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In vitro protection against dental erosion afforded by commercially available, calcium-fortified 100 percent juices

Rachael E. Davis, DDS; Teresa A. Marshall, PhD, RD/LD; Fang Qian, PhD; John J. Warren, DDS, MS; James S. Wefel, PhD

ABSTRACT

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Background. Calcium in acidic beverages can decrease a person's risk of experiencing dental erosion. The authors compared the pHs and titratable acidities of commercially available calcium-fortified and unfortified 100 percent juices, and enamel and root surface lesion depths after they were exposed to different juices.

Methods. The authors measured the pH and titratable acidity of calcium-fortified and unfortified 100 percent juices. They exposed enamel and root surfaces to different 100 percent juices for 25 hours and measured lesion depths. They used the Spearman rank correlation test and the two-sample *t* test to identify associations between the juices' properties and lesion depths and to compare lesion depths between fortified and unfortified juices.

Results. The authors found that fortifying apple, orange and grapefruit juices with calcium prevented enamel erosion and decreased root surface erosion ($P < .01$). They also found that fortifying white grape juice with calcium decreased enamel erosion ($P < .001$) but not root surface erosion. They observed that mean lesion depths were greater in root surfaces than in enamel surfaces after exposure to unfortified orange juice and all fortified juices ($P < .001$).

Conclusions. Calcium concentrations in commercially available, calcium-fortified 100 percent juices are sufficient to decrease and prevent erosion associated with extended exposure to a beverage.

Clinical Implications. People at risk of experiencing erosion could decrease their erosion risk by consuming calcium-fortified juices.

Key Words. Juice; erosion; calcium; fortification.

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Dental erosion is defined as the progressive, chemical removal of mineral from the enamel or exposed root surface of the tooth.[1,2] Erosion differs from caries in that caries is a site-specific lesion associated with bacterial fermentation of carbohydrates. Erosion is classified as extrinsic (that is, from the person's diet) or intrinsic (that is, gastroesophageal) in origin.[1-3] Dental erosion is considered to be a significant oral health concern in European and Middle Eastern countries; however, it has received much less attention in the United States.[4-6]

European investigators have studied acidic foods and beverages to determine if they are risk factors for enamel erosion; most investigations have focused on acidic beverages.[7-9] The pH, titratable acidity (that is, quantity of base required to bring a solution to neutral pH), acid composition and mineral concentrations contribute to the beverage's erosion potential. Danish researchers Larsen and Nyvad[8] reported that in vitro erosion was minimal for beverages with a pH higher than 4.2, but it was more evident for beverages with pHs lower than 4.0. They also found that the extent of erosion was not associated with titratable acidity. In a study in England, Hemingway and colleagues[9] found progressive enamel loss when teeth were exposed continuously to

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a variety of juices and juice drinks. They also found that enamel loss was associated with initial pH but not with titratable acidity. Thus, pH is considered a stronger determinant of erosion potential than is titratable acidity.

The presence of calcium, phosphorous or fluoride in a solution has the potential to prevent or limit the extent of erosion.[10] Larsen and Nyvad[8] reported that calcium and phosphorous supplementation of orange juice prevented in vitro enamel erosion. The addition of calcium citrate or calcium triphosphate to orange juice also has been associated with a lower in vitro erosion potential.[11] Similar to the situation with orange juice, which has a pH of 3.8, the addition of calcium to black currant juice, which also has a pH of 3.8, resulted in a decreased amount of observed erosion both in vitro and in situ.[12]

Although dental erosion has received little attention in the United States, increased consumption of 100 percent juices and juice drinks could increase the potential for erosion. In the United States, some 100 percent juices and juice drinks are fortified with varying concentrations of calcium with the goal of increasing dietary calcium intakes for bone accretion and osteoporosis prevention. We hypothesized that calcium fortification decreases the erosion potential of 100 percent juices. We conducted a study to compare the pHs and titratable acidities of commercially available 100 percent juices with and without calcium fortification and to compare the extent of enamel and root surface erosion after juice exposure.

