# MICROBIAL AND BEHAVIORAL DETERMINANTS OF DENTAL CARIES IN PERMANENT DENTITION AMONG THAI SCHOOLCHILDREN

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**Abstract.** Identifying the causes of dental caries is important to develop effective prevention strategies. This study aimed to identify factors associated with dental caries in permanent dentition of Thai schoolchildren. We conducted a cross section study to evaluate the microbial and behavioral factors associated with caries by comparing children with and without caries. We randomly selected study subjects to determine their surface-specific dental caries counts using the decayed, missing and filled surfaces (DMFS) index: socio-demographic data, snacking and oral care behaviors were determined using a self-administered questionnaire. The saliva of each subject was examined and tested for buffer capacity and level of salivary cariogenic bacteria [*Streptococcus mutans* (SM) and Lactobacilli (LB)]. Multiple linear regression analysis was used to determine the potential determinants of the DMFS score. A total of 139 subjects were included in the study. The mean [± standard deviation (SD)] age of subjects was 13.4 (± 0.93) years. The study subjects had different brushing and snacking behaviors. The average (±SD) DMFS score was 7.65 (± 5.46). The factors significantly associated with caries were buffering capacity, level of salivary cariogenic bacteria and some oral care behaviors (brushing after lunch, brushing before bed and using fluoride toothpaste) ($p$ <0.001). Caries prevention education in the study population should include education about quality of tooth brushing. Further studies are needed to determine if these prevention control measures can be effective.

**Keywords:** caries, determinants, DMFS, schoolchildren, Lactobacilli, *Streptococcus mutans*

## INTRODUCTION

Dental caries result from the imbalance between remineralization and demineralization of the tooth structure (Selwitz *et al*, 2007). Interactions between bacterial fermentation of dietary carbohydrates over time and host factors relating to the teeth and saliva are associated with demineralization of tooth structures (Caufield *et al*, 2005). An ecological imbalance between oral microbial biofilm and tooth minerals can cause dental caries (Selwitz *et al*, 2007). Microorganisms can produce acid in the carbohydrate substrate. Patients with dental caries typically have high levels of *Streptococcus mutans* (SM) (Arino *et al*, 2015) and Lactobacilli (LB) (Borgstrom *et al*, 2000; Arino *et al*, 2015). The levels of

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these bacteria are associated with dental caries (Drucker *et al*, 1995). SM initiate fissures and root caries (Fitzgerald and Keyes, 1960; Tanzer *et al*, 2001) and LB are important subservient bacteria (Tanzer *et al*, 2001). Higher levels of SM are found in people with greater numbers of decayed teeth (Dasanayake *et al*, 1995). Caries free subjects have been found to have few or no SM (Singh *et al*, 2015). LB can be found in carious lesions later than SM (Crossner *et al*, 1989; Tanzer *et al*, 2001).

Saliva plays an important role in demineralization and remineralization of teeth. The buffer capacity of saliva can alter the salivary pH (Voelker *et al*, 2013). Saliva with a higher buffering capacity can raise an acidic pH up to normal faster than saliva with a low buffering capacity (Aiuchi *et al*, 2008). Caries free individuals tend to have saliva with a higher buffering capacity (Vitorino *et al*, 2006; Singh *et al*, 2015).

Determinants of caries include age (Attin *et al*, 1999; Baelum *et al*, 2009), gender (Crossner and Unell, 2007; Molina-Frechero *et al*, 2009), socio-economic status (SES) (Källestål and Wall, 2002; Hobdell *et al*, 2003; Alm, 2008; Al Agili and Alaki, 2014), oral hygiene (Tayanin *et al*, 2005), fluoride use (Tayanin *et al*, 2005; Alm, 2008), frequency of tooth brushing (Alm, 2008; Molina-Frechero *et al*, 2009) and a high sugar diet (Marshall *et al*, 2005; Molina-Frechero *et al*, 2009). Other factors associated with dental caries include increasing age (Attin *et al*, 1999; Baelum *et al*, 2009) and consumption of a sugary diet (Marshall *et al*, 2005; Molina-Frechero *et al*, 2009). People with good oral hygiene, who use fluoride regularly and brush their teeth frequently tend to have fewer dental caries (Tayanin *et al*, 2005; Alm, 2008; Molina-Frechero *et al*, 2009). Most studies have reported associations between cavitated caries lesions and various studied factors (Zhang and Helderman, 2006; Alm, 2008). The Bureau of Dental Health (2013) reported the prevalence of dental caries among Thai children aged 12 years to be 52.3% and among Thai children aged 15 years to be 62.4%; 22.0% of Thai children aged 15 years had initial caries and 36.0% had untreated caries with an average number of decayed, missing and filled teeth (DMFT) of 1.9 (Bureau of Dental Health, 2013).

