THE EFFECT OF HONEY ON HUMAN TOOTH ENAMEL IN VITRO OBSERVED BY ELECTRON MICROSCOPY AND MICROHARDNESS MEASUREMENTS

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Summary - Various fruit juices with relatively low pH are known to have erosive effects on human tooth enamel in a reasonably short time. Honey, also with a relatively low pH, could do the same, but scanning electron microscopy showed no erosion of enamel by honey over a period of 30 min, neither did Knoop microhardness tests show any deterioration of enamel structure. The absence of any effect could be only partially attributed to the calcium, phosphorus and fluoride levels in honey.

Key words: erosivity, analysis, honey, microhardness, scanning electron microscopy.

INTRODUCTION

Honey has been used by man for many centuries—written records dating back to 3000 BC describe the practice of loading beehives on to barges and moving them up and down the Nile depending on the season, enabling the bees to collect nectar where it was most abundant. Circa 2000 BC, the Assyrians covered the bodies of their dead with wax and buried them in honey. King Solomon, 986-933 BC, extolled the goodness of honey, as is evident in Proverbs 24:13. Production of honey in our present society is an important economic factor in many parts of the world, as it is extensively used as a sweetening agent and is considered by some to be a 'health food' (Root, 1983; Vogel, 1980).

The dissolution of dental enamel can be investigated, amongst other methods, by scanning electron microscopy as well as microhardness tests (Craig and Peyton, 1958; Purdell-Lewis, Groeneveld and Arends, 1976; Rensema and Arends, 1987; Zero et al., 1990). The demineralizing effect of the excessive intake of fruit drinks, especially of citrus fruits with low pH values, and other acidic beverages is well documented (Grobler, Jenkins, and Kotze, 1985; Grobler, Senekal and Van Wyk Kotze, 1989; Grobler, Senekal and Laubser, 1990). Honey also has a relatively low pH, averaging 3.9 in South Africa (Anderson, Buys and Janssensmeier, 1983) and the USA (Root, 1983). However, to our knowledge, the possible erosive effect of honey on human dental enamel has not been evaluated and we have now investigated this.

MATERIALS AND METHODS

The honey sample

The honey was harvested in the South Western Cape Province of South Africa during summer (January–March) 1990, and consisted mainly of nectar gathered from the blossoms of Eucalyptus trees. The honey was used after it had been extracted from the combs and did not contain artificial preservatives or diluents, neither had it been heated by any artificial method. Extraction and storage was in stainless-steel containers. Smaller quantities were kept in polyethylene tubes.

Tooth specimen preparation

The crowns of 25 human maxillary incisors were brushed with warm detergent solution, washed with distilled water and air-dried. Each crown was embedded in epoxy resin and positioned with the buccal bulge exposed above the level of the liquid resin. After the resin had set, the buccal aspects of the crowns were ground on a Metaserv polishing machine, firstly with 600-grade and finally with 1200-grade silicon carbide paper. The surfaces were ground until an area of enamel approx. 3 mm in diameter at the mid-central region had been smoothed. The crowns were then washed with distilled water and stored in saline with 0.001% thymol until required for testing.

Three crown specimens were used for the pilot experiment, phase 1. The polished areas were covered with plastic adhesive tape (3M), and the tape then cut with a surgical scalpel (No. 11) to the extent that the enamel surface also scored. A central square was cut with arms radiating from each corner of the square to the outer edge of the polished area [Fig. 1(a)]. The first specimen was prepared and one outer segment of tape removed, leaving three outer segments and the central square still covered with tape. Honey was poured into a Petri dish and the polished surface of the specimen immersed in the honey and continuously agitated for 60 s. A second outer segment of tape was then removed and the specimen again agitated in the
honey for 60 s. Similarly, the third and the fourth segments were removed at 60-s intervals and the immersion procedure repeated. The specimen was then rinsed in deionized water during which time the central segment (the control) of tape was removed. The procedure was repeated with the second and the third specimens, the difference being that while the segments on the first specimen were exposed to the honey for 0, 1, 2, 3 and 4 min, those on the second specimen were exposed for 3, 6, 9, 12 and 15 min and the segments of the third specimen exposed for 18, 21, 24, 27 and 30 min. After rinsing, the three specimens were evaluated for evidence of erosion by SEM. As no erosion could be detected it was decided to extend the study to phases 2 and 3.

