

## Demineralization of Pigs Teeth

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### Abstract

Fresh pigs teeth were extracted and were cut into approximately 35mm<sup>2</sup> pieces. These pieces were placed in orange juice, milk, Kool-aid, tap water, hot chocolate, or Pepsi and vortexed for a specific length of time. The amount of demineralization that occurred was measured by weighing and by Atomic Flame Spectrophotometry. The samples were tested to see if exposure time made a difference in the amount of demineralization that occurred and if the pH of the beverages had an effect. The only conclusive evidence was that certain beverages are more destructive than others orange juice > milk > hot chocolate > water > Pepsi.

### Introduction

It is known that when teeth lose calcium, they are being demineralized and as a result, caries may develop. Demineralization can occur in two ways. One is by the direct action of foods that are acidic when taken into the mouth; fermentation is not involved. The other is by acid that is formed in the mouth as the result of fermentative activity of mouth bacteria (Bibby 1983). Acidic beverages leach calcium out of the enamel therefore making teeth weak so bacteria can set in and develop a carie. In this study it will be determined which beverages may attribute to the demineralization process. It was expected that a low pH would have a significant effect and even more so if let to interact for long periods of time. Therefore it was predicted that Pepsi, orange juice, and Kool-aid would have a greater demineralizing effect because they contain a lot of sugar and are acidic.

The demineralizing effects of various beverages has been widely studied. Many studies have been done using rats, in which water was replaced with some beverage (Touyz et al). These studies along with others on bovine enamel and human teeth have also been done (Featherstone and Mellberg 1981). In all these studies certain beverages were determined to be more destructive than others.

The focus of this study was to test a select group of beverages available to college students and determine which are the most harmful to the enamel of pigs teeth and to if the exposure time to the beverages had any effect. The beverages used in this study were Pepsi (pH 3.05), orange juice (pH 3.9), two percent milk (pH 6.55), tap water (pH 7.6), hot chocolate (pH 6.7), and grape Kool-aid (pH 3.9).

### Materials and Methods

Fresh pigs teeth were extracted from the jaws of slaughtered animals. The teeth were immediately stored in two percent milk to prevent a large loss in calcium. The teeth were cut into approximately 37mm<sup>2</sup> pieces and stored in a 0.1% thymol solution. The teeth were cleaned with a slurry of pumice flour, and rinsed with distilled water.

The enamel block, with the exception of the enamel surface that had been exposed to the oral cavity, was coated with nail varnish and weighed. The pH of the beverages was measured using a pH meter. The coated block was then placed into 120 ul of a specific beverage (carbonated beverages were totally degassed) and placed in 1.5 ml polypropylene microfuge tube and agitated with a vortex for 5, 10, 20, or 30 minutes.

After exposure, the enamel block was taken out of the beverage and held above the surface of the tube and rinsed with 30 ul of distilled water and after drying reweighed and stored at 20°C. Concentrated nitric acid (120 ul) was then added to each tube. The samples were then left to sit uncovered for approximately two hours to allow excess gas to escape. The contents of the tube were then wet-ashed by sealing them lightly and placing them into a 60°C incubator for 12 hours. After cooling, 50 ul of a one mol/L KCL solution and 680 ul of distilled water were added.

Controls of each beverage were treated identical to the experimental groups. Solutions of calcium carbonate were prepared in concentrations of 25, 12.5, 6.25, 3.15, and .78 ppm and were used as standards for the atomic absorption spectrophotometer (Perkin Elmer model 306). The wavelength for the spectrophotometer was set at 422.7nm and a slit setting of 1.4nm. An air acetylene oxidizing flame was used with a calcium hollow cathode lamp. The spectrophotometer measured the calcium content of each sample in parts per million (ppm).

Scheffe's test was used to analyze the variation among the beverages and among the times.

### Results

There was no significant change in the weight of the enamel blocks after treatment in all beverages (Table 1). There was, however a significant effect as to the amount of calcium extracted from the enamel (Table 2).

The time the enamel block was left in the beverage had no affect upon the amount of demineralization that

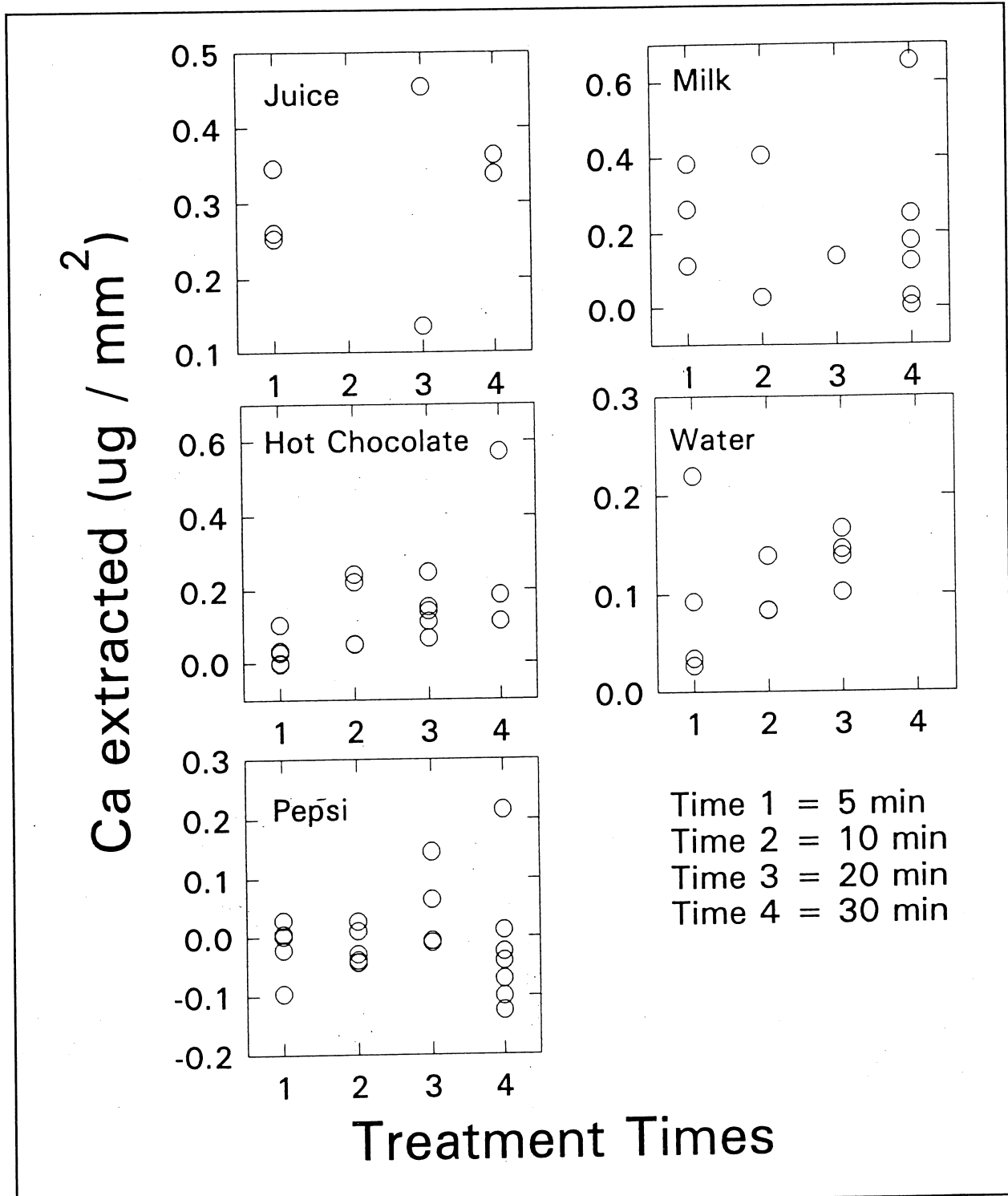


Figure 1. Demonstrates the extreme variation between beverages and selected times.

occurred (Fig. 1). All beverages demonstrate a large amount of variation (Fig. 1).

