Comparison of the cariogenicity of some processed cheeses

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ABSTRACT

Aim: Cheeses have been investigated for their potential cariogenicity in several studies and have been shown to produce little change in the resting pH in dental plaque and little or no demineralisation of enamel in most intra-oral cariogenicity studies. The aim of the present study was to investigate the cariogenicity of four processed cheese formulations. Methods: Enamel demineralisation was measured intra-orally in bovine enamel, and aliquots of 10g of each test cheese were used to assess plaque pH using the plaque harvesting technique after the San Antonio criteria. In a second experiment, the same cheeses were assessed for their effects on enamel using the intra-oral cariogenicity test (ICT) with bovine enamel. Results: None of the four cheeses caused pH drops below the critical pH and two of the cheeses raised the pH slightly. The effects on pH were all significantly different from those of the sucrose saliva control. None of the cheeses produced microhardness changes that were statistically significantly different from the saliva control. Conclusion: None of these cheeses as tested were found to lead to acidogenicity and by inference to be cariogenic. They were therefore deemed to be safe for teeth when used as a food.

KEYWORDS: Cheese, Cariogenicity, Plaque pH

Introduction

Dental caries is a multifactorial disease involving tooth enamel, bacterial plaque and fermentable carbohydrates. Studies have shown that the sequence in which foods are eaten makes a significant impact on the amount of acid produced by the plaque bacteria and on the degree of demineralisation of enamel. If fermentable carbohydrates are consumed at succinct eating occasions, there is, in a healthy mouth, time between these events for enamel to remineralise. If, however, food or drink is consumed frequently by ‘grazing’, it is thought that demineralisation of enamel is prolonged and little or no remineralisation may occur [Burgess, 1985]. Foods that are safe for teeth, or have a low cariogenic potential, have been investigated by several methodologies including animal, human, plaque pH and enamel demineralisation studies [Harper et al., 1986]. Of particular interest is the role of dairy food in the dental caries process. Dairy foods, when eaten alone, before or after fermentable carbohydrates, or combined with other foods, have been shown to have protective effects against dental caries [Vehkalahti and Paunio, 1996]. These include buffering of bacterial acids, acceleration of the pH rise after eating, bacterial inhibition, decrease in demineralisation with casein phosphoproteins and/or calcium and phosphates, and less dental caries with lactose when compared with other sugars [Edgar et al., 1975].

Laboratory investigations of the effect of milk and milk derivatives on the solubility of enamel reported a greater reduction in enamel solubility with high protein milk products, in particular casein [Weiss and Bibby, 1966]. Cheddar cheese had the lowest enamel demineralisation potential of foods and was found to have the lowest cariogenic potential when compared with biscuits, yogurt, chocolate milk and milk chocolate [Pearce and Hampton, 1987]. The effect of dairy foods has also been evaluated in animal studies. Reynolds and Johnson [1981] found that supplementing a cariogenic diet with pasteurised bovine milk in rats...
substantially reduced the incidence of dental caries. Reynolds and Del Rio [1984] reported a significant reduction in dental caries in rats fed casein in their drinking water. Rosen et al. [1984] found very little dental decay in animals fed cheese alone; caries increased when 20% sucrose was added to the cheese, but was not as severe as with sucrose alone. Bowen et al. [1997] found less caries in desalivated rats fed milk based infant formulae compared with animals fed non-milk formulae or milk formulae with added sucrose.

Changes in human plaque pH with dairy food use have also been studied. Foods containing milk products do not tend to drop the pH below the critical level and chewing cheese appears to increase pH and decrease plaque acid concentrations [Edgar et al., 1975; Rugg Gunn et al., 1975]. The lowered pH of human dental plaque increased if cheese was eaten after drinking sweet coffee or fruit [Rugg Gunn et al., 1975]. It was suggested that this occurred because of the increased salivary flow and enhanced buffering related to increased calcium concentration in the dental plaque. If the sweet coffee was drunk after cheese, the pH rise was not as great, suggesting that the timing of eating cheese in a meal or its combination with other foods is important.

Enamel demineralisation studies have also been carried out to study the effect of dairy foods. Casein phosphoproteins were shown to prevent subsurface enamel demineralisation in a human intra-oral study using bovine enamel. This was thought to be due to an increase in plaque calcium and phosphate and the acid buffering capacity of the phosphoproteins [Reynolds, 1987]. In a survey looking at the relationships between cheese in the diets of adults and their dental caries levels, men who reported eating cheese at least once daily had fewer decayed teeth and more intact teeth. This relationship was not as clear in women [Vehkalahti and Paunio, 1996].

