# Cariostatic Effect of Various Cheeses on the Degree of Mineralisation of Enamel in Situ

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## ABSTRACT

**Objective:** To investigate the effect of 9 cheeses with different compositions on the extent of demineralisation/ remineralisation of enamel slabs in situ.

**Methods:** Sucrose and sorbitol solutions were used as controls. Ten subjects were required to wear removable appliances with human enamel slabs cut from white spot lesions created *in* vitro. The subjects were required to wear their appliances, for two days to allow plaque to grow. Over the following 5 days, they were asked to immerse their appliances 4 times per day for 10 minutes each time in a suspension of the test cheese or control solution. De- or remineralisation was then measured using a combination of microradiography and an image analysis system.

**Results:** The results for lesion depth (μm) tested by paired t-test revealed significant net remineralisation ($p<0.05$) with two types of cheeses. As for mineral loss or gain (vol.%μm), significant net remineralisation was seen with 6 types of cheeses.

**Conclusion:** Some cheeses can cause remineralisation of early lesions.

**Key words:** Cheese, Intra-oral cariogenicity test, Microradiography

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## Introduction

The importance of diet in the production of dental caries has long been acknowledged.[1] Despite improved trends in levels of dental caries in developed countries, dental caries remains prevalent and is increasing in some developing countries undergoing nutrition transition.[2] Findings from experimental animal studies and human investigations suggest that several varieties of cheese do not contribute to dental caries and that they may even help reduce the risk of caries.[3] Components of cheese such as protein (e.g., casein, bioactive peptides).[4] fat, calcium, and phosphorus may contribute to this food's caries protective effect. The potential mechanisms by which cheese may play a protective role in dental caries include its ability to stimulate saliva flow, inhibit plaque bacteria, and provide calcium and inorganic phosphate, which reduce demineralization and enhance remineralization of tooth enamel.[5,6] While evidence to date suggests a protective effect of cheese against dental caries, the active chemical or physical characteristics of cheese, which are involved in this relationship, are not fully understood.

Toumba and Curzon investigated the potential cariogenicity of seven New Zealand cheeses using the plaque sampling method. The tested cheeses were all shown to have low acidogenic

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**Table I:** Cheese Compositions

| Cheese | Moisture % | Fat % | Salt % | pH | Calcium mg/100g | Protein % | CPP * % |
|---|---|---|---|---|---|---|---|
| Control 1 (cheese 1) | 46.4 | 26.5 | 1.9 | 5.7 | 550 | 19.7 | - |
| Cook 1 Ca 1200mg/100gA (cheese 2) | 43.5 | 26.5 | 2.0 | 5.7 | 1430 | 19.8 | - |
| Cook 2 Ca 1800mg/100gA (cheese 3) | 40.9 | 26.5 | 1.9 | 5.7 | 2140 | 20.1 | - |
| Cook 3 Ca 1200mg/100gT (cheese 4) | 43.7 | 27.0 | 2.0 | 5.7 | 1420 | 19.8 | - |
| Cook 4 Ca 1800mg/100gT (cheese 5) | 42.5 | 27.0 | 1.9 | 5.6 | 2060 | 19.9 | - |
| Cook 1 CP 2% HCP101 (cheese 6) | 51.2 | 25.0 | 2.1 | 6.1 | 250 | 19.8 | 0.4 |
| Cook 2 CP 2% HCPIV (cheese 7) | 48.8 | 25.5 | 2.7 | 5.4 | 390 | 19.5 | 1.4 |
| Cook 3 CP 5% HCPIV (cheese 8) | 50.2 | 29.5 | 3.5 | 5.0 | 200 | 19.0 | 2.4 |
| Cook 1 CP 2% HCP101 (cheese 9) | 48.4 | 27.5 | 1.9 | 5.7 | 536 | 18.4 | 1.2 |

* Casein Phosphopeptide

and cariogenic potential.[7] However, it was not possible to say that cheeses caused remineralisation. So the authors recommended further research in enamel demineralisation and remineralisation testing to establish the mineral loss or gain with the cheese.