Ten crowns were prepared for the second phase of the study. The polished areas were, however, divided into four segments by a horizontal and a vertical score [Fig. 1(b)], and the exposure times for the specimens were 0, 10, 20 and 30 min per segment. The effects of the treatments of these specimens were evaluated by SEM.

The third phase consisted of six crowns, which were similarly divided. However, three specimens were exposed to a solution of 50% honey/50% deionized water by volume, while three were exposed to a solution of 25% honey/75% deionized water; immersion times were 0, 10, 20 and 30 min per segment. These specimens were evaluated by hardness test and SEM.

In the fourth phase, nine crowns were also divided into four segments each. In this case, three specimens were exposed to a solution of 100% honey, three to 50% honey/50% water, and the other three to 25% honey/75% deionized water; immersion times were 0, 1, 2 and 3 h per segment. These specimens were evaluated by hardness tests.

In the fifth phase, six crowns were also divided into segments as explained above. In this investigation, however, three specimens were exposed to an artificial honey solution, prepared according to the analytical results in Table 1 and those reported by Root (1983), containing: 38% d-fructose, 31% d-glucose, 7% maltose, 1.5% sucrose, 100 mg calcium/l, 308 mg phosphorus/l (as NaH₂PO₄), 0.7 mg F/l (as NaF) and the pH adjusted to that of natural honey (4.24) with HCl solution. The other three specimens were exposed to a solution containing 25% of the above artificial honey and 75% deionized water. In this fifth phase the immersion times were 0, 0, 30 and 30 min per segment. These specimens were also evaluated by SEM.

**Microhardness test and SEM**

For the hardness tests, seven indentations about 25 μm apart were made on each of the embedded enamel segments. Knoop hardness tests were made with a Leitz Wetzlar microhardness tester with a load of 200 g applied for 15 s. From the depth of each diamond indentation the Knoop hardness values were calculated. For SEM, the crowns were cut out of the resin, dried in an oven at 37°C and prepared by covering them with a 300 Å of gold in a Balzars ion-sputter coater. The specimens were examined with a Cambridge 180 SEM operating at a 15 kV accelerating voltage.

**pH Measurement**

The pH readings of honey and its dilutions were taken on a Radiometer PHM 84 pH-meter with an Orion combination pH electrode at 25°C.

**Buffering capacity**

The buffering capacity (Lilienthal, 1955) of pure honey was calculated by measuring the volume of a standard sodium hydroxide solution (three tests) that had to be added to 15 g honey diluted with 10 ml of water in order to increase the pH by one unit.

Table 1. The calcium, phosphorus, ionic fluoride and buffering capacity of pure honey and the pH of pure and diluted honey

<table>
<thead>
<tr>
<th>pure honey</th>
<th>50% honey/50% water</th>
<th>25% honey/75% water</th>
<th>Mean fluoride (mg/l)</th>
<th>Mean buffering capacity (mol base/l)</th>
<th>Calcium (mg/l)</th>
<th>Phosphorous (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.24</td>
<td>4.24</td>
<td>4.25</td>
<td>0.70</td>
<td>0.0149</td>
<td>100</td>
<td>308</td>
</tr>
<tr>
<td>(0.02)*</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.03)</td>
<td>(0.0005)</td>
<td>(2)</td>
<td>(6)</td>
</tr>
<tr>
<td>n = 3</td>
<td>n = 3</td>
<td>n = 3</td>
<td>n = 3</td>
<td>n = 3</td>
<td>n = 3</td>
<td>n = 3</td>
</tr>
</tbody>
</table>