MATERIALS AND METHODS

Beverage selection. We purchased ready-to-feed 100 percent apple, orange, grape and grapefruit juices with and without calcium fortification from local grocery stores. These 100 percent juices were Mott's Apple Juice (Stamford, Conn.), Minute Maid Apple Juice With Calcium (Atlanta), Tropicana Pure Premium Orange Juice (Bradenton, Fla.), Tropicana Pure Premium Pure Orange Juice With Calcium and Vitamin D, Tropicana Pure Premium Ruby Red Grapefruit Juice, Florida's Natural Grapefruit Juice With Calcium (Lake Wales, Fla.), Welch's White Grape Juice (Concord, Mass.) and Old Orchard White Grape Juice With Calcium (Sparta, Mich.). We stored the juices at room temperature or refrigerated them according to manufacturer's recommendations.

Physiochemical properties. We measured the pH and titratable acidity of each juice by using an automatic titrator (Metrohm E512 analog pH meter, Brinkmann Instruments, Westbury, N.Y.). We measured the titratable acidity of each juice by adding 1 molar of potassium hydroxide (KOH) to 50 milliliters of the juice until the pH reached 7.0. We took measurements three times for each juice.

Tooth preparation. We disinfected 64 extracted, uncavitated molars and premolars. We then removed any soft tissue and debris by scraping the teeth with a razor blade to remove gross debris, sonicating them in water for one to two minutes and brushing them individually with an electric brush. We painted the teeth with fingernail polish, leaving an unexposed 1- × 4-millimeter window of tooth structure on a flat, smooth surface. We prepared the teeth with one enamel or root surface window.

Juice exposure. We suspended the teeth (four enamel surface windows and four root surface windows on the basis of pilot data per beverage) in 250 mL of juice with the windows submerged for 25 hours at room temperature. We stirred the juices by using a magnetic stir bar, and we replaced the juices with fresh juices every five hours. After the teeth were submerged for 25 hours, we removed the teeth from the juice, rinsed them in water and air-dried them.

Measurements. After juice exposure, we mounted the teeth in a mandrel with sticky wax, leaving the window exposed and protruding from the mandrel. We sectioned the teeth through the painted and exposed surfaces by using a microtome (Series 1000 Hard Tissue Microtome, SciFab, Lafayette, Colo.). The sections were 100 to 150 micrometers deep, and we took approximately eight sections per tooth. We removed the sections from the tooth and stored them in water.

We removed the sections from the water and used a polarized light microscope (Olympus BX-50, Olympus America, Center Valley, Pa.) at ×10 and ×5 magnification to visualize the sections to identify any changes in the exposed surfaces. We photographed three representative windows or lesions per tooth surface.

We used image analyses software to measure the depths of both the enamel and root surface

ABBREVIATION KEY. DV: Daily value. **%DV:** Percent daily value. **KOH:** Potassium hydroxide. **NaOH:** Sodium hydroxide.

erosion lesions. We defined lesion depth as the average distance between a straight line representing the original tooth structure and a line drawn at the base of demineralization. We calculated the average of the three lesion depths per tooth surface to create a tooth value.

Statistical analysis. We conducted statistical analyses by using a statistical software package (SAS for Windows, Version 9.1, SAS Institute, Cary, N.C.). Physiochemical properties and lesion depths were reported as means and standard deviations. We used the Spearman rank correlation test to identify relationships between physiochemical properties and lesion depths. We used the two-sample *t* test to compare the mean lesion depths between fortified and unfortified juices for enamel and root surfaces. We also used the two-sample *t* test to compare mean lesion depths between enamel and root surfaces exposed to the same juice. The level of significance was $P < .05$.

RESULTS

Table 1 shows the physiochemical properties of the 100 percent juices. We found that the percent daily value (%DV) of calcium was not associated with either the pH or the quantity of KOH required to increase the pH to neutral. The daily value (DV) is defined as the recommended intake of a nutrient on the basis of a 2,000-calorie diet, and the %DV is the percentage of the recommended intake provided by one serving.[13]

Table 2 shows the mean lesion depths in enamel surfaces after they were exposed to calcium-fortified or unfortified 100 percent juices; photographic examples are presented in Figure 1. We noted an absence of enamel erosion in the calcium-fortified apple, orange and grapefruit juice samples, while we noted a decrease in enamel erosion in the calcium-fortified white grape juice samples. Enamel surface lesion depths decreased as %DV for calcium increased ($r = -0.83$; $P = .010$) and pH increased ($r = -0.74$; $P = .037$). We found that titratable acidity was not associated with enamel lesion depth.