The most destructive beverage was orange juice > milk > hot chocolate > water > Pepsi (Fig. 2).

**Discussion**

Kool-aid was taken out of the analysis because its blank calcium reading was too high to be detected

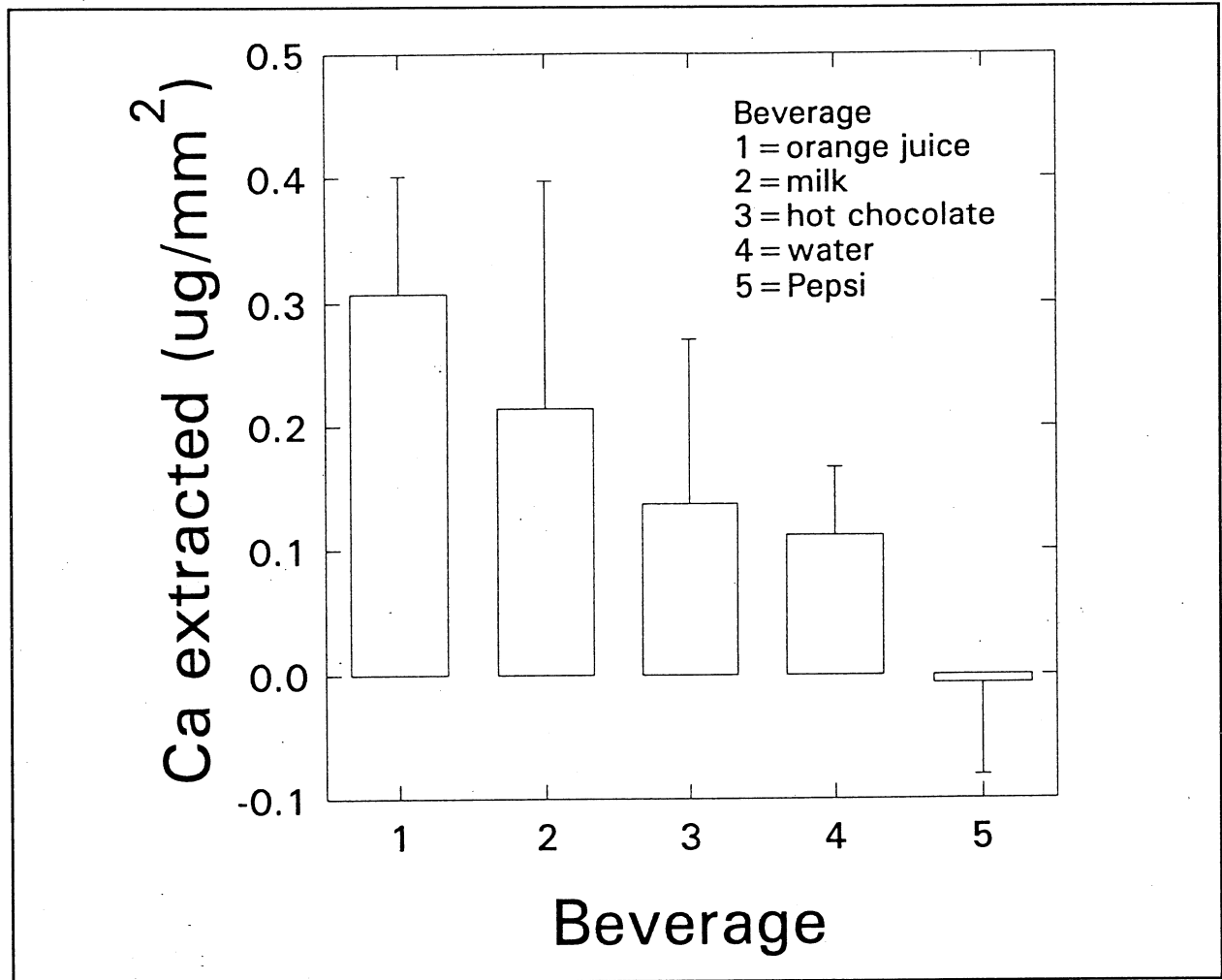


Figure 2. Demonstrates which beverage was the most destructive to the enamel. The standard deviation is expressed on each bar.

Table 1. Mean weights of enamel blocks before and after demineralization.

|        | Beverage |       |       |       |       |
|--------|----------|-------|-------|-------|-------|
|        | 1        | 2     | 3     | 4     | 5     |
| Before | .1254    | .0983 | .1216 | .1212 | .1379 |
| After  | .1254    | .0983 | .1216 | .1212 | .1379 |

accurately by the spectrophotometer and a standard calibration curve.

This study supports the results by Wynn and Haldi (1947) that there was not a strict and invariable correlation between the acidity and the amount of tooth destruction. The lack of a strict correlation between the extent of demineralization and the degree of acidity of the beverages was also reported by

Table 2. Means of ppm Ca present in comparison to times.

|      | Beverage |       |       |       |        |
|------|----------|-------|-------|-------|--------|
| Time | 1        | 2     | 3     | 4     | 5      |
| 1    | .2867    | .2542 | .0330 | .0934 | -.0164 |
| 2    | .0000    | .2166 | .1418 | .1021 | -.0195 |
| 3    | .2954    | .1400 | .1453 | .1385 | .0485  |
| 4    | .3502    | .2066 | .2900 | .0000 | -.0195 |

McClure and Ruzicka (1946). The results of this experiment were orange juice pH 3.9 > milk pH 6.55 > hot chocolate pH 6.7 > water pH 7.6 > Pepsi pH 3.9. In

contradiction, Theuns, et al, (1985) showed that pH did have an effect on the amount of demineralization that occurred to human teeth.

Orange juice contains very high amounts of citric acid (Asher and Read 1987). It has been shown by Stafne et al (1947) that because of its high amounts of citric acid it is very destructive to the enamel of the teeth. The destructive effects of citric acid on enamel is a result of a calcium citrate complex which forms when the citrate ions come into contact with the enamel surface (Asher and Read 1987). However, if the orange juice contains a small amount of oxalate the degree of demineralization will be less (Miller 1950). Holloway (1958) also states that sodium fluoride reduces the demineralizing properties of acidic drinks.

Milk was shown to be considerably demineralizing also. This result is backed by studies done by Featherstone and Rodgers (1981) which show that lactic acid under experimental conditions does cause cavities and demineralization. Their study also confirmed that lactic acid seems to have the greatest demineralizing affect upon tooth enamel when it is at a pH of 5.0 and higher. The pH of the milk in this study was 6.55 thus following Featherstone and Rodgers 1981 study and having extreme demineralizing potential. Other studies that have been done used buttermilk and other milk products and show that milk products don't cause any demineralization and that buttermilk has no erosive properties (Rytomaa, et al., 1988). However Rytomaa, et al., (1988) noted the opposite to be true.

Most studies indicate that when using a non-diet cola carbonated beverage the demineralization of the enamel will be severe (Grobler, et al, 1990). However, in this study Pepsi was the least destructive of all the beverages used (Fig. 2). The lack of demineralization may be attributed to the fact that the Pepsi used was from a fountain machine and not from a can where it is more concentrated (Grobler, et al, 1990).

Time was shown to have very little affect upon the amount of demineralization that occurred (Fig. 1). The lack of response to time is in agreement with Theuns, et al, (1985) who showed that time had little if no affect and actually demineralization decreased as the amount of time increased.

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#### Acknowledgements

I would like to thank Ellenwood Packing Plant for the donation of the pig jaws. I would also like to thank Dr. D. Hoagland, Dr. R. Zerger, Prof. A. Dutrow, and Dr. J. Frye for answering questions and assisting with the operation of equipment. Thanks to Dr. W. DeCoursey for help cutting the teeth. Thank you also to E. Cassidente and M. Blair for help in vortexing samples.