*Standard deviation (in parentheses).
Table 2. Knoop microhardness indentation lengths in μm (SD in parentheses) before and after exposure to honey

<table>
<thead>
<tr>
<th>Dilution factor</th>
<th>Time exposed (min)</th>
<th>0</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>60</th>
<th>120</th>
<th>180</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% honey</td>
<td></td>
<td>96 (2)</td>
<td>98 (2)</td>
<td>100 (3)</td>
<td>96 (2)</td>
<td>108 (8)</td>
<td>89 (12)</td>
<td>110 (14)</td>
</tr>
<tr>
<td>50% honey/50% water</td>
<td></td>
<td>94 (2)</td>
<td>93 (2)</td>
<td>92 (5)</td>
<td>96 (3)</td>
<td>102 (10)</td>
<td>104 (14)</td>
<td>109 (13)</td>
</tr>
<tr>
<td>25% honey/75% water</td>
<td></td>
<td>97 (2)</td>
<td>96 (3)</td>
<td>92 (2)</td>
<td>94 (2)</td>
<td>106 (12)</td>
<td>91 (9)</td>
<td>99 (9)</td>
</tr>
</tbody>
</table>

Table 3. The solubility products (Ksp) and ion-activity products (pl) of the two artificial honey solutions with respect to fluoroapatite (FAP) and hydroxyapatite (HAP)

<table>
<thead>
<tr>
<th>Artificial honey solution</th>
<th>pKsp (HAP)</th>
<th>pKsp (FAP)</th>
<th>pl (HAP)</th>
<th>pl (FAP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% honey</td>
<td>54.6</td>
<td>63.2</td>
<td>57.9</td>
<td>62.9</td>
</tr>
<tr>
<td>25% honey/75% water</td>
<td>59.6</td>
<td>67.6</td>
<td>62.9</td>
<td></td>
</tr>
</tbody>
</table>

Calculations done by courtesy of Professor B. ten Cate, Department of Cariology and Endodontology, ACTA, Amsterdam, The Netherlands.

Fluoride concentration

One part (per volume) of honey was diluted with 1 part TISAB with CDTA (Nicholson and Duff, 1981) to obtain a pH of approx. 5.1. After 24 h the fluoride was measured with a combination fluoride ion-selective electrode. The calibration curve was constructed with fluoride solutions between 0.50–5.00 parts/10^6 also containing, for background purposes, 35.5% glucose and 4.5% sucrose. The density of the honey was also determined by weighing a fixed volume of 10.00 ml at 20°C.

Calcium and phosphorus concentration

The calcium concentration of the honey was determined by flame (N2O/C2H2) atomic-absorption spectrophotometry (Chakrabarti, 1981). Phosphorus was analysed by a flameless atomic-absorption method employing zirconium-treated graphite tubes (Havezov, Russeva and Jordanov, 1979).

RESULTS

The average density of our honey sample was 1.45 (SD = 0.03) g/ml (five determinations).

pH, Fluoride, calcium, phosphorus and buffering capacity

Table 1 gives the mean and standard deviation of the pH, calcium, phosphorus, ionic fluoride and buffering capacity of the pure honey. The pH values of diluted honey are also given.

Microhardness

Table 2 indicates the Knoop microhardness indentation lengths (in μm) before and after exposure to pure and diluted honey. The Friedman non-parametric test showed no significant differences (p > 0.05) in the Knoop hardness values.

Table 3 gives the calculated solubility products and ion-activity products of hydroxy- and fluoroapatite. This was done for the artificial honey containing the carbohydrate, calcium, phosphorus, fluoride and pH levels found in pure honey, as well as for the diluted (4 x) artificial honey.

