF: Solids-Not-Fat	Water, almond (2.3%), Calcium (calcium carbonate), Sea salt, Stabilisers (Guar gum, Gellan gum), Natural flavouring Emulsifier (Lecithins), Vitamins B12, D2, E, Potassium Iodide. May contain traces of nuts (no peanuts). Free from dairy and gluten, naturally lactose free	Water, Coconut milk (7%) (Coconut cream, Water), Coconut water (2.6%), Calcium (Tri-calcium phosphate), Natural coconut flavour, Stabilisers (Guar gum, Xanthan gum, Gellan gum), Sea salt, Vitamins (B12, D2). May contain traces of nuts (no peanuts). Free from dairy and gluten.
pH	6.39	6.93	6.80

2.6. Surface Microhardness

Each mounted specimen was placed under the indenter of a Vickers microhardness tester (Qness, QATM, Golling, Austria) and subjected to a 200 g load for 10 s. Three adjacent indentations were made on the same native, unpolished labial enamel surface used for the other surface assessments. Indentations were measured using Qpix T12 hardness testing software at 40× magnification, where the indentation margins were clearly visualized. The three technical readings were averaged to obtain one specimen-level Vickers hardness value for statistical analysis.

2.7. Statistical Analysis

Data was analyzed using GraphPad Prism version 10.2.3 (GraphPad Software, Boston, MA, USA). The tooth was the experimental unit (n = 10 per group), because every specimen underwent the erosive and treatment exposures in a separate 250 mL container. Technical measurements were averaged within each tooth before inference: the two EDS sampling points were combined to obtain one specimen-level value for each elemental outcome, the image-derived Ra measurements were averaged to obtain one specimen-level roughness

index, and the three Vickers indentations were averaged to obtain one specimen-level microhardness value. Continuous outcomes were summarized as mean $\pm$ standard deviation. Normality within groups was assessed using the Shapiro–Wilk test, and homogeneity of variance was assessed using the Brown–Forsythe test; the reported assumption checks were satisfied for all outcomes (Brown–Forsythe $p \geq 0.248$). Differences among the five groups were examined using one-way ANOVA, with eta-squared (η^2) reported as the effect size. When the omnibus test was significant, all 10 pairwise comparisons were evaluated using Tukey’s honestly significant difference (HSD) procedure, controlling the family-wise error rate at 0.05. Statistical significance was set at $p < 0.05$.

A descriptive recovery index was calculated as $100 \times (\text{treatment-group mean} - \text{eroded-control mean}) / (\text{never-eroded-control mean} - \text{eroded-control mean})$. An index of 100% indicates that the treatment mean equals the never-eroded-control mean, and 0% indicates equality with the eroded-control mean. This index expresses a between-group contrast at the endpoint; it is not a percentage of mineral mass regenerated or a within-specimen estimate of recovery. No equivalence test was performed.

3. Results

3.1. Surface Morphology

Examination of the negative control group revealed a smooth, regular enamel surface with no evidence of cracking, erosive areas, or other surface defects. Well-defined perikymata grooves, ridges, and areas of rodless enamel were clearly observed (Figure 1A–C). The positive control group showed an irregular enamel surface with widespread porosity, consistent with dissolution of the enamel’s calcium and phosphorus content; darkened areas with uneven outline and texture; and irregular depressions of variable length (Figure 1D–F).

The three treatment groups showed different surface appearances superimposed on the eroded substrate. In the bovine milk group, residual porosities were frequently bordered or partly occluded by bright outlines (Figure 1G–I). The almond group showed less confluent bright borders around exposed rods, with residual porosity and occasional crack lines (Figure 1J–L). The coconut group showed exposed rods and comparatively sparse bright outlines (Figure 1M–O). These observations are compatible with surface deposits or coverage, but SEM brightness alone does not establish their chemical identity or prove mineral deposition.

3.2. Elemental Composition

Calcium and phosphorus content differed significantly among the five groups (Ca: $F(4,45) = 167.12$, $p < 0.001$, $\eta^2 = 0.937$; P: $F(4,45) = 126.78$, $p < 0.001$, $\eta^2 = 0.918$). At the endpoint, the positive control group had 21.9% lower calcium and 15.9% lower phosphorus atomic percentages than the never-eroded control (both $p < 0.001$). All three milk groups retained significantly more calcium and phosphorus than the positive control (all $p < 0.001$) while remaining significantly below the negative control (all $p < 0.001$).