It is important to identify the causes of dental caries in order to develop caries prevention strategies. There are few studies of the association between bacterial and behavioral factors and non-cavitated caries among schoolchildren. The aim of this study was to identify microbial and behavioral factors associated with dental caries in permanent dentition among Thai schoolchildren aged 11-17 years.

## MATERIALS AND METHODS

**Participants**

A total of 192 schoolchildren studying in grades 6-8 at Wat Patumwanaram School, a public school under the administration of the Bangkok Metropolitan Administration, Bangkok, Thailand were included in the study. Participants who did not have the salivary or microbial tests were excluded from the study.

**Oral examination**

Each participant was examined using a periodontal probe and mouth mirror under light without drying the teeth. Dental caries were classified using modified World Health Organization (WHO) criteria (WHO, 2013; Warren *et al*, 2016): normal enamel surface (S), carious lesion with or without loss of tooth structure (D), filled surface without evidence of secondary caries (F) and missing teeth due to caries (M). This decayed, missing and filled teeth surfaces (DMFS) index (Burt and Eklund, 2005) and the prevalence of dental caries were calculated for each subject.

**Salivary and microbial testing**

A 5-minute paraffin chewing procedure was used to obtain saliva to examine for the stimulated salivary flow rate, saliva buffering capacity and the level of salivary SM and LB.

The Dentobuff® Strip was used to measure saliva buffering capacity (Orion diagnostica, Espoo, Finland). The strip contains dry acids and color indicators. When saliva is added, the acids are dissolved and the pH drops. If the saliva can buffer, the pH will increase. The strip shows the final pH. The Dentobuff® Strip was soaked in saliva and the final color was compared to standard color chart to determine the saliva buffer capacity. There are 3 possible results: 1) poor buffering capacity (PBC) (final pH $\leq$ 4.0); 2) inadequate buffering capacity (IBC) (final pH 4.5-5.5), and 3) normal or good buffering capacity (GBC) (final pH $\geq$ 6.0).

The SM level was determined using the Dentocult®SM Strip (Orion diagnostica). The Dentocult®SM Strip was soaked in saliva and inserted into the mitis-salivarius-bacitracin media solution (Orion diagnostica) with an indicator and incubated at 35-37°C for 48 hours. Colony growth was then assessed by comparing the colony density to a comparison scale. The results were classified into 4 levels: 1) none detected (0 CFU/ml), 2) low (< $10^5$ CFU/ml), 3) medium ($10^5$-$10^6$ CFU/ml), and 4) high (> $10^6$ CFU/ml).

The LB level was determined using the Dentocult®LB (Orion diagnostica). The Dentocult®LB strip was soaked in saliva and incubated at 35-37°C for 48 hours. LB colony growth was assessed by comparing the colony density with a comparison scale. The results were classified into 5 levels: 1) none detected (0 CFU/ml), 2) low (<$10^3$ CFU/ml), 3) medium ($10^3$-$10^4$ CFU/ml), 4) medium high ($10^4$-$10^5$ CFU/ml), and 5) high ($10^5$-$10^6$ CFU/ml).

**Characteristics and behaviors related to caries development**

Participants were asked to complete a self-administered questionnaire about their socio-demographic factors (age, gender, monthly family income, father's occupation and use of antibiotics), timing and frequency of cariogenic food consumption, timing and frequency of tooth brushing and dental service experience.

