Short Communication

Caries Research

Caries Res 2010;44:20–23
DOI: 10.1159/000271593

Received: April 2, 2009
Accepted after revision: November 4, 2009
Published online: December 31, 2009

Dental Erosion Protection by Fermented Shrimp Paste in Acidic Food

C. Chuenarrom P. Benjakul

Faculty of Dentistry, Prince of Songkla University, Hat Yai, Songkhla, Thailand

Key Words
Erosion protection · Fermented shrimp paste · Microhardness · Tooth erosion

Abstract
The aim of this study was to determine the extent to which fermented shrimp paste (which has a high calcium concentration) reduces dental erosion in vitro. In experiment 1, enamel specimens were exposed to various concentrations of shrimp paste in tamarind juice for 15 min, once a day, for a total of 29 days. In experiment 2, pre-softened enamel specimens were exposed to different concentrations of shrimp paste in water, using an exposure method similar to experiment 1. Profilometry and a microhardness test were used to assess changes in enamel loss and softening. The results showed that shrimp paste can reduce the erosive potential of tamarind juice and re-harden softened enamel.

Many previous investigations have reported that the consumption of low-pH beverages or foods is the main extrinsic factor in dental erosion. Although these substances can erode enamel, when one considers their popularity, it is difficult to imagine consumption would be reduced for dental health protection. One solution, which has been investigated in many previous reports, is to raise the calcium concentration of drinks [Hughes et al., 1999; Davis et al., 2007; Hooper et al., 2007] or to consume high-calcium foods, such as cheese, which can protect against dental erosion [Gedalia et al., 1991].

The present study focuses on fermented shrimp paste (SP), as it is also high in calcium. The calcium concentration of some shrimp byproducts (heads, shells and tails) has been found to be as high as 3,000 mg/100 g [Heu et al., 2003]. Essentially, fermented SP consists of whole tiny shrimp, salted, fermented and dried until they break down into a paste. It has a very salty taste and a strong smell. Fermented SP is used as a general condiment and seasoning in Southeast Asian and Southern Chinese cooking. It is known as 'kapi' in the Thai, Khmer and Lao languages, 'terasi' in Indonesian, 'ngapi' in Burmese, 'belacan' in Malay, 'mam tom' in Vietnamese, 'bagoong alamang' in Filipino and 'hom ha/hae ko' in Min Nan Chinese.

In Thailand, fermented SP is an ingredient in many sour foods, or is cooked as a sauce to eat with tart fruits. It is an important ingredient in curry paste. The most popular curry in southern Thailand is 'kaeng som', a hot and sour curry with a tamarind juice (TM) base. Therefore, 'kaeng som' is a source of concern regarding tooth erosion [Tiantananurak, 2004]. Unexpectedly, the erosion potential of 'kaeng som' decreases at elevated temperatures, unlike that of simple acid solutions [Eisenburger and Addy, 2003]. However, Barbour et al. [2006] found that for a drink containing calcium, temperature had no statistically significant impact on erosion prevention. Therefore, the chemical reaction between 'kaeng som' and enamel, which decreases as temperature increases, may be related to the calcium content in SP.

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0008–6568/10/0441–0020$26.00/0

Accessible online at:
www.karger.com/cre

Chanya Chuenarrom
Department of Prosthetic Dentistry, Faculty of Dentistry
Prince of Songkla University
Hat Yai, Songkhla 90110 (Thailand)
Tel. +66 7428 7562, Fax +66 7442 9874, E-Mail chanya.ch@psu.ac.th

Table 1. Characteristics and baseline surface microhardness (SMH) values of enamel before immersion, percentage of SMH change (%SMHC), and enamel loss