The aim of this study was to investigate the effect of nine New Zealand cheeses with different compositional components on the extent of demineralisation/remineralisation of enamel slabs as determined using the intra-oral cariogenicity test (ICT).

## Methods

This study was conducted at Leeds Dental Institute / UK during the year 2001. This ICT study design was that of Koulourides *et al*.[8] and later modified by Pollard.[9]

The study involved subjects using various cheeses in a double-blind randomised 11-leg cross-over design study. Nine New Zealand cheese products with different compositions, and 10% sucrose and 10% sorbitol controls were tested. The exact compositions of cheeses are shown in Table I.

### *Subject Selection*

Following approval by the Research Ethics Committee, at Leeds General Infirmary, 10 healthy dentate subjects participated in the study after giving their informed consent. The volunteers comprised of 9 females and one male with a mean age of 35.1 years ± 8.09, mean DMFS of 22.8 ±13.37, and mean salivary flow rate of 1.55 ml/minute ±0.69.

Subjects were healthy, not taking any medications and with no active periodontal disease. A DMFS score ≥ 12 was required and a minimum unstimulated salivary flow rate of 0.25 ml/min to participate in the study.

### *Enamel Slab Preparation*

Human premolar teeth, which were extracted for orthodontic purposes, were collected and stored in distilled water and thymol (Sigma Aldrich, Thymol 98%). Suitable teeth were

selected and lightly abraded with pumice and fine wet and dry abrasive paper (English Abrasives P320A) to remove the outermost enamel and any remnants of pellicle from the buccal surface.

The teeth were then painted with two coats of an acid resistance coloured nail varnish (Max Factor® "Diamond Hard"), except for a window of exposed enamel measuring approximately 6mm x 2mm in a mesial distal orientation on the buccal surface. The apical end of each tooth was covered in inlay wax and attached to a suitable length of solid glass rod to hold the tooth in the demineralising gel. The rod was secured to the lid of a "Sterilin" type universal tube so that when the top was screwed onto the tube, the tooth was suspended in the centre of the tube's free space.

The acidified gel that was used for the creation of the artificial white spot lesions was based on that described by Edgar.[10] The gel was then poured into the tubes in which the mounted teeth were submerged. The teeth were left in the gel for four to seven days until a white spot was clearly visible.

On removal from the gel, the teeth were washed thoroughly in de-ionised water, and the varnish carefully removed with acetone (GPR, BDH, Poole, England). Once cleaned the teeth were air dried and mounted in "greenstick" impression compound (Kerr) on plates that fitted into the cutting machine, a "Well" diamond wire saw (Well® Walter EBNER, CH-2400 Le Loche). Each tooth was carefully sectioned to give the white spot lesion measuring 6 mm length x 2mm width x 1.5 mm thickness. This was then further divided to give three equal sized enamel portions of approximately 2mm x 2mm x 1.5mm. Two were used as "test slabs" that were mounted within the *in situ* appliances while the third was retained to serve as the control.

The enamel slabs were stored damp in sealed containers and exposed to gamma radiation for sterilisation (4080 Gy) in the Department of Chemistry of the University of Leeds.

### *Experimental Protocol*

A mandibular removable Hawley appliance with a labial arch wire and U clasps and acrylic flanges buccally to the first permanent molars were made for each volunteer. The enamel slab with an artificial white spot lesion was randomly assigned to the left side of the appliance, where a well was made in the buccal surface of the acrylic flange.

The selected enamel slab was secured in its position with sticky wax, care being taken to ensure that the wax did not cover the exposed surface of the enamel. The slab was then covered by 0.15 mm Dacron gauze (Meadox Medicals, Oakland, NJ, USA) to promote plaque accumulation. The enamel slab was placed so that its surface was level with that of the acrylic.[9]

The subjects were asked to wear their appliances continuously (except for eating and drinking) for two days to allow plaque to grow. Over the following 5 days, they were asked to immerse their appliance 4 times per day for 10 minutes each time in a suspension of the test food or control solution.