**Statistical analyses**

The characteristics of the participants were analyzed using descriptive statistics calculated with the Statistical Package for Social Science (SPSS, version 22.0; IBM, Armonk, NY). The various studied factors were compared between those with and without caries using cross-tabulation. 2-D dot plot was used to provide the graphical representation of the DMFS scores of participants with different saliva buffering capacity and microbial properties. The independent sample *t*-test and ANOVA were used to compare means.

Multiple linear regression analysis was used to investigate determinants of dental caries among participants. The variable included in the multiple regression analysis were based on literature review (Keyes, 1968) and bivariate analysis. The tests of normality, linearity, homoscedasticity and colinearity diagnosis were employed to ensure no violation of the assumptions. The variables used for analyses were : (1) saliva buffering capacity and SM and LB levels, (2) timing of and frequency of tooth brushing (per day) and fluoride use; (3) amount and frequency of cariogenic food consumption per day and (4) subject age. The timing of cariogenic food consumption and tooth brushing were analyzed as seperate variables. Correlation coefficients and cross-tabulations were employed to assess associations among variables.

Due to the small sample size and number of variables on multiple regression being limited, variables were recategorized into fewer categories. Saliva buffering capacity was recategorized into two levels: low buffering capacity (LBC)(final pH ≤5.5) and good buffering capacity (GBC) (final pH ≥6.0). The SM levels were recategorized into three levels: none (0 CFU/ml), low (<$10^5$ CFU/ml) and high (≥$10^5$ CFU/ml). The LB levels were recategorized into three levels: none (NLB)(0 CFU/ml), low (LLB)(≤$10^4$ CFU/ml) and high (HLB)(>$10^4$ CFU/ml) (Drucker *et al*, 1995). Since the ordinal variables were treated as nominal variables, dummy variables of the ordinal variables were generated prior to multiple regression analyses. Two dummy variables for SM were generated: low SM level (LSM) (<$10^5$ CFU/ml) and high SM level (HSM) (≥$10^5$ CFU/ml). Two dummy variables for LB were generated: low LB level (LLB) (≤$10^4$ CFU/ml) and high LB level (HLB) (>$10^4$ CFU/ml). Four groups of variables were used for the regression model. The regression model was constructed to identify the variables associated with dental caries (DMFS) using the enter method.

**Ethical considerations**

This study received ethical approved by the Human Research Ethics Committee of the Faculty of Dentistry, Chulalongkorn University (HREC-DCU 2011078). The parents, students and school principal all gave informed consent prior to participation in this study.

## RESULTS

**Participants**

A total of 139 participants were included in the study, of whom 79 (56.8%) were male. The mean age of participants was 13.4±0.9 years. Forty-eight point two percent of participants' fathers were privately employed and 55.4% had a monthly family income of <15,000 Baht (<USD455). Sixty-one point two percent of participants went to the dentist only when they had a problem and 51.8% of participants had their last dental visit within the previous 6 months (Table 1).

**Dental caries**

The prevalence of dental caries (both cavitated and non-cavitated) among the 139 participants was 89.9%. Fourteen participants (10.1%) had no caries. The mean [± standard deviation (SD)] DMFS score was 7.65 (± 5.46).

**Salivary and microbial testing**

Fifty point four percent of participants had IBC and 46.0% had GBC. The mean (± SD) DMFS scores among the groups of participants with IBC and GBC were 6.88 (± 5.73), and 8.55 (± 5.02), respectively. Forty-eight point nine percent of paticipants had no SM and 39.6% had no LB. The mean (± SD) DMFS scores among those with no SM, LSM and HSM levels were 6.04 (± 4.97), 8.35 (± 5.45) and 10.5 (± 5.41), respectively. The mean (± SD) DMFS scores among those with no LB, LLB and HLB level were 5.56 (± 4.85), 8.28 (± 5.65) and 10.8 (± 4.50), respectively. Participants with different LB and SM levels had significantly different DMFS scores ($p$ =0.001) (Table 2).