The three milks showed different results. Bovine milk gave the highest calcium content of the treated groups (32.02 ± 0.57 at%), significantly greater than almond milk (30.05 ± 0.71 at%, $p < 0.001$) and coconut milk (28.96 ± 0.74 at%, $p < 0.001$); almond milk in turn exceeded coconut milk ($p = 0.009$). For phosphorus, bovine and almond milk did not differ significantly ($p = 0.272$), and both exceeded coconut milk ($p < 0.001$ and $p = 0.006$, respectively). Expressed as a recovery index, the bovine group had calcium and phosphorus recovery indices of 69.7% and 62.9%, compared with 43.4% and 53.6% for almond milk and 28.8% and 36.7% for coconut milk.

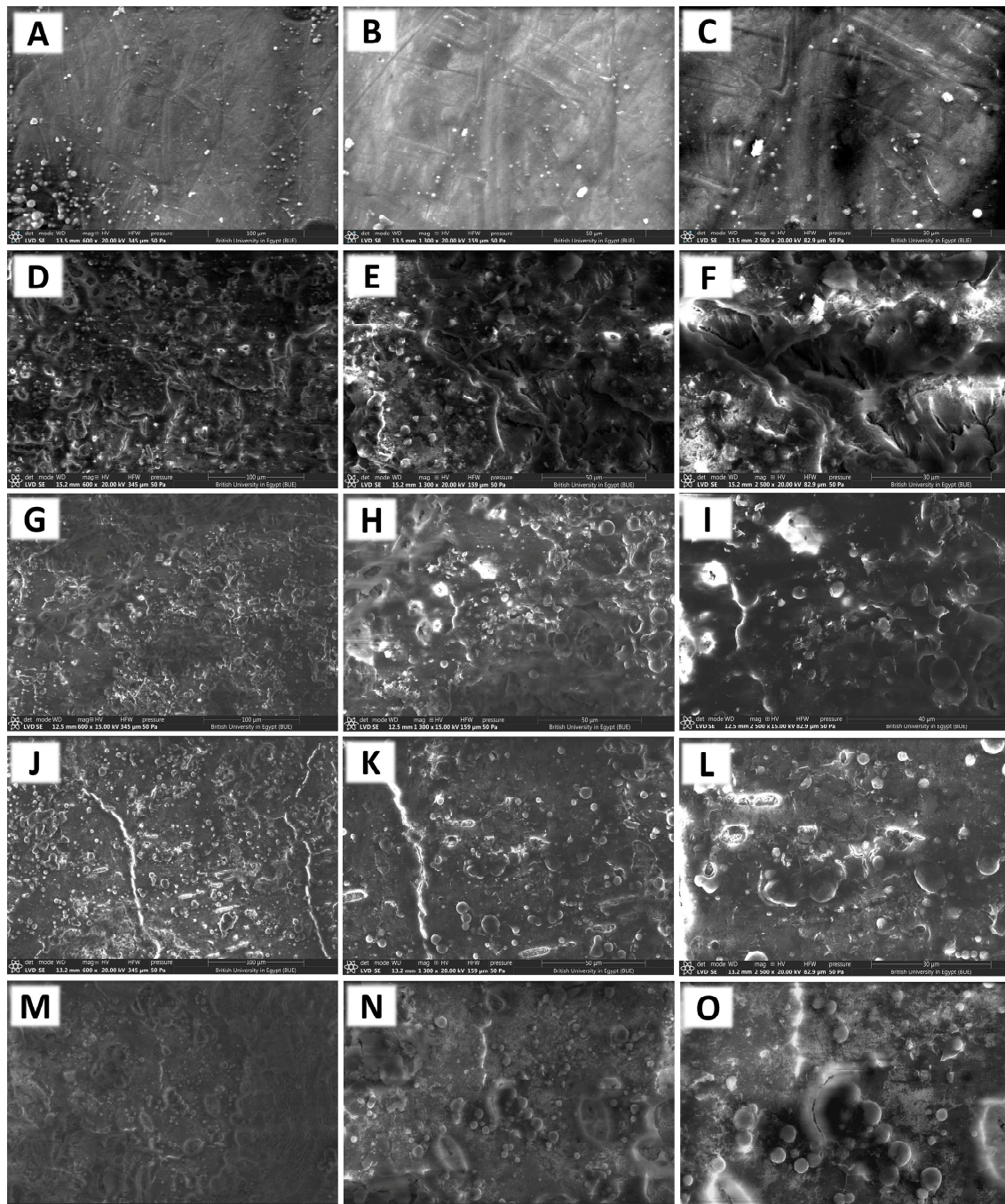


Figure 1. Scanning electron micrographs of the enamel surface at different magnifications. Negative control (A–C): perikymata ridges and grooves with areas of rodless enamel. Positive control (D–F): irregular surface with widespread porosities and depressions. Bovine milk (G–I): residual porosities largely bordered by bright outlines. Almond milk (J–L): exposed enamel rods with bright borders and persisting porosity. Coconut milk (M–O): multiple exposed enamel rods with sparse bright outlines. Brightness is not a phase-specific marker of mineral deposition.

The Ca/P ratio also differed significantly among groups ($F(4,45) = 14.20$, $p < 0.001$, $\eta^2 = 0.558$), and the pattern differed from that of the individual elements. Sound enamel returned a ratio of 1.711 ± 0.045 , close to the stoichiometric atomic ratio of hydroxyapatite, whereas the eroded control had a value of 1.588 ± 0.047 ($p < 0.001$), indicating a lower relative calcium-to-phosphorus signal, consistent with the greater proportional reduction in calcium atomic percentage. The bovine milk group had a ratio of 1.697 ± 0.047 , which did not differ significantly from sound enamel ($p = 0.970$) and corresponded to a recovery index

of 89.2%. In contrast, neither almond milk (1.618 ± 0.047) nor coconut milk (1.605 ± 0.048) differed significantly from the eroded positive control ($p = 0.629$ and $p = 0.931$, respectively), and both differed significantly from bovine milk ($p = 0.004$ and $p < 0.001$). A nonsignificant difference from the never-eroded control is not evidence of equivalence, and the Ca/P ratio does not identify a mineral phase (Figure 2).