Cheeses are good dairy foods in children and adolescent diets. Cheese provides calcium to the diet and the wide range of low fat and processed cheeses can be considered useful snacks or main meal foods. Dairy foods are an important energy source and research suggests they can play a role in reducing dental caries. By 2 months of age, children in a UK study were shown to be receiving 33% of their energy from dairy products, which included cheese and yogurt [Mills and Tyler, 1992]. Cheese consumption was found to be higher in children with less dental caries in an earlier study [Rugg Gunn et al., 1984]. The results of this previous work suggest that when food containing dairy products is included in eating occasions, the pH drop is less. However, only a small range of dairy products has been tested, compared with the range that are available including different cheeses, yogurts and milk based drinks and desserts.

The aim of this study was to investigate the cariogenic potential of four different processed cheeses using a plaque sampling method [Pollard, 1995] and an intraoral cariogenicity test [Koulourides and Volker, 1964], in order to determine if these cheeses could be supported as being safe foods for teeth.

**Materials and methods**

Ethical approval was obtained from the New Zealand Southern Regional Health Authority’s Ethics Committee. Subjects were given written information and gave their informed consent. The cheeses were tested using two different methods of testing, as recommended by the 1985 San Antonio Consensus Conference on Assessment of the Cariogenic Potential of Foods [Hefferren, 1986] and the updated workshop proceedings on the methods to evaluate cariogenic and erosive potential [Curzon and Hefferren, 2001].

**Determination of plaque pH.** Seven volunteers aged 20 to 30 years were screened according to the guidelines described by Harper et al. [1986]. All were healthy with a high past caries experience determined by the presence of at least 12 decayed missing and filled teeth surfaces (DMFS). The subjects demonstrated a drop in plaque pH to <5.5 following a one minute challenge with 10% (w/v) sucrose solution. All subjects continued their usual oral hygiene routines during the study period, but refrained from brushing for the 48 hours before each plaque-sampling occasion. They also avoided having anything to eat or drink (except water) for 2.5 hours before each experimental session. All appointments were scheduled for the same time of day to avoid variations in circadian rhythm. There was a gap of 3 days before the subjects ceased oral hygiene for the next test.

A pooled sample of plaque was removed with a blunt probe from six smooth tooth surfaces, representing all quadrants of the mouth but excluding the lower incisors. Care was taken to avoid contamination with food remnants or saliva/blood. Plaque was sampled at time 0 and at 3, 7, 11, 15, 19, 23 and 27 minutes from the start of the chewing or
rinsing. Each plaque sample was mixed with 20 µl of sterile deionised water and the pH measured using an ion-sensitive field effect transistor with a diameter of 1.0 mm and a pH meter (ISFET, Sentron, Sentron Inc., BV, Roden, The Netherlands) [Harper et al., 1986]. The electrode was standardised using buffers at the beginning of each subject's plaque collection. It was also rechecked at the end of the collection period.

Intraoral cariogenicity testing (ICT). Seven healthy dentate volunteers aged 20 to 44 years with a DMFS of at least 12, normal salivary function and not taking any medication, took part in the study. Custom-designed, cast chrome-cobalt intraoral appliances with flanges buccal to the premolars and molars were made for each volunteer (Fig. 1). Appliances carried six removable bovine enamel blocks in recesses in the buccal flanges. To avoid site bias in the mouth, the colour coded enamel blocks were moved around the appliance to occupy each site for the same amount of time during the study. All subjects carried out their usual oral hygiene routines with the appliances out of the mouth, brushing their teeth and the fitting surfaces of their appliances twice per day with fluoride toothpaste containing 1,000 ppmF.

Bovine enamel blocks (3x4 mm and 2 mm thick) were cut from freshly extracted bovine incisors. They were sterilised in ethylene oxide, highly polished using diamond abrasives and mounted on composite bases to fit into the flanges of the appliance. Blocks were colour coded (Kerr Kolors, Kerr, Michigan, USA) to match the colours on the test food containers, and then covered with Dacron gauze to facilitate formation of dental plaque. Subjects wore their appliances continuously (except for eating and drinking) for 48 hours to allow plaque growth. For the following five days the enamel blocks were removed from the appliances and placed in saliva and test substrate slurries four times daily for 10 minutes at room temperature. All test substrates were kept refrigerated until used and were allowed to come to room temperature before being mixed with saliva. Subjects did not wear their appliances for five days between each test period.

Demineralisation was assessed from the surface microhardness of the enamel before and after testing using a Knoop diamond loaded with a 50g weight for 15 seconds (Shimadzu Microhardness Tester, Kyoto, Japan). Five indentations were made in each block before and after the experiment. The mean changes in indentation lengths were recorded and expressed as enamel softening or hardening. The enamel blocks for each experiment were all from one animal and all pre-test indentation values were very consistent. This allowed the results to be expressed as changes in the lengths of the indentations rather than as percentage changes.