For the cheese samples this was achieved by having the subjects chew 10 gm portions of the test cheeses for one minute to obtain cheese/saliva slurry. This slurry was used to cover the enamel slabs for the 10 minutes immersion periods. After the 10 minutes immersion periods, the appliances were removed, rinsed with water, and replaced in the mouth. This procedure was repeated four times during the day.

The subjects were assigned randomly to either one of the test groups or to the control groups according to a Latin square table. The volunteers had a washout period of at least seven days between each test cheese and the control solutions, to prevent any carry-over effect.

### *Specimen Preparation and Microradiography Processing of the Lesion*

After the intra-oral periods, the enamel slabs were removed from the appliances and mounted in "green stick" impression compound (Kerr) onto stubs. The enamel slabs were sectioned using the diamond wire saw, which was described earlier, to give specimens with thickness of approximately 250 μm.

The sections were then placed on a brass anvil and secured using the nail varnish. This in turn was suspended above a diamond disc which was impregnated with 15 μm diamond particles (Beuhler, Illinois) on three accurately milled

bearings (Spheric Engineering, Ltd., Crawley, UK) in circular movement.

The anvil and the diamond plate were a total of 11.00 mm thick, so when the smallest ball bearing was running free it produced a section of enamel that was 80-100 μm thick. This thickness is recommmended for optimum analysis by transverse microradiography.

### *Microradiography*

The control and experimental sections were placed in a specially fabricated radiographic plate-holding cassette, incorporating an aluminium step-wedge (steps of 25 μm thickness). The mounted sections were scanned using a Mustek 8000 SP, A4 colour scanner, running at 600 d.p.i. and the digitised image retained to aid identification of individual sections on the exposed plate. Following scanning, and processing, the microradiographs were then subjected to image analysis using a computer programme written by de Josselin de Jong.[11] The mineral content of the specimens was expressed as mineral loss, and lesion depth. These parameters are useful to describe important features of a lesion. A fall in mineral loss represents remineralisation of the body of the surface of the lesion and a reduction in depth represents remineralisation at the former advancing front of the lesion.

### *Statistical analyses*

For each lesion, the lesion depth (LD) and mineral loss (ML), assessed by image analysis, was compared. Comparisons between baseline controls (C) and exposure to the test cheeses and positive and negative control (T) were examined using student paired t- tests.

As different tooth samples (enamel slabs) were used on each occasion and hence different controls, the comparisons between the different samples were made with the percentage change (DLD% and DML%) in lesion depth and mineral loss (which is a change relative to the control).

$$\text{The percentage difference} = \frac{\text{Control-Test}}{\text{Control}} \times 100$$

## Results

### *Changes in Lesion Depth*

When the subjects used the cheeses as the test group, the lesion depth (μm) revealed a reduction between test and control with cheese 2 ($p< 0.001$) and cheese 3 ($p<0.05$) which was statistically significant. With cheese 1, cheese 3, cheese 4, cheese 5, cheese 6 and cheese 8, the lesion depth also revealed a reduction between test and control slabs, but it was statistically not significant ($p>0.05$). When the subjects used the negative control (10% sorbitol) there was a reduction in the lesion depth, and it was statistically not significant.

On the other hand, with cheese 7 and cheese 9, the lesion depth revealed an increase between the test and control slabs, but it was statistically not significant. There was also an increase in the lesion depth with the positive control (10% sucrose) and it was statistically not significant. Table II shows the results of the paired t-test. Figure 1 shows the differences in the lesion depth in the control and test samples.

### *Percentage Difference in Lesion Depth*

Similar results were evident when the percentage differences in lesion depth were analysed. Remineralisation was evident in the following order: with cheese 2, cheese 3, cheese 4, cheese 8, cheese 1, cheese 5, cheese 6 and was also evident with 10% sorbitol. Demineralisation was evident with cheese 7, cheese 9, and 10% sucrose.