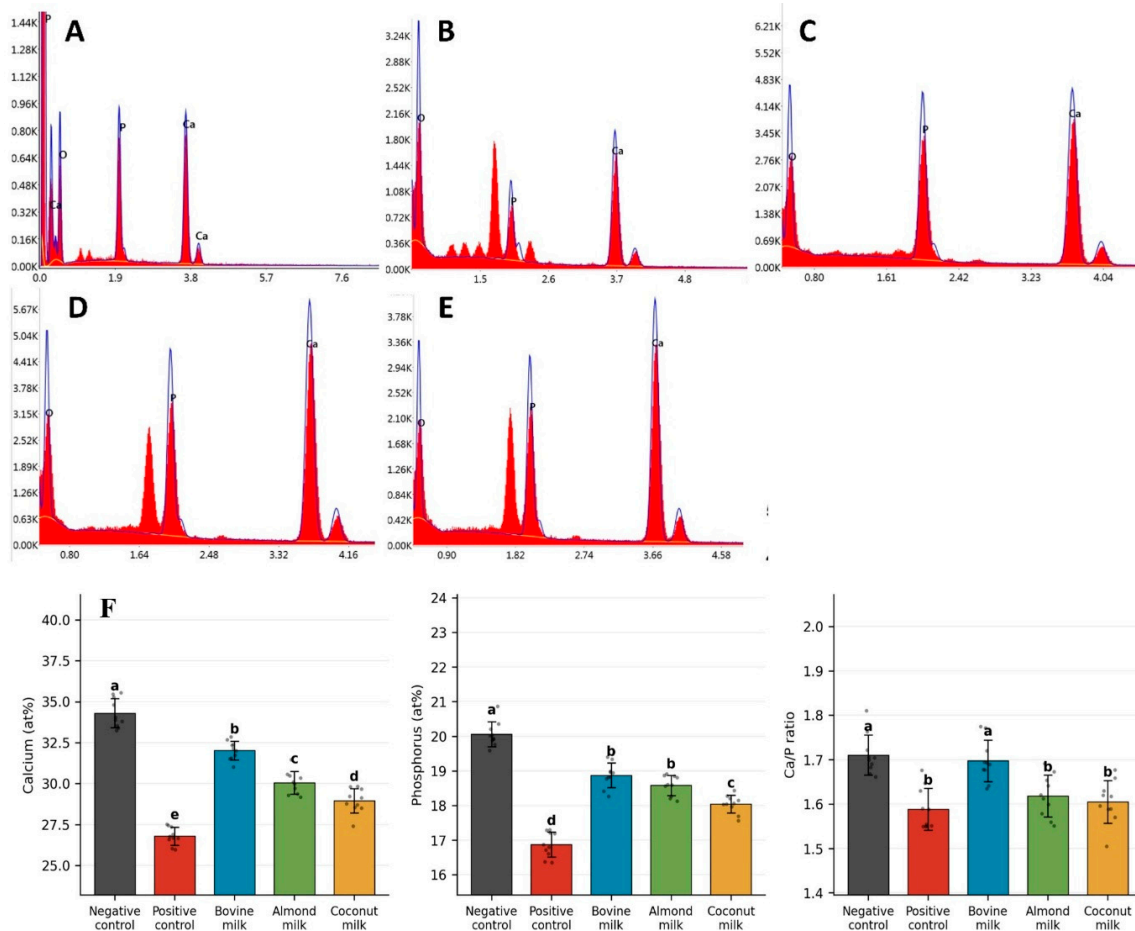


Figure 2. Representative energy-dispersive X-ray spectra of the negative control (A), positive control (B), bovine milk (C), almond milk (D), and coconut milk (E) groups, and mean calcium and phosphorus content and Ca/P ratio for all groups (F). Groups not sharing a superscript letter differ significantly (Tukey HSD, $p < 0.05$).

3.3. SEM Image-Derived Surface Roughness

The image-derived Ra roughness index differed significantly among groups ($F(4,45) = 125.90$, $p < 0.001$, $\eta^2 = 0.918$). The eroded positive control showed the highest value (0.459 ± 0.034 a.u.), compared with 0.191 ± 0.033 a.u. for the never-eroded control ($p < 0.001$). All three milk groups showed significantly lower Ra values than the eroded control and significantly higher values than the never-eroded control (all $p < 0.001$). The bovine milk group showed the lowest image-derived roughness among the treated groups (0.284 ± 0.030 a.u.), significantly lower than almond milk (0.331 ± 0.019 a.u.; $p = 0.004$) and coconut milk (0.362 ± 0.019 a.u.; $p < 0.001$); almond and coconut milk did not differ significantly from each other ($p = 0.119$). The corresponding descriptive recovery indices were 65.3%, 47.6%, and 36.2% for bovine, almond, and coconut milk, respectively. These values are comparative image-derived indices and should not be interpreted as absolute profilometric surface heights (Figure 3).

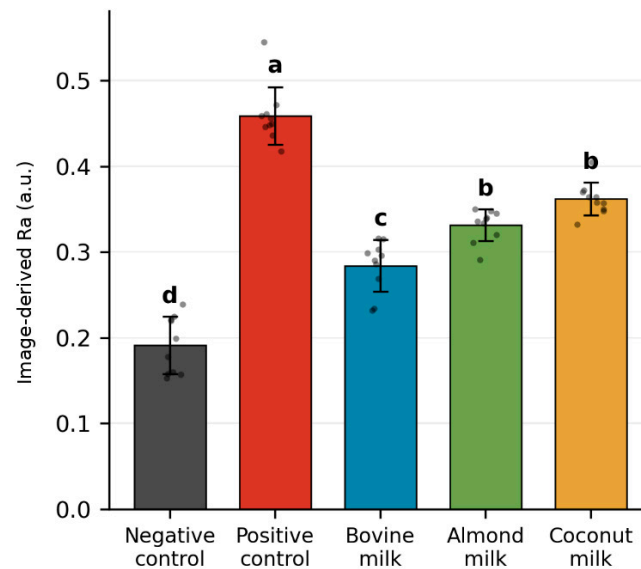


Figure 3. Mean SEM image-derived Ra roughness index for all study groups. Bars show mean $\pm$ standard deviation; points represent individual specimens. Ra is reported in arbitrary units and represents a comparative grayscale-derived surface-texture index rather than calibrated vertical surface height. Groups not sharing a letter differ significantly (Tukey HSD, $p < 0.05$).

3.4. Surface Microhardness

Microhardness differed significantly among groups ($F(4,45) = 188.52$, $p < 0.001$, $\eta^2 = 0.944$), and every pairwise comparison reached significance. Sound enamel recorded 339.88 ± 6.67 HV, whereas the eroded control was 26.6% lower at 249.49 ± 11.61 HV ($p < 0.001$). The treated groups ranked bovine milk (315.45 ± 5.46 HV) above almond milk (304.15 ± 6.27 HV; $p = 0.016$), which in turn ranked above coconut milk (291.49 ± 6.92 HV; $p = 0.005$). Recovery indices were 73.0%, 60.5%, and 46.5%, respectively. The ordering of group means was identical to that observed for calcium content and inverse to the image-derived Ra pattern (Figure 4). Table 2 summarizes the specimen-level enamel surface outcomes.

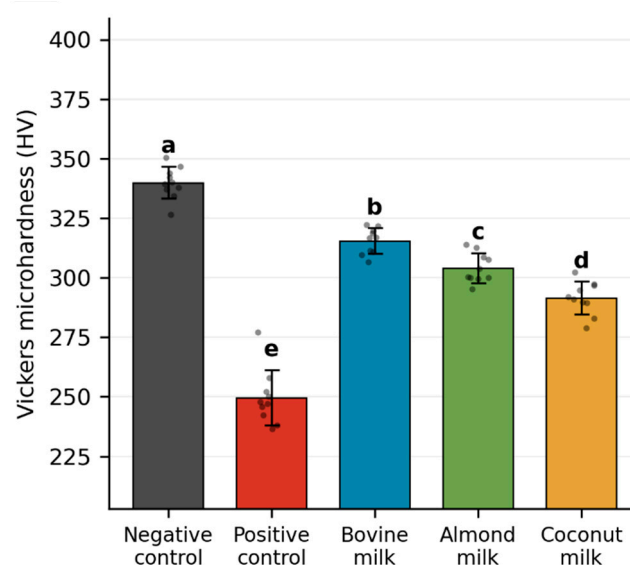


Figure 4. Mean Vickers microhardness of all study groups. Bars show mean $\pm$ standard deviation; points represent individual specimens. Groups not sharing a letter differ significantly (Tukey HSD, $p < 0.05$).

Table 2. Enamel surface outcomes by group (mean $\pm$ SD, n = 10 independent specimens per group).

Outcome	Negative Control	Positive Control	Bovine Milk	Almond Milk	Coconut Milk	F	p	η^2
Ca (at%)	34.30 $\pm$ 0.90 ^a	26.79 $\pm$ 0.54 ^e	32.02 $\pm$ 0.57 ^b	30.05 $\pm$ 0.71 ^c	28.96 $\pm$ 0.74 ^d	167.12	<0.001	0.937
P (at%)	20.06 $\pm$ 0.36 ^a	16.87 $\pm$ 0.36 ^d	18.88 $\pm$ 0.35 ^b	18.58 $\pm$ 0.29 ^b	18.04 $\pm$ 0.26 ^c	126.78	<0.001	0.918
Ca/P ratio	1.711 $\pm$ 0.045 ^a	1.588 $\pm$ 0.047 ^b	1.697 $\pm$ 0.047 ^a	1.618 $\pm$ 0.047 ^b	1.605 $\pm$ 0.048 ^b	14.20	<0.001	0.558
SEM image-derived Ra (a.u.)	0.191 $\pm$ 0.033 ^d	0.459 $\pm$ 0.034 ^a	0.284 $\pm$ 0.030 ^c	0.331 $\pm$ 0.019 ^b	0.362 $\pm$ 0.019 ^b	125.90	<0.001	0.918
Microhardness (HV)	339.88 $\pm$ 6.67 ^a	249.49 $\pm$ 11.61 ^e	315.45 $\pm$ 5.46 ^b	304.15 $\pm$ 6.27 ^c	291.49 $\pm$ 6.92 ^d	188.52	<0.001	0.944

Groups not sharing a superscript letter differ significantly (Tukey HSD, $p < 0.05$).