Test and control substrates. Four commercially available processed cheese formulations were tested. Saliva and 10% sucrose (w/w) mixed with saliva were used as controls. The processed cheeses tested were a cheddar cheese in block form for slicing (BLOCK), a cheese packaged in slices for eating alone or in sandwiches (SLICE), a cheese spread packaged in a plastic tub (SPREAD), and a cheese snack packaged in individual toy-shaped wrappers (SNACK). For each test, 10 g of cheese was mixed to a slurry with 10 ml of saliva.

Analysis of data. The effect of the test substrates on plaque pH was investigated for each substrate by calculating the minimum plaque pH reached, maximum drop in plaque pH from baseline and the area under the pH curve. The effect of the substrates on enamel softening in the ICT experiment was investigated by comparing the mean change in enamel microhardness for each substrate. Differences among substrates were examined using paired t-tests.

Results

Determination of plaque pH. There were no major differences between the subjects in the degree of depression of plaque pH related to their previous dental caries experience or salivary flow as determined by selection at baseline. There were also no statistically significant relationships in the results with respect to age or gender. The mean pH curves for each of the
substrates are shown in Figure 2. The mean minimum pHs reached for each test cheese and each subject are summarised in Figure 3. All the cheeses and saliva were statistically significantly different from sucrose: BLOCK (P<0.001), SLICE (P<0.001), SPREAD (P<0.007), SNACK (P<0.02). The critical pH for enamel demineralisation was not reached for any of the cheeses tested. The maximum changes in the pH are shown in Figure 4. All cheeses and saliva were statistically significantly different from sucrose in the maximum drop in pH produced: BLOCK, SLICE, SNACK (P<0.0001) SPREAD (P<0.002). All produced statistically significantly smaller areas under the curves than sucrose saliva: SNACK (P<0.005), BLOCK (P<0.006), saliva (P<0.016), SLICE (P<0.027), SPREAD (P<0.037). This is shown in Figure 5.

Intraoral cariogenicity testing (ICT). Table 1 shows the mean changes in enamel microhardness for each substrate. Sucrose saliva produced a greater decrease in microhardness when compared with the cheeses and saliva. However, there were no statistically significant differences found in the degree of enamel softening between any of the substrates when all the subjects were compared together. For some subjects the cheeses resulted in no softening or even hardening of the enamel during the experiments.

Discussion
These results support previous studies showing low cariogenic potential of cheese. The present study mostly followed recommendations of the San Antonio Conference on the Scientific Consensus on Methods for Assessment of the Cariogenic Potential of Foods [Hefferren, 1986; Curzon and Pollard,
Two changes were made. Sucrose mixed with saliva was used as the positive control because the cheeses were also mixed with saliva, as done previously [Rosen et al., 1984]. Saliva alone was used as the negative control; sorbitol (a non-cariogenic substrate) mixed with saliva may, however, be more appropriate.

Human intra-oral measurement of demineralisation in bovine enamel and harvested plaque pH testing have both been used in many previous studies. [Chandler et al., 1995; Pollard, 1995; Hussein et al., 1996]. In our study the pH did not drop below the critical pH for any of the cheeses. None were found to be statistically different to that produced by saliva alone in the degree of enamel softening produced. In some instances, not unexpectedly, there appeared to be an increase in microhardness, as reported previously with natural cheeses [Thomson, 1988; Jensen and Schachtele, 1983]. Only one study using processed cheeses has reported reduction of buccal caries in rats [Harper et al., 1986].

It is interesting to consider possible effects of the different cheese formulations (provided by New Zealand Dairy Research Centre, Palmerston North, New Zealand) on acid production in plaque or on demineralisation of enamel. Several protective factors may be involved. SNACK, which had the highest lactose content, produced a slightly greater pH drop. BLOCK and SLICE had almost twice as much calcium and phosphate as the other two cheeses. This has been shown previously to be related to degree of demineralisation and acid production [Rugg Gunn et al., 1975]. BLOCK and SLICE had almost double the protein content and produced the least enamel softening, maintained the pH near resting levels and produced smaller areas under the pH curves. Previous researchers have suggested that the protein content is possibly the most important factor in the effect of dairy products on dental caries [Reynolds and Del Rio, 1984; Harper et al., 1986]. The zinc content also varied between the cheeses. BLOCK and SLICE had a higher zinc content, which may have an inhibitory effect on dental plaque [Jones et al., 1988; Giertsen et al., 1989]. This may partly explain the smaller drop in pH with these cheeses. Variations found in pH testing and microhardness may also be due to factors not investigated in this study, including the retention time of the cheeses. Our subjects commented on the different textures of the cheeses when using them for the plaque pH tests. Previous researchers have commented on the effects of texture and/or gustatory stimuli of cheese in promoting food clearance and salivary buffering [Jensen et al., 1984].