### *Changes in Mineral Loss*

When the subjects used cheese as the test group, remineralisation was evident with cheese 2, cheese 3, cheese 4, cheese 5, cheese 8 and cheese 9, this was statistically significant ($p<0.05$). With cheese 1, cheese 6 and cheese 7, remineralisation was also evident but this was not significant. When the subjects used the positive and negative controls as test group, there was evidence of remineralisation but it was not significant. Table III shows the results of the paired t-test. Figure 2 shows the differences in the mineral loss in the control and test samples.

### *Percentage Difference in Mineral Loss*

Analysing the percentage differences in mineral loss also revealed similar results where remineralisation was evident in the following order: with cheese 3, cheese 2, cheese 4, cheese 5, cheese 8, cheese 1, cheese 9, cheese 6, and cheese 7.

**Table II.** Results of the Paired t-Test for the Lesion Depth

| Cheese | Mean CLD° | Mean TLD°° | Paired Differences: Mean | S.D.• | S.E. •• Mean | 95% confidence Interval of the Difference: Lower | Upper | Significance (2-tailed) |
|---|---|---|---|---|---|---|---|---|
| Cheese 1 | 62.2 | 54.7 | 7.4 | 22.1 | 7.0 | -8.4 | 23.2 | 0.315 (N.S.)* |
| Cheese 2 | 71.8 | 51.6 | 20.2 | 13.1 | 4.2 | 10.8 | 29.6 | 0.001 (S) ** |
| Cheese 3 | 69.4 | 54.7 | 14.7 | 16.3 | 5.1 | 3.1 | 26.3 | 0.019 (S) |
| Cheese 4 | 63.4 | 51.8 | 11.6 | 22.3 | 7.1 | -4.3 | 27.6 | 0.133 (N.S.) |
| Cheese 5 | 59.5 | 54.5 | 5.0 | 13.7 | 4.3 | -4.9 | 14.8 | 0.284 (N.S.) |
| Cheese 6 | 57.4 | 54.6 | 2.9 | 17.1 | 5.4 | -9.4 | 15.1 | 0.610 (N.S.) |
| Cheese 7 | 63.6 | 63.7 | -0.1 | 13.4 | 4.2 | -9.7 | 9.5 | 0.985 (N.S.) |
| Cheese 8 | 71.0 | 62.3 | 8.7 | 12.4 | 4.0 | -0.2 | 17.6 | 0.055 (N.S.) |
| Cheese 9 | 59.7 | 62.3 | -2.7 | 8.7 | 2.8 | -8.9 | 3.5 | 0.354 (N.S.) |
| 10% Sucrose | 58.1 | 58.9 | -0.7 | 12.7 | 4.0 | -9.8 | 8.4 | 0.865 (N.S.) |
| 10% Sorbitol | 60.2 | 50.7 | 9.5 | 17.0 | 5.4 | -2.6 | 21.6 | 0.110 (N.S.) |

°= control lesion depth °°= test lesion depth •= standard deviation ••= standard error
* = non significant ** = significant