4. Discussion

This study compared one bovine, one almond, and one coconut milk product after repeated cola exposure, using never-eroded and eroded water-treated controls. Each tooth was exposed independently in its own 250 mL container, allowing the tooth to serve as the experimental unit. Endpoint elemental composition and Vickers microhardness were more favorable in all milk groups than in the eroded water control, while the SEM image-derived Ra index was significantly lower. Significant differences were also present among the tested products. These findings describe product-specific mineral/property and relative surface-texture recovery under the tested conditions; they do not demonstrate regeneration of enamel lost to erosion.

The contribution of this study is the combination of a mechanical endpoint, a standardized comparative SEM image-derived Ra index, and EDS measurements in a controlled product comparison. Surface Ca/P ratios describe relative elemental composition; they do not independently identify an apatitic phase. Earlier work examined bovine and plant-based milks on eroded human enamel using microscopy and X-ray microanalysis [17]. The present controls allow endpoint differences to be placed on a descriptive common scale, but they do not provide the original condition of each treated tooth. Conclusions must therefore account for unmeasured baseline variation.

Relative to the never-eroded control, the eroded control had 21.9% lower calcium atomic percentage, 15.9% lower phosphorus atomic percentage, 26.6% lower microhardness, and a higher SEM image-derived Ra index (0.459 $\pm$ 0.034 versus 0.191 $\pm$ 0.033 a.u.), alongside porous and irregular surface morphology. These endpoint findings are consistent with an erosive effect of cola-type beverages [29,30]. EDS atomic percentages are relative compositional measurements, not direct measurements of the mass of mineral lost, and the Ra values are comparative image-derived indices rather than calibrated surface heights.

Among the tested products, the bovine group had the highest endpoint calcium and microhardness and the lowest image-derived Ra, the almond group showed intermediate values, and the coconut group had the lowest calcium and microhardness and the highest Ra among the treatments. Phosphorus did not differ significantly between the bovine and almond groups. Recovery indices describe the position of treatment-group means between the two control means, rather than within-tooth restoration. Together, the elemental, image-derived roughness, and hardness results support differences among these products, while their interpretation remains limited by the endpoint-only design.

The Ca/P ratio requires cautious interpretation. The lower ratio in the eroded control reflects a greater proportional reduction in calcium than phosphorus atomic percentage. The bovine-group ratio did not differ significantly from the never-eroded control, whereas the almond- and coconut-group ratios did not differ significantly from the eroded control. Neither result establishes equivalence. EDS cannot determine whether surface-associated calcium and phosphorus occur in apatite, another calcium phosphate phase, or residual

deposits. Although casein-associated mineral interactions offer a plausible explanation for dairy-related effects [12,31], this study did not quantify casein-derived peptides, dissolved ion activity, or deposited crystalline phases. Accordingly, apatite restoration and non-apatitic deposition cannot be inferred from Ca/P ratios alone.

Published compositional comparisons show substantial variation among dairy and plant-based beverages, including their mineral and protein content and fortification [15,32–34]. The lower endpoint calcium and hardness in the tested coconut group cannot be attributed specifically to lipid content, phosphate deficiency, or calcium bioavailability without compositional measurements of that formulation. Similarly, the almond-group results do not establish the mechanism of a fortification effect. A commercial coconut drink should not be assumed to have the composition of undiluted coconut milk.

These results do not establish that plant-based beverages as a class are inferior dental substitutes for dairy milk. Only one formulation in each category was examined, and product pH, mineral speciation, buffering capacity, and additives may influence the response. Soy, oat, and other commercial formulations were not evaluated. Clinical dietary recommendations require evidence that also addresses salivary exposure, habitual drinking patterns, and longer-term outcomes.

Saliva and the acquired enamel pellicle are particularly relevant to interpretation. Mutahar et al. found lower pellicle total protein and statherin on eroded than non-eroded surfaces within the same participants [35]; this association does not establish the direction of causation. Zaheer et al. reported short-term changes in salivary lubrication, film behavior, and selected proteins after apple-juice exposure [36]. Their findings concern apple juice and dynamic salivary responses, not the effects of cola or milk on enamel. Together, these studies show why a saliva-free immersion model omits potentially important biological interactions. Distilled water avoids an additional salivary mineral and protein source but cannot reproduce buffering, clearance, or pellicle-mediated surface interactions.

Several limitations constrain interpretation. The present experiment specifically evaluated two commercially formulated plant-based beverages, almond and coconut, alongside bovine milk. Soy, oat, and other plant-based formulations were outside the scope of this comparison, and the selected products should not be interpreted as representative of plant-based beverages as a class. In addition to that, bovine enamel is a laboratory surrogate, and the native unpolished surfaces retained variation in curvature, morphology, and mineralization [22], which can influence Vickers indentation, local EDS sampling, and grayscale-derived surface-texture measurements. Random allocation and individual exposure vessels support independent between-group comparison, but no within-specimen baseline or immediate post-erosion measurements were available; therefore, endpoint contrasts cannot quantify change within the same tooth. The 3 h continuous cola exposure followed by 3 h treatment on each of four days is an accelerated challenge rather than a simulation of normal beverage consumption. The model excludes saliva, acquired pellicle, oral clearance, and biofilm. The SEM image-derived Ra analysis also has an important methodological limitation: grayscale intensity is influenced by surface geometry, composition, and detector response and is not independently calibrated as vertical height. The Ra values should therefore be interpreted only as comparative image-derived indices obtained under standardized acquisition and processing conditions, not as absolute profilometric roughness. No fluoride-based or CPP-ACP reference treatment was included. In addition, batch-specific product composition, including measured pH, buffering capacity, mineral speciation, protein content, fluoride, and fortification chemistry, was not characterized; mechanistic differences among products therefore cannot be assigned to individual formulation components. The group size was modest, and the sensitivity calculation for $f = 0.50$

was 77.3%, slightly below the conventional 80% target. Longer-term, in situ, and clinically relevant models are required before dietary recommendations can be made.

Future studies should combine saliva or validated artificial-saliva conditions with a pellicle-forming step, shorter repeated beverage exposures, repeated independent experimental runs, and validated height-resolved surface measurements by contact or optical profilometry or atomic force microscopy to confirm the image-derived Ra findings. Baseline, post-erosion, and post-treatment measurements on the same teeth would allow within-specimen changes to be quantified. Product characterization should include study-batch pH, buffering capacity, calcium and phosphate chemistry, fluoride where relevant, protein content, and fortification salts. A fluoride-based reference arm with a specified formulation, concentration, contact time, and application schedule would provide a benchmark. Spectroscopic or diffraction methods selected for surface sensitivity would be needed to characterize mineral phases. Multiple dairy and plant-based formulations, including soy and oat products and more than one commercial batch, should be evaluated before making category-level conclusions.

5. Conclusions

Under this accelerated in vitro protocol with independent specimen immersion, the tested bovine, almond, and coconut milk products were associated with significantly higher endpoint calcium, phosphorus, and microhardness and significantly lower SEM image-derived Ra values than the eroded water control. The bovine product produced higher calcium and microhardness and a lower image-derived Ra than the almond and coconut products, whereas phosphorus did not differ significantly between the bovine and almond groups. The bovine-group Ca/P ratio did not differ significantly from the never-eroded control; however, this does not demonstrate equivalence or restoration of an apatitic mineral phase. The Ra findings represent relative grayscale-derived surface-texture differences rather than absolute profilometric roughness. The results support product-specific differences in post-erosive enamel mineral/property recovery under laboratory conditions and should not be generalized to clinical efficacy or to plant-based beverages as a class.

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