Agents	pH	TA mmol/l	Composition	Baseline SMH kg/mm^2	%SMHC	Enamel loss μm
Experiment 1						
TM	2.74	55.5	TM	335.3 (17.8)	–82.5 (8.1)	122.2 (14.1)[a]
0.5× SP + TM	2.84	54.0	0.33 g SP in 100 ml TM	337.4 (18.1)	–82.1 (6.7)	99.0 (5.6)[b]
1× SP + TM	2.96	53.5	0.65 g SP in 100 ml TM	320.8 (22.2)	–84.6 (4.9)	79.4 (7.0)[c]
2× SP + TM	3.17	50.5	1.33 g SP in 100 ml TM	325.1 (36.2)	–83.2 (4.8)	46.6 (8.9)[d]
Experiment 2						
Saliva	6.75	n.a.	artificial saliva	241.5 (13.9)	17.0 (11.8)[a, b]	0.4 (0.4)
0.5× SP + DW	5.95	0.75	0.33 g SP in 100 ml DW	246.6 (26.9)	12.0 (7.2)[a]	0.5 (0.4)
1× SP + DW	5.86	1.5	0.65 g SP in 100 ml DW	243.0 (11.8)	21.0 (9.1)[a, b]	0.5 (0.5)
2× SP + DW	5.90	3.0	1.33 g SP in 100 ml DW	242.3 (11.8)	27.8 (8.5)[b]	0.5 (0.3)

Figures in parentheses are SD. Values within columns sharing the same superscript letter are not significantly different ($p > 0.05$).

The aim of this in vitro study was to investigate the protection against dental erosion afforded by fermented SP. TM was selected as the erosive agent because it is an acidic juice that is used in many Asian dishes. The investigation was divided into 2 independent experiments. First, different concentrations of SP and TM were used to investigate the effect of SP on the erosive potential of TM. In the second experiment, mixtures of SP and distilled water (DW) were used to determine the effect of SP on the re-hardening of softened enamel.

Materials and Methods

Assigning Agents for Analysis

The average SP and TM contents from 10 'kaeng som' recipes from cookbooks were (for 100 ml of 'kaeng som') 0.65 g SP and 10.3 g concentrated TM. TM was prepared from 10.3 g concentrated TM (Taladthai, Thai Market Agriculture, Thailand) dissolved in 100 ml water. The fermented SP (Pantainorasingh Manufacturing, Thailand) was a grey cake type, composed of 90% shrimp and 10% salt. Calcium concentration of the SP was analyzed by the ICP-OES technique using an optical emission spectrometer (Optima 4300 DV; PerkinElmer, Waltham, Mass., USA).

Three different concentrations of SP in TM and DW were used in this study (table 1). The abbreviations 0.5× SP, 1× SP and 2× SP refer to 50, 100 and 200% of the average amount of SP used in a 'kaeng som' recipe. The pH of the assigned agents at 37°C were determined using a pH meter (pH900, Precisa Instruments, Dietikon, Switzerland). Next, 20 ml of each agent were titrated with 0.1 N NaOH to raise their original pH to 7.0. The volume of 0.1 N NaOH used in the titration determined the titratable acidity.

Enamel Specimen Immersion

Enamel specimens derived from surgically removed caries-free human third molars were used for the study. A total of 160 longitudinal enamel sections were cut from 40 molars using a water-cooled diamond saw (IsoMet 4000, Buehler, Lake Bluff, Ill., USA). Specimens were then embedded in acrylic resin blocks. An outer enamel surface of each specimen was ground flat using 320, 600 and 1,200 grit silicon carbide paper, then polished with a 1-μm diamond suspension (Wirtz-Buehler, Düsseldorf, Germany) to produce an approximately 1.5 × 1.5 mm^2 flattened window of enamel.

The investigation was divided into 2 independent experiments. Each experiment was composed of 4 treatment groups of 20 specimens. Ten specimens in each group were prepared to measure enamel loss, and the other 10 for microhardness determination.

Experiment 1. This study was conducted to determine the erosive potential of TM, and TM containing SP. TM was used as a positive control. The 3 concentrations of SP in TM already mentioned were used to test the ability of SP to protect against erosion.