**Table III.** Results of Paired t-Test for Mineral Loss

| Cheese | Mean CML° | Mean TML°° | Paired Differences: Mean | S.D. • | S.E. •• Mean | 95% confidence Interval of the Difference: Lower | Upper | Significance (2-tailed) |
|---|---|---|---|---|---|---|---|---|
| Cheese 1 | 1319.7 | 846.6 | 473.1 | 836.6 | 264.6 | -125.4 | 1071.6 | 0.107 (N.S.) * |
| Cheese 2 | 1687.2 | 669.1 | 1018.1 | 964.3 | 304.9 | 328.3 | 1707.9 | 0.009 (S) ** |
| Cheese 3 | 1398.3 | 592.8 | 805.5 | 312.6 | 98.9 | 581.9 | 1029.2 | 0.000 (S) |
| Cheese 4 | 1792.9 | 805.5 | 987.4 | 1080.3 | 341.6 | 214.6 | 1760.2 | 0.018 (S) |
| Cheese 5 | 1305.5 | 666.2 | 639.3 | 526.3 | 166.4 | 262.9 | 1015.8 | 0.004 (S) |
| Cheese 6 | 1125.1 | 757.9 | 367.2 | 584.9 | 1854.0 | -51.3 | 785.6 | 0.078 (N.S.) |
| Cheese 7 | 1359.6 | 927.1 | 432.5 | 608.4 | 192.4 | -2.7 | 867.8 | 0.051 (N.S.) |
| Cheese 8 | 1496.1 | 858.4 | 637.71 | 697.1 | 220.4 | 139.1 | 1136.4 | 0.018 (S) |
| Cheese 9 | 1823.0 | 1296.4 | 525.8 | 463.7 | 146.6 | 194.9 | 858.3 | 0.006 (S) |
| 10% Sucrose | 1039.3 | 768.5 | 270.9 | 632.6 | 200.1 | -181.7 | 723.5 | 0.209 (N.S.) |
| 10% Sorbitol | 1272.5 | 618.1 | 654.4 | 1104.7 | 349.4 | -135.9 | 1444.7 | 0.094 (N.S.) |

°= control mineral loss °°= test mineral loss •= standard deviation ••= standard error * = non significant ** = significant

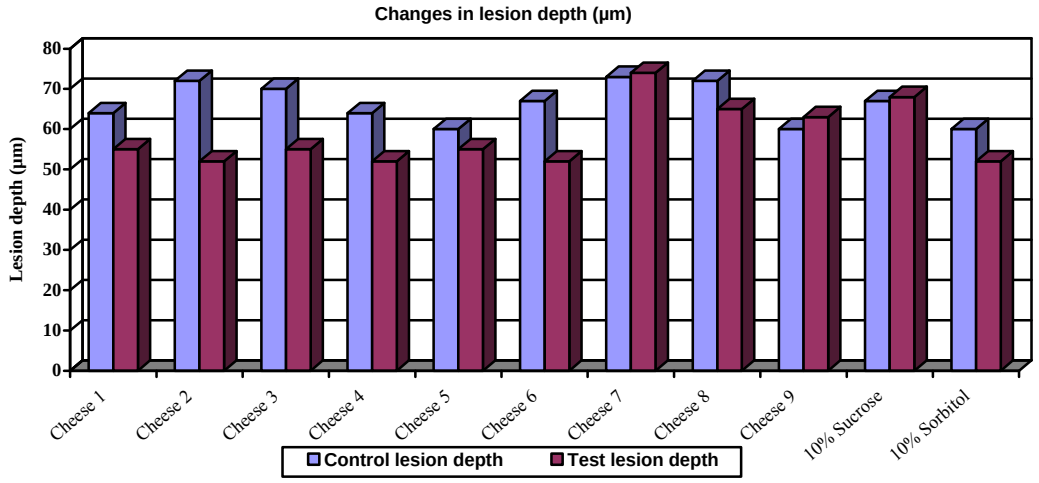


**Fig 1:** Changes in lesion depth

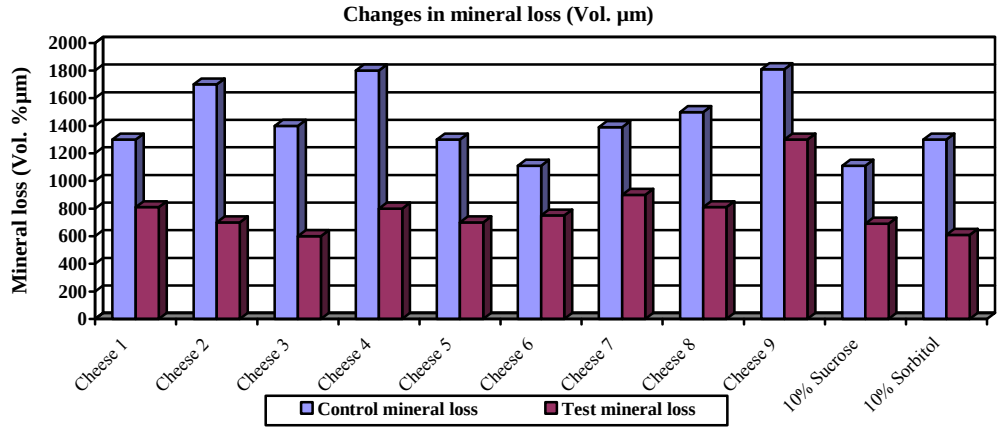


**Fig 2.** Changes in mineral loss

## Discussion

It was interesting to note that the cheeses that caused significant remineralisation, had higher calcium and protein content. These results support previous reports of the cariostatic effect of cheese. Harper *et al*.[12] in their study found that the most caries inhibiting cheeses were the highest in protein and calcium phosphate content. Eating cheese increases plaque calcium concentration.[13,14,15] Higher concentration of ionic calcium and phosphate would produce a loading of the plaque with calcium and phosphate. Calcium concentration in dental plaque is an important determinant of the balance between enamel de- and remineralisation since the rate of dissolution of enamel is determined mainly by the level of saturation with calcium and phosphate ions of the tooth environment.[16] Elevated levels of calcium and/or possibly phosphorus, in dental plaque might inhibit demineralisation through a common-ion effect, or might enhance remineralisation during periods of high pH.[15-17] In addition, there is evidence that high extracellular free calcium concentrations may have bacteriostatic or even bactericidal effects.[18] Protein present in the cheese might prevent caries by adsorbing to the enamel surface and interfering with ionic diffusion at the plaque enamel interface. This would be possible during the first few hours before the formation of pellicle and plaque.[15] The majority of protein in milk and cheese is highly phosphorylated and with marked affinity for the hydroxyapatite. This affinity could be so strong that the casein could displace non-phosphorylated protein and high molecular weight dextrans. The phosphoproteins could also have a regulatory role in the mineral process by regulating the movement of calcium

and phosphate between the crystal lattice and the hydration layer.[19] On the other hand, other cheeses such as cheese 8, which contained the highest percentage of casein phosphopeptides (CPP) also, caused remineralisation. CPP by stabilising calcium phosphate could facilitate high concentration of calcium and phosphate ions, which could diffuse into the enamel subsurface lesion. CPP could also maintain the high activities of the free calcium and phosphate ions during remineralisation through the reservoir of bound amorphous calcium phosphate.[20] Another possible explanation for the protective capacity may be related to the fat content. Fat might have a protective role both physically and possibly by inhibition of microbial metabolism.[21] Fat might also exert a beneficial effect by acceleration of oral clearance of the carbohydrates, thereby decreasing the cariogenic potential.[22] The remineralisation seen with sucrose could be explained by the use of fluoridated toothpaste by the volunteers. The study of (Duggal *et al*., 2001)[23] for the investigation of the extent of demineralisation of enamel slabs in situ, using a sugar based solution, consumed in constant amounts but varying frequency in subjects using fluoride toothpaste, showed a net remineralisation of enamel slabs in subjects using sucrose solution with the once, 2, and 5 times/day. So in this study it is not surprising to get remineralisation with sucrose used 4 times/day. The results support previous reports of the cariostatic potential of cheese.[15,19,24,25] and suggest that a substantial portion of protection may be afforded through prevention of enamel demineralisation and promotion of remineralisation, which may be related to casein-calcium content of the cheese.

## Recommendations

- Further research in enamel demineralisation and remineralisation to establish the mineral loss or gain with the cheeses, using different methods of assessment.
- Further research in enamel demineralisation and remineralisation to establish the mineral loss or gain with the cheeses, using non fluoridated toothpaste.
- Further research in remineralisation testing to investigate the exact mechanism by which cheeses can cause remineralisation.

## Conclusion

Cheese can cause remineralisation of early lesions.

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