An unexpectedly low group mean for the softening of enamel with sucrose saliva was found. Changes in microhardness of 10 µm in indentation length are associated with microradiographic evidence of enamel caries [Pearce and Gallagher, 1975]. In the present study the mean changes were 4.7 µm for sucrose saliva and 1.6 µm for the cheeses. These changes are consistent with enamel softening with saliva alone [Chandler et al., 1990]. A possible reason for variation in enamel softening is the variability in hardness of bovine enamel. Pre-experiment measurements of hardness showed little variation and the enamel was all from one dairy herd.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Slice</th>
<th>Block</th>
<th>Spread</th>
<th>Snack</th>
<th>Saliva</th>
<th>Sucrose saliva</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>2.2</td>
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<td>1.9</td>
<td>1.03</td>
<td>6.3</td>
</tr>
<tr>
<td>2</td>
<td>2.5</td>
<td>-0.3</td>
<td>5.5</td>
<td>2.3</td>
<td>2.1</td>
<td>1.6</td>
</tr>
<tr>
<td>3</td>
<td>0.1</td>
<td>0.6</td>
<td>1.1</td>
<td>5.0</td>
<td>4.6</td>
<td>9.8</td>
</tr>
<tr>
<td>4</td>
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<tr>
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<td>3.6</td>
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<tr>
<td>6</td>
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<tr>
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<td>0.1</td>
<td>2.2</td>
<td>-1.0</td>
<td>4.7</td>
<td></td>
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<tr>
<td>MEAN</td>
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<td>1.6</td>
<td>2.5</td>
<td>1.9</td>
<td>4.7</td>
</tr>
<tr>
<td>+SE</td>
<td>+0.4</td>
<td>+0.4</td>
<td>+1.5</td>
<td>+0.8</td>
<td>+0.6</td>
<td>+1.4</td>
</tr>
</tbody>
</table>

Table 1 - Changes in microhardness of bovine enamel for each cheese tested and for each subject (µm).
It suggests that the variation was related to the subjects’ dental plaque activity and possibly salivary buffering. While all subjects had a DMFS of at least 12 and were able to depress plaque pH below the critical pH (pre-testing), these measurements alone do not determine the severity of the plaque activity or the dental caries risk. Subjects with the ability to depress plaque pH may well have other mechanisms in place that control demineralisation such as well-controlled eating patterns and fluoride use. In future ICT testing it may be important to select subjects who have evidence of new carious lesions and to lengthen the testing time, although seven days or more of testing would require significant commitment from the subjects.

The results suggest these processed cheeses have a low cariogenic potential. It is important to consider the significance of this in terms of dietary advice. The current study does not consider the effect of cheese eaten with, before or after other foods. It would be interesting to study the effect of these cheeses combined with other foods to determine whether they truly have an overall protective effect or can only be considered protective when eaten alone as a snack. Previous studies have partly addressed this. Dental caries was reduced by an average of 71% where aged cheddar cheese was eaten after sucrose rinses [Silva et al., 1986]. Rising plaque pH was reported when cheese was eaten after sweet foods or drinks [Rugg-Gunn et al., 1975, Imfeld et al., 1978; Schachtiele et al., 1982]. Moynihan et al. [1999] reported an increase in plaque calcium when meals containing cooked cheese were eaten. Present evidence suggests that cheese tends to be consumed at main meal times [García-Closas et al., 1997] and other evidence suggests that regular consumption of cheese over a long period is associated with less caries [Vehkalahti and Paunio, 1996]. These aspects warrant further investigation, as overall advice may not be just to replace snack foods with cheese, but also to include cheese or other dairy foods in several eating occasions each day.

The present study has demonstrated that the processed cheeses investigated were significantly different from sucrose saliva when assessed for their ability to cause acid production in dental plaque. They produced no more enamel softening than saliva under normal oral conditions and resulted in less enamel softening than sucrose mixed with saliva. Cheese does appear to have dental protective effects and consideration should be given to recommending it be included in children’s meals and as a safe snack.

Acknowledgements
The authors acknowledge the cooperation of the participants in the process of testing over many weeks, the financial support of the New Zealand Dairy Board, the expert technical help of Mr C.W. Thomson, the statistical support of Associate Professor W.M. Thomson and the illustration support of Dr S. Koshy.

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