Participants with GBC had no SM and LB; participants with IBC had no SM and low LB level (Fig 1). Most participants had no or low SM and LB level (Fig 1).

Table 1
Association between DMFS scores and selected socio-demographic characteristics.

| Characteristics | Number | Percent | Mean DMFS scores | SD | $p$-value |
|---|---|---|---|---|---|
| Gender | | | | | |
| Male | 79 | 56.8 | 7.14 | 5.35 | 0.209[a] |
| Female | 60 | 43.2 | 8.32 | 5.57 | |
| Age (years) | | | | | |
| 10 | 1 | 0.7 | 9 | | $r$ =0.104 |
| 11 | 6 | 4.3 | 5.00 | 2.76 | 0.233[b] |
| 12 | 9 | 6.5 | 5.22 | 3.03 | |
| 13 | 59 | 42.4 | 7.9 | 6.07 | |
| 14 | 58 | 41.7 | 8.17 | 5.12 | |
| 15 | 5 | 3.6 | 4.8 | 6.72 | |
| 17 | 1 | 0.7 | 13 | | |
| Paternal occupation | | | | | |
| Government employee | 26 | 18.7 | 8.04 | 5.34 | 0.923[c] |
| Privately employed | 67 | 48.2 | 7.50 | 5.44 | |
| Business owner | 27 | 19.4 | 8.23 | 5.62 | |
| Unemployed | 5 | 3.6 | 8.75 | 4.65 | |
| Others | 14 | 10.1 | 7.54 | 6.63 | |
| Monthly family income in Thai Baht | | | | | |
| < 15,000 | 77 | 55.4 | 7.68 | 5.43 | 0.513[c] |
| 15,000-30,000 | 42 | 30.2 | 8.5 | 6.11 | |
| > 30,000 | 20 | 14.4 | 6.71 | 4.24 | |
| Frequency of visiting dentist | | | | | |
| Twice a year | 20 | 14.4 | 7.35 | 6.42 | 0.947[c] |
| Once a year | 21 | 15.1 | 8.29 | 6.71 | |
| Less than once a year | 13 | 9.40 | 7.69 | 5.19 | |
| When having a problem | 85 | 61.2 | 7.55 | 4.99 | |
| Last dental visit | | | | | |
| < 6 months | 72 | 51.8 | 7.82 | 5.20 | 0.169[c] |
| 6-12 months | 21 | 15.1 | 9.29 | 6.47 | |
| > 12 months | 46 | 33.1 | 6.63 | 5.27 | |

[a]Independent *t*-test; [b]Pearson correlation coefficient; [c]Analysis of variance (ANOVA).
DMFS, decayed, missing and filled surface index score; SD, standard deviation.

Most participants without caries had neither SM nor LB and inadequate buffering capacity. All participants with a poor buffering capacity had caries (Fig 1). Participants with caries had varying SM and LB levels, but only a few had high levels (Fig 1).

**Behaviors related to dental caries development**

The timing and frequency of snacking and tooth brushing were analyzed. Participants ate snacks up to 11 times daily. The mean (± SD) frequency of eating snacks per day was 1.63 (± 2.32)