Fig. 2. Scanning electron micrograph of etched enamel surface exposed to orange juice for 10 min. × 5100
Fig. 3. Scanning electron micrograph of intersection dividing the enamel surface into four segments. (a) Enamel exposed to honey for 30 min; (b) exposure for 20 min; (c) exposure for 10 min; (d) control unexposed. × 150

SEM

For comparative purposes we include an illustration (Fig. 2) showing erosion of human tooth enamel after 10 min immersion in orange juice (magnification: 5100).

Phase 1. Each section of the three specimens was viewed at 5100 magnification and none showed signs of erosion. Scanning electron photomicrographs for this phase were identical to those shown in phase 2 and, to avoid repetition, have not been reproduced.

Phase 2. Figure 3 illustrates the intersection between exposed areas at 150 magnification. No signs of erosion were noticeable for exposure times of 30, 20, 10, 0 min (starting clockwise from the upper left section). The central enamel surface, which was not exposed to honey, is illustrated in Fig. 4 at 5100 magnification. At 5100 magnification of exposure times of 10, 20 and 30 min (Fig. 5) there were no signs of erosion. No difference could be seen when these surfaces were compared to those of the control surface.

Phase 3. No signs of erosion were noticed for pure as well as for any of the diluted honey samples.

Phase 5. Figure 6 (5100x) shows a low degree of erosion (30 min) for the undiluted artificial honey. However, the artificial honey diluted four times (30 min) was associated with a higher degree of enamel erosion (Fig. 7, magnification: 5100).

DISCUSSION

The variance of the pH of honey is dependent on the blossoms from which nectar is collected (Root, 1983). So it is found that honey made in one region, but from spring blossoms, will have a slightly different pH from that made by the same bees from summer blossoms; this will naturally be from plants differing from those that flower earlier. The honey used here was made from Eucalyptus blossoms, which were in blossom at the time. Another matter which must be considered is that bees need water, the fluoride content of which varies from region to region.

Overheating of honey can cause deterioration in quality and care was taken that this honey had not been heated in any artificial manner.

The term 'pure' honey when used in this study denotes honey as it had been extracted from the honeycomb without the addition of artificial preservatives or diluents.

The tooth enamel specimens were ground with 1200-grade silicon carbide paper so that the smooth surface would highlight the slightest signs of erosion should any take place. To simulate conditions in the mouth the specimens were kept moist until the moment they were immersed in honey. The specimens were dried only before preparation for SEM.

Fig. 4. Scanning electron micrograph of the control enamel section, which was not exposed to honey. × 5100
It is well known that the density of honey varies (Root, 1983) depending mainly on the moisture content. However, our value of 1.45 g/ml corresponds very well with the values found in the literature (Root, 1983).

The buffering capacity of honey (0.015 mol base/l) is higher than that of saliva (Lilienthal, 1955) (approx. 0.005 mol acid/l) but lower than that of fruit juices (0.05 mol base/l) (Grobler and Van der Horst, 1982). Thus, less saliva would be needed to neutralize the same volume of honey with its low pH (4.24). On the other hand, most of the fruit juices also have a lower pH (Grobler and Van der Horst, 1982; Grobler, Van Wyk and Muller, 1984; Grobler et al., 1985) (approx. 3.5) than honey, suggesting that honey would be less harmful to enamel.

The buffering power of honey can be attributed to the presence of at least 18 different organic acids (Root, 1983). The strength of the buffering capacity of honey will depend mainly on the types and amounts of acids and the minerals present. In agreement with the SEM studies, the microhardness

Fig. 5. Scanning electron micrograph of enamel section exposed to honey for 30 min. × 5100

Fig. 6. Scanning electron micrograph of enamel section exposed to artificial honey for 30 min. × 5100
profiles did not show any signs of enamel demineralization. We diluted the honey with water (Table 2) to reduce the viscosity and composition of the solution, but even with only a 25% honey content, and when exposed for 3 h, no significant difference could be demonstrated in the microhardness indentation lengths or SEM studies. The mean indentation lengths (Table 2) correspond to an enamel hardness value of 325 KHN, which in turn corresponds very well with the values found for undamaged abraded human enamel (Craig and Peyton, 1958; Purdell-Lewis et al., 1976; Reintsema and Arends, 1987; Zero et al., 1990).

Concerning the solubility of a solid (the enamel) in an aqueous medium, the most relevant factors are pH, ion-activity coefficient and dissociation constants (McCann and Brudevold, 1966). The occurrence of many different components in the honey renders the relation between the solubility and the solubility product of biominerals very complex. From kinetic studies it is clear that enamel dissolution is decreased by increasing the concentration of the total buffer and by the presence of phosphate or calcium ions (Lilienthal, 1977; Driessens, 1982; Grobler et al., 1990). Amongst other minerals, (Root, 1983) our honey sample contained 100 parts/106 calcium and 0.7 parts/106 fluoride (Table 1). It is reported by Driessens (1982) that fluorapatite is 10,000 times less soluble than hydroxyapatite. The decrease in enamel solubility from fluoride incorporation is well documented (McCann and Brudevold, 1966; Brown, Gregory and Choco, 1977). Wong, Cutress and Duncan (1987) showed that trace amounts of fluoride in solution (0.10 parts/106), even without calcium and phosphate, are equally as effective in decreasing the rate of demineralization as structurally incorporated fluoride (Sorvari, Kiviranta and Luoma, 1988; Page, 1991). Margolis, Varughese and Moreno (1982) observed protection of human enamel by fluoride concentrations in excess of about 0.024 parts/106 in using a pH 4.3 buffer containing about 470 parts/106 calcium and 186 parts/106 phosphorus. On the other hand, artificial honey with similar calcium, phosphorus, fluoride and pH levels did produce enamel erosion after 30 min (Figs 6 and 7). This could be expected because the p ionic product (pIP) value with respect to hydroxyapatite is higher (undersaturated) than the solubility product value, while the pIP with respect to fluorapatite is supersaturated (Table 3). A stronger degree of erosion, however, was found (Fig. 7) for the artificial honey diluted four times. This can be explained by the higher pIP values of this solution (Table 3); the solution is now undersaturated with respect to both hydroxyapatite and fluorapatite.

Thus, it is clear that the calcium, phosphorus and fluoride levels could be only partially responsible for the non-erosive action of honey and that something more out of the following renders it non-destructive. Honey contains colloidal particles, which appear to be very heterogeneous and to vary in composition (Root, 1983). There are always present appreciable quantities of protein material, enzymes, wax particles, pollen grains, silica and other extraneous matter. Edgar and Jenkins (1974) suggested that the solubility-reducing agent of honey is an organic phosphorus ester that is degradable by salivary enzymes. This fact makes it unlikely that the phosphorus ester would exert a protective effect in vivo. However, if the agent is able to bind to the enamel surface, then it might resist degradation while protecting the enamel. Furthermore, it can also be accepted that the high concentration of the carbohydrates will decrease the solubility of enamel. Sucrose reduces the dissolution rate of enamel in buffered acid solutions because of (1) viscosity effects, (2) coating effects, (3) chelation

Fig. 7. Scanning electron micrograph of enamel section exposed to diluted (4x) artificial honey for 30 min. x 5100
Effect of honey on tooth enamel

or complexing effects and (4) hydration effects, according to Blackwell and Fosdick (1956). On the other hand, König (1967) observed that dentinal fissure lesions in rats increased dramatically with the addition of jam, honey or sucrose to their basal bread diet. In their rat study (Shannon, Edmonds and Madison, 1979) concluded that honey is at least as cariogenic as sucrose and it may be that it retains its highly cariogenic character even at very low concentrations when ingested with natural food.

We conclude that our in vitro tests show that pure honey with a relatively low pH does not have an erosive effect on human tooth enamel, even after an exposure of 180 min. Honey also has no effect on the surface hardness of enamel. Studies with artificial honey indicated that this effect can only be partially attributed to its calcium, phosphorus and fluoride levels.

REFERENCES