Experiment 2. The aim was to study the effect of SP on the re-hardening of softened surface enamel. All specimens were eroded by TM for 15 min to create softened enamel, and then immersed in 1 of 4 agents: artificial saliva [McKnight-Hanes and Whitford, 1992] (a negative control) or SP in DW at the 3 concentrations described above.

In both experiments, each group of specimens was exposed to the assigned agent, shaken at 100 rpm in a continuously vibrating water bath (WNB22; Memmert, Büchenbach, Germany) at 37°C for 15 min. Specimens were then rinsed with tap water, and stored in artificial saliva at 37°C in an incubator until the next day. Each group was immersed in its assigned agent once a day for a total of 29 days. The agents and artificial saliva were changed daily.

Microhardness and Enamel Loss Measurement

Surface microhardness (SMH) of specimens was measured using a microhardness tester (Micromet II, Buehler) with a load of

200 g for 10 s. Each specimen was indented 3 times in different areas, both before and after exposure to the assigned agents, to ascertain the average SMH on days 1, 8, 15, 22 and 29. The percentage SMH change (%SMHC) was calculated from the difference between the baseline SMH (SMH_0) and SMH after exposure to the agents (SMH_1), as: $100(SMH_1 - SMH_0)/SMH_0$.

Enamel loss was measured using a contact stylus profilometer (Surfcorder SE-2300, Kosaka Laboratory, Tokyo, Japan) with a 5-μm radius stylus tip under a 4-mN load. The stylus traveled at a velocity of 0.5 mm/s perpendicular to the specimen surface, across the reference (the acrylic resin surface surrounded the exposed areas) and exposed areas for a tracing of 2.5 mm in length. The difference in height in relation to the baseline and the eroded enamel was measured and averaged from 3 equally spaced positions across the profile using the image analysis software (Adobe Photoshop CS, Adobe, San Jose, Calif., USA). The accurate length measurement of the software was calibrated by measuring the vertical magnification marker of the profile. The enamel loss of each specimen was obtained from 2 tracings and then averaged.

Statistical Analysis

The normal distribution and the homogeneity of variance of each response variable were examined using a Kolmogorov-Smirnov test and Levene's test, respectively. Hardness values for each group at different times were analyzed using repeated-measures ANOVA. Independent statistical analyses of enamel loss and %SMHC variables at day 29 were evaluated by ANOVA, followed by Tukey's test. The level of significance was set at $p < 0.05$.

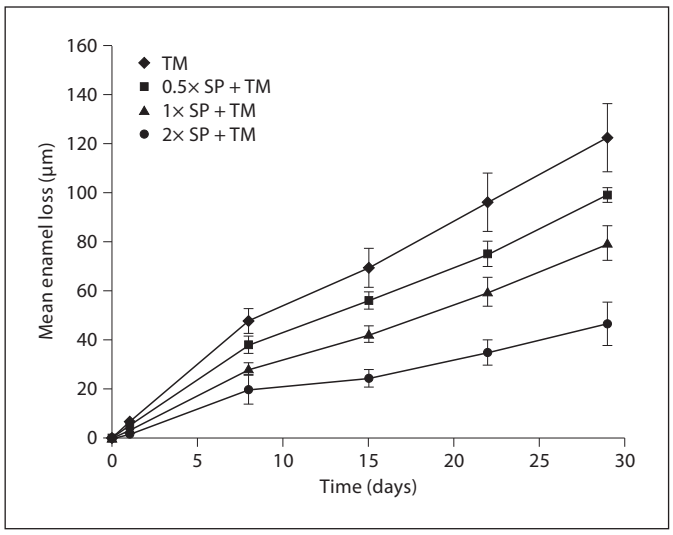

Fig. 1. Mean enamel loss of specimens exposed to either TM or SP + TM agents for 15 min daily over a 29-day period.

Results

The calcium concentration of SP was 13.7 mg/g. The pH and titratable acidity of each of the agents are given in table 1. The addition of SP to the TM increased pH and reduced titratable acidity, but these effects were small. Figure 1 shows the enamel loss in experiment 1 over 29 days. This reveals that the addition of SP to TM had the effect of reducing enamel loss. Among the 4 assigned agents, the order (greatest to least) of enamel loss was: TM, 0.5× SP + TM, 1× SP + TM, and 2× SP + TM. On day 29, all groups were significantly different from each other ($p < 0.05$), whereas there were no statistically significant differences ($p > 0.05$) in %SMHC between the groups (table 1).

In experiment 2, hardness of enamel decreased to 241.5–246.6 kg/mm^2 after the preliminary softening. On day 29, enamel loss among the treatment groups showed no statistically significant differences ($p > 0.05$). Repeated-measures ANOVA showed that the hardness of softened enamel significantly increased after exposure to artificial saliva or the SP solutions. On day 29, %SMHC in the 0.5× SP + DW, 1× SP + DW and 2× SP + DW groups were not significantly different from the control group (saliva), whereas 2× SP + DW significantly re-hardened the softened enamel in comparison with 0.5× SP + DW ($p < 0.05$).

Discussion

Lussi et al. [2004] found that the calcium concentration of a foodstuff or beverage seems to be one of the most important factors for the prediction of erosive potential. For example, yoghurt is a food with a low pH, but it hardly has an erosive effect due to its high calcium and phosphate content. Therefore, product modification to reduce the erosive potential may require the addition of calcium and/or phosphorus. Hooper et al. [2007] found that 100 mg/l of calcium inhibited enamel erosion by an acidic beverage (pH 3.4) during 10 days of in situ exposure. Davis et al. [2007] studied the prevention of erosion by 4 juices: orange and grapefruit juices, which were fortified with 1,480 mg/l of calcium; and apple and white grape juices, which were fortified with 423 mg/l of calcium. The results indicated that the calcium concentration in orange, grapefruit and apple juices was sufficient to prevent erosion, but was insufficient in the case of white grape juice. Larsen and Nyvad [1999] reported that orange juice supplemented with 40 mmol/l of calcium (or 1,600 mg/l) prevented enamel erosion. In the present study, adding 0.65 g SP to 100 ml of water or TM resulted in calcium concentrations of 89 mg/l. These studies indicate that the

appropriate calcium content for erosion prevention varies for specific drinks or foodstuffs.

Experiment 1 revealed that if TM were considered to have 100% erosive potential, then 0.5× SP + TM, 1× SP + TM and 2× SP + TM would have only 80, 61 and 36% erosive potential, respectively. This indicated that the erosive inhibition of SP in TM depends on its concentration. Interestingly, the %SMHC among the treatment groups was not statistically significantly different during the experimental period. The pattern of surface softening behaves in a different way than enamel loss, as was shown in a previous study [Eisenburger and Addy, 2003]. These researchers explained that enamel loss progresses faster than surface demineralization, and that an upper limit is reached for the latter process. The study also revealed that a microhardness test was not appropriate for studying the erosive potential of an acidic agent over long experimental periods.

Yoshida et al. [2001] explained the concept of adhesion/decalcification resulting from carboxylic acid interacting with hydroxyapatite. Likewise, the calcium ions in SP could interact with the carboxyl group of tartaric acid to create calcium salt; hence, the number of available carboxyl groups of tartaric acid would decrease, and calcium would be extracted from the enamel surface at a lower rate. In addition, the small increase in the pH of TM caused by adding SP would result in a decrease in erosive potential.

Under the designated experimental conditions, the results of experiment 2 showed that the enamel surfaces did not return to their original state, but all softened enamel groups were capable of re-hardening softened enamel. Although %SMHC of 3 test groups were not significantly different from the control group (saliva), the levels of re-hardening of softened enamel surfaces by the assigned agents were (in the order greatest to least): 2× SP + DW, 1× SP + DW, 0.5× SP + DW, although the statistical evidence for a dose-response effect was not conclusive. Several in vitro studies have shown that calcifying solutions can re-harden the enamel surface [Collys et al., 1991; Eisenburger et al., 2001a, b] and Gedalia et al. [1991] found that cheese consumption significantly increased the hardness of softened enamel. SP may demonstrate a different re-hardening potential in the oral environment due to the acquired pellicle, but this possibility was not incorporated into the present in vitro study. Further research in situ is necessary to study the enamel surface re-hardening potential of SP.

Finally, this study demonstrated that a tiny amount of SP added to sour foods has a high efficiency in reducing enamel loss. Experiment 2 supported the finding that the more SP added to foods, the greater the re-hardening of softened enamel. Thus, Asian food containing SP could be introduced as a healthy food for consumers who have enamel erosion problems.

Acknowledgments

This study was supported by research grant DEN5122020021S from Prince of Songkla University, Songkhla, Thailand. The authors would like to thank Dr. Alan Geater for statistical advice and Mr. Mitchell Atkins for language editing.

References

Barbour ME, Finke M, Parker DM, Hughes JA, Allen GC, Addy M: The relationship between enamel softening and erosion caused by soft drinks at a range of temperatures. J Dent 2006;34:207–213.

Collys K, Cleymaet R, Coomans D, Slop D: Acid-etched enamel surfaces after 24 h exposure to calcifying media in vitro and in vivo. J Dent 1991;19:230–235.

Davis RE, Marshall TA, Qian F, Warren JJ, Wefel JS: In vitro protection against dental erosion afforded by commercially available, calcium-fortified 100 percent juices. J Am Dent Assoc 2007;138:1593–1598.

Eisenburger M, Hughes J, West NX, Shellis RP, Addy M: The use of ultrasonication to study remineralisation of eroded enamel. Caries Res 2001a;35:61–66.

Eisenburger M, Addy M, Hughes JA, Shellis RP: Effect of time on the remineralisation of enamel by synthetic saliva after citric acid erosion. Caries Res 2001b;35:211–215.

Eisenburger M, Addy M: Influence of liquid temperature and flow rate on enamel erosion and surface softening. J Oral Rehabil 2003; 30:1076–1080.

Gedalia I, Ionat-Bendat D, Ben-Mosheh S, Shapira L: Tooth enamel softening with a cola type drink and rehardening with hard cheese or stimulated saliva in situ. J Oral Rehabil 1991;18:501–506.

Heu M, Kim J, Shahidi F: Components and nutritional quality of shrimp processing by-products. Food Chem 2003;82:235–242.

Hooper S, Hughes J, Parker D, Finke M, Newcombe RG, Addy M, West N: A clinical study in situ to assess the effect of a food approved polymer on the erosion potential of drinks. J Dent 2007;35:541–546.

Hughes JA, West NX, Parker DM, Newcombe RG, Addy M: Development and evaluation of a low erosive blackcurrant juice drink in vitro and in situ. 1. Comparison with orange juice. J Dent 1999;27:285–289.

Larsen MJ, Nyvad B: Enamel erosion by some soft drinks and orange juices relative to their pH, buffering effect and contents of calcium phosphate. Caries Res 1999;33:81–87.

Lussi A, Jaeggi T, Zero D: The role of diet in the aetiology of dental erosion. Caries Res 2004; 38(suppl 1):38–44.

McKnight-Hanes C, Whitford GM: Fluoride release from three glass ionomer materials and the effects of varnishing with or without finishing. Caries Res 1992;26:345–350.

Tiantananurak N: Evaluation of acidic Thai food on dental enamel; thesis, Prince of Songkla University, 2004.

Yoshida Y, Van Meerbeek B, Nakayama Y, Yoshioka M, Snauwaert J, Abe Y, Lambrechts P, Vanherle G, Okazaki M: Adhesion to and decalcification of hydroxyapatite by carboxylic acids. J Dent Res 2001;80:1565–1569.