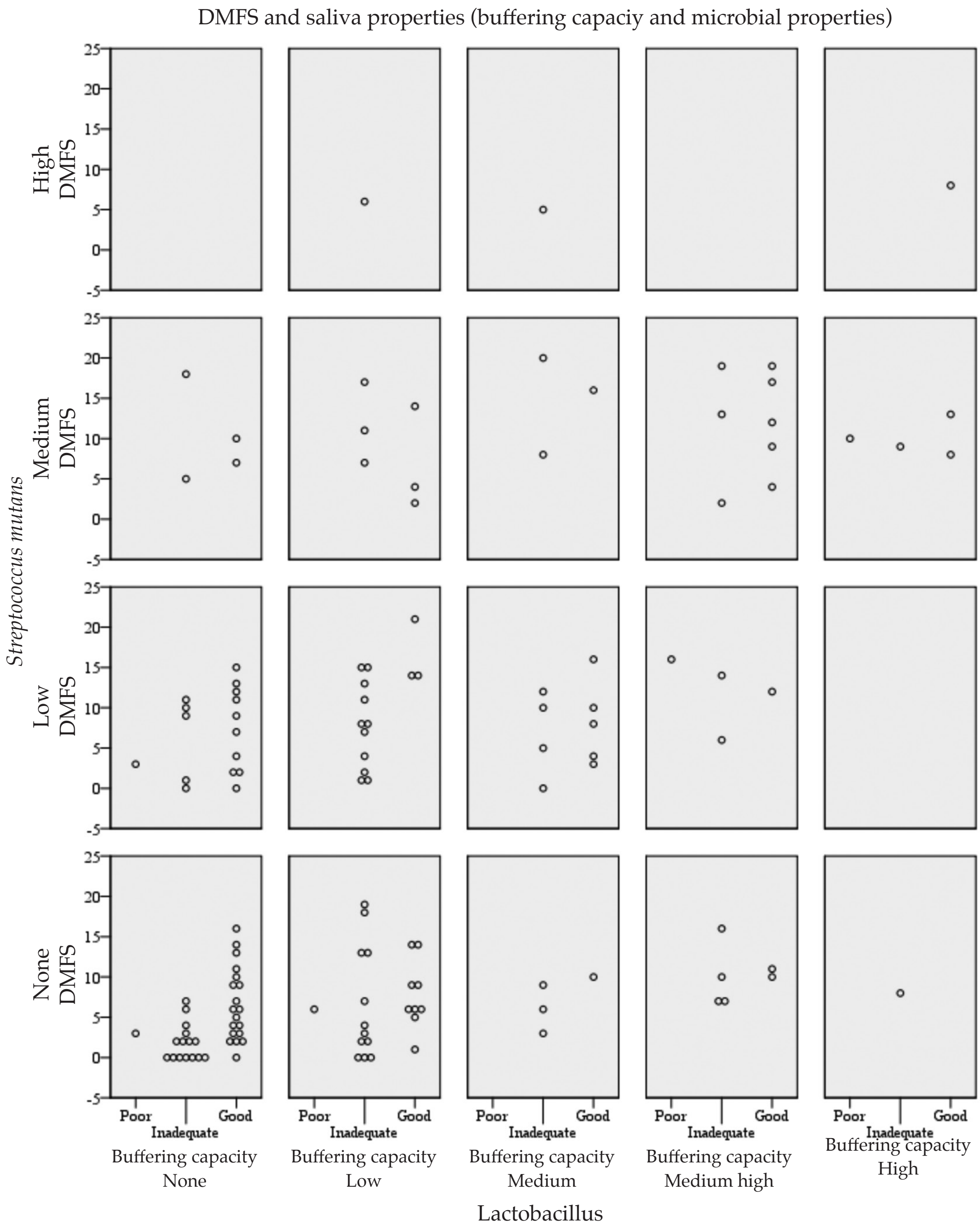


Fig 1–Decayed, missing and filling surface (DMFS) scores of each participant (each dot) with different buffering capacity and microbial properties. Buffering capacity: Poor (final pH ≤4.0); Inadequate (final pH 4.5-5.5); Good (final pH ≥6). Lactobacillus: None (0CFU/ml); low (≤$10^3$ CFU/ml); Medium ($10^3$-$10^4$ CFU/ml); Medium high ($10^4$-$10^5$ CFU/ml); High ($10^5$-$10^6$ CFU/ml). *Streptococcus mutans*: None (0CFU/ml); Low (<$10^5$ CFU/ml); Medium ($10^5$-$10^6$ CFU/ml); High (>$10^6$ CFU/ml).