Table 3 shows mean lesion depths in root surfaces after exposure to calcium-fortified or unfor-

TABLE 1

Physiochemical properties of 100 percent juices.

JUICE*	CALCIUM (%DV†)	pH (MEAN ± SD‡)	TITRATABLE ACIDITY (MILLILITERS OF 1 MOLAR OF KOH§ [MEAN ± SD])
Apple			
Mott's Apple	2	3.60 ± 0.17	3.18 ± 0.02
Minute Maid Apple With Calcium	10	3.93 ± 0.06	2.65 ± 0.02
Orange			
Tropicana Pure Premium Orange	0	4.00 ± 0.00	5.39 ± 0.05
Tropicana Pure Premium Pure Orange With Calcium and Vitamin D	35	4.33 ± 0.06	5.17± 0.01
Grapefruit			
Tropicana Pure Premium Ruby Red Grapefruit	2	3.40 ± 0.00	9.36 ± 0.14
Florida's Natural Grapefruit With Calcium	35	3.80 ± 0.00	9.12 ± 0.14
White Grape			
Welch's White Grape	0	3.20 ± 0.00	3.95 ± 0.03
Old Orchard White Grape With Calcium	10	3.20 ± 0.00	4.46 ± 0.02

* Mott's is located in Stamford, Conn.; Minute Maid is located in Atlanta; Tropicana is located in Bradenton, Fla.; Florida's Natural is located in Lake Wales, Fla.; Welch's is located in Concord, Mass.; Old Orchard is located in Sparta, Mich.
† %DV: Percent daily value, which is the percentage of a nutrient's daily requirement provided by one serving of the beverage.
‡ SD: Standard deviation.
§ KOH: Potassium hydroxide.

TABLE 2

Lesion depths of enamel* after 25 hours of exposure to 100 percent juice with or without calcium fortification.

JUICE	ENAMEL SURFACE LESION DEPTH (MEAN MICROMETERS ± SD†)		*P* VALUE
	Juice Without Calcium	Juice With Calcium	
Apple	106 ± 13	0 ± 0	< .001
Orange	69 ± 9	0 ± 0	< .001
Grapefruit	187 ± 29	0 ± 0	< .001
White Grape	176 ± 39	71 ± 20	< .001

* Four teeth were exposed to each juice.
† SD: Standard deviation.

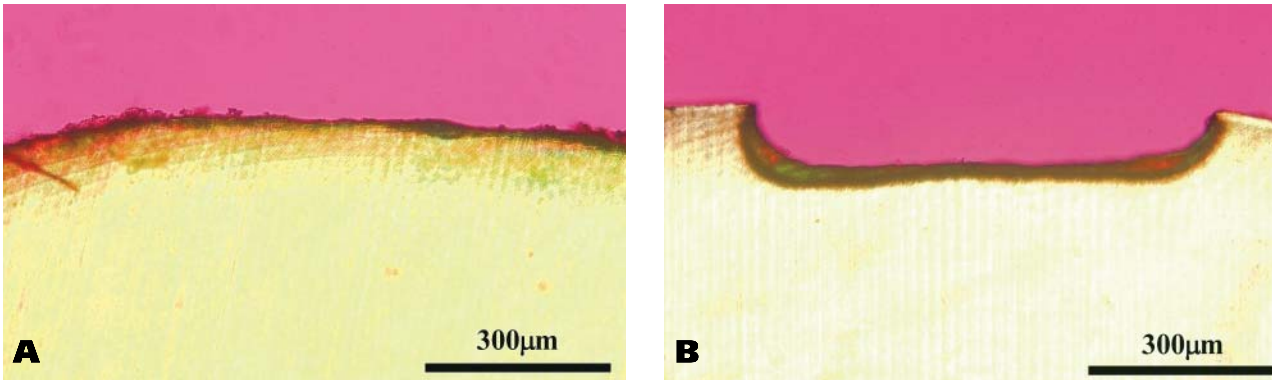

Figure 1. Enamel surface lesions produced during 25 hours of exposure to apple juice with calcium (**A**) or without calcium (**B**) (original magnification ×10). µm: Micrometers.

TABLE 3

Lesion depths of root surfaces* after 25 hours of exposure to 100 percent juice with or without calcium fortification.

JUICE	ROOT SURFACE LESION DEPTH (MEAN MICROMETERS ± SD†)		*P* VALUE
	Juice Without Calcium	Juice With Calcium	
Apple	121 ± 12	78 ± 4	< .001
Orange	139 ± 12	85 ± 10	< .001
Grapefruit	181 ± 19	123 ± 3	< .008
White Grape	168 ± 21	156 ± 9	.346

* Four teeth were exposed to each juice.
† SD: Standard deviation.

tified 100 percent juices; photographic examples are presented in Figure 2. We observed a decrease in root surface erosion in calcium-fortified apple, orange and grapefruit juice samples. Although marginally significant, root surface lesion depths increased as pH decreased ($r = -0.71$; $P = .050$). We found that neither the %DV for calcium nor the titratable acidity were associated with lesion depths in root surfaces.

We compared the lesion depths between enamel and root surfaces. We found that mean lesion depths after exposure to unfortified orange juice were greater in root surfaces than in enamel surfaces ($P < .001$) and that lesion depths did not differ between enamel and root surfaces for other unfortified juices. We found that lesion depths were greater in root surfaces than in enamel surfaces for all calcium-fortified juices ($P < .001$).

DISCUSSION

Our data suggest that calcium fortification of 100 percent juices can prevent enamel surface erosion and decrease the severity of root surface erosion associated with prolonged exposure to 100 percent juices. These data are consistent with the results from a recent report by Hooper and colleagues,[14] who found that calcium fortification of 100 milligrams of calcium per liter inhibited enamel erosion by an acidic beverage (pH 3.4) during 10 days of in situ exposure. The orange and grapefruit juices in our study were fortified with 35 percent (1,480 mg/L) of the %DV for calcium, and the apple and white grape juices were fortified with 10 percent (423 mg/L) of the %DV for calcium. The pH of white grape juice was lower than the pHs of orange and apple juices and did not increase with calcium supplementation, suggesting that the calcium concentration in white grape juice was insufficient to prevent demineralization.

The erosion we observed was associated inversely with pH in enamel and root surfaces and inversely with the %DV for calcium in enamel surfaces. These data are consistent with those reported by other investigators. Larsen and Nyvad[8] reported that pH was associated inversely with the depth of enamel lesions after exposure to soft drinks (that is, carbonated beverages, juice drinks, 100 percent juices) and that juice supplemented with 40 millimoles per liter of calcium prevented enamel erosion. Jensdottir and colleagues[11] reported an inverse association between the pH of soft drinks and erosion potential assessed by means of weight loss or soluble cal-

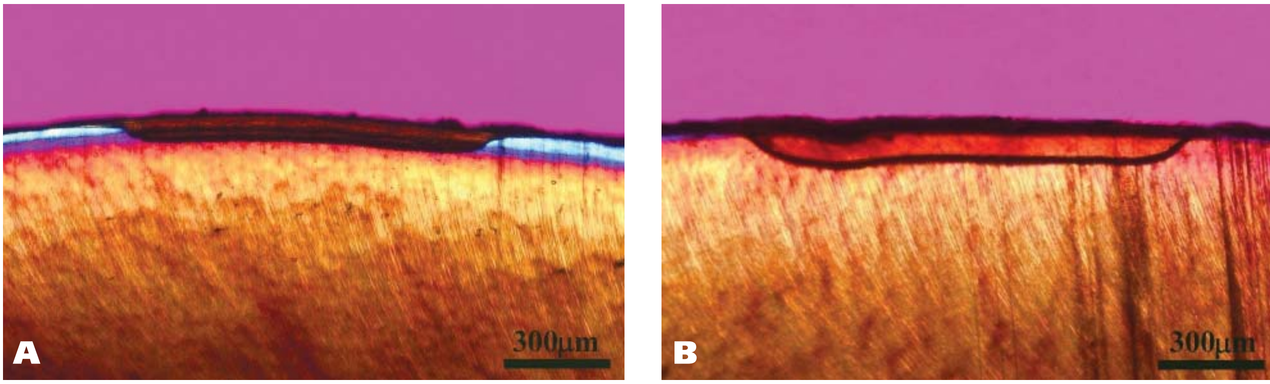

Figure 2. Root surface lesions produced during 25 hours of exposure to apple juice with calcium (**A**) or without calcium (**B**) (original magnification ×10). µm: Micrometers.

cium increase in solution after 24 hours of incubation. They also noted that addition of calcium phosphate to orange juice decreased the observed erosion potential.

The mechanisms by which calcium could prevent erosion in juice are twofold.[10] First, the acid typically dissolves the surface of the enamel, dentin or both until a calcium-saturated solution is reached. This saturated solution prevents further dissolution or erosion. Addition of sufficient calcium to the initial solution, as in the calcium fortification of juice, can saturate the initial solution and prevent subsequent dissolution.[10] Second, the calcium added to the solution can bind to citrate and prevent citrate from chelating calcium from enamel.[10]

Although the titratable acidity is hypothesized to prolong the decrease in oral pH and increase risk of experiencing dental erosion,[15] the titratable acidities of juices in our study were not related to enamel or root surface erosion. Jensdottir and colleagues[11] reported a significant correlation between the titratable acidities of selected soft drinks and the percentage of tooth section weight lost during 24 hours of incubation. In another study, they reported that erosion that occurred during the first three minutes of exposure (that is, immediate erosive potential) was associated with pH and not with titratable acidity.[16] Hemingway and colleagues[9] also reported that enamel erosion was associated with the pHs of and calcium concentrations in 100 percent juices and juice drinks but not with titratable acidity.

The pHs of the 100 percent apple and grapefruit juices we tested were similar to the those of the apple and grapefruit juices (3.59 and 3.36, respectively) studied by Jensdottir and colleagues[11] in Denmark, while the pH of the orange juice we studied was higher than the pH of the orange juice (3.83) they studied. The titratable acidities for the apple and orange juices in our study were similar to those for the apple and orange juices in Jensdottir and colleagues'[11] study (2.79 and 5.05 mL sodium hydroxide [NaOH], respectively), but the titratable acidities were higher for our grapefruit juice compared with theirs (5.92 mL NaOH). Lussi and colleagues[17] in Switzerland reported lower pHs for apple and orange juices (3.30 and 3.74, respectively), with correspondingly higher titratable acidities (4.50 and 6.20 mL 1 normal NaOH, respectively). Although one would expect similar pHs and titratable acidities for all 100 percent juices from the same type of fruit, variation among different varieties produced or marketed for juice in different countries could explain these results. Furthermore, exposure to juices with different pHs could explain part of the differences in erosion rates observed in European countries and in the United States.

Both the artificial time of exposure (25 hours) and the in vitro nature of the study design are limitations. The artificial time likely exacerbated the potential for erosion, and the in vitro nature did not allow for consideration of a person's rate of beverage consumption, manner of swallowing, salivary clearance, remineralization or any combination of these between exposures. Given that these limitations could exacerbate the erosion we observed with all beverages, the protection afforded by calcium against erosion is likely to be observed in clinical studies.

The objective of calcium fortification of 100 percent juices and juice drinks in the United States is to increase dietary calcium intake for osteoporosis prevention. The ability to prevent enamel erosion is, perhaps, an incidental benefit. The 100 percent juices contain significant amounts of energy (for example, 110-160 kilocalories per 8-ounce serving), while orange and grapefruit juices also serve as important sources of vitamin C and folate. Whole fruit is less energy-dense than is juice, and it contains fiber; thus, current dietary guidelines recommend that consumption of 100 percent juice be limited.[18] In addition to excessive energy, excessive intake of calcium-fortified juices potentially could increase the risk of developing calcium toxicity. Therefore, recommendations of calcium-fortified juices, other fortified beverages or both for erosion prevention should take into consideration other dietary sources of energy and calcium.

CONCLUSIONS

The concentration of calcium in fortified 100 percent juices available in the United States appears sufficient to decrease or eliminate the risk of experiencing erosion. In beverages with a pH close to 4, 10 percent of the %DV for calcium prevented erosion in enamel and decreased erosion in root surfaces. In beverages with lower pHs, 35 percent of the %DV also reduced risk of experiencing erosion. Thus, people at risk of experiencing erosion could decrease their risk by consuming calcium-fortified juices. ■

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