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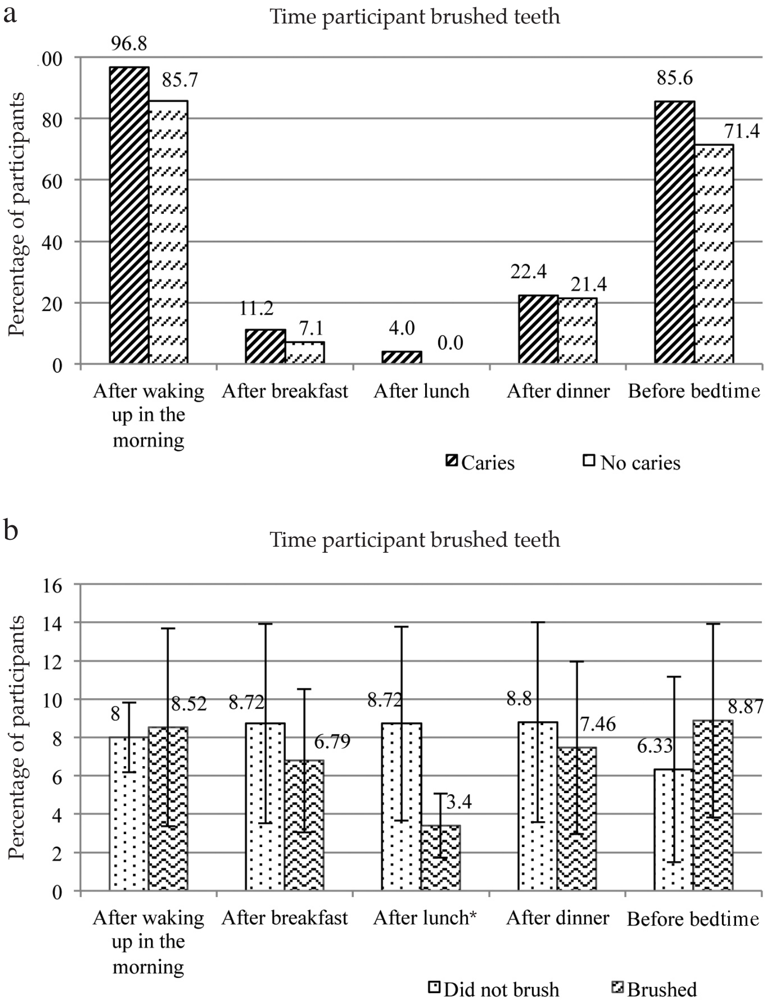


Fig 2–Comparison between time of brushing with: a) Participants with ($n$=125) and without ($n$=14) caries. b) The mean DMFS (±SD) scores of participants with caries; *$t$- test; $p$< 0.05.

times. Forty-one percent of participants ate snacks between lunch and dinner and 23.7% ate snacks before bedtime. Seventy-nine point nine percent of participants brushed twice daily, 95.7% brushed after waking up in the morning, 84.2% brushed before bedtime and 96.4% did not brush their teeth after lunch. The mean (± SD) DMFS scores among those who did and did not brush their teeth before bedtime were 8.11 (± 5.43) and 5.18 (± 5.03), respectively. Seventy-five point five percent used fluoride toothpaste during the previous 6 months. The mean (± SD) DMFS scores among participants who did and did not use fluoride toothpaste during the previous 6 months were 7.06 (± 5.29) and 9.47 (± 5.64), respectively (Table 2).

Most participants with and without caries brushed their teeth after waking up in the morning and before bedtime (Fig 2a). None of caries-free children brushed after lunch. Among participants with caries, the mean DMFS score was significantly ($p$<0.001) higher among those who did not brush after lunch (8.72) than those who did brush (3.40) (Fig 2b). Most participants snacked between lunch and dinner (Fig 3a). Among participants with caries, there were no significant difference in DMFS scores by time of snacking (Fig 3b). Twenty-seven point two percent of participants with caries and 14.3% without caries brushed after snacking. Nearly half of participants did not brush after snacking (Fig 4a). Among participants with caries, there was no significant difference between those who did and did not brush their teeth after snacking (Fig 4b).

**Bivariate analyses of DMFS scores and related factors**

We found no significant associa-

Table 2
Association between mean DMFS scores and potential caries causing factors.

| Factors related to caries development | Frequency | Percent | Mean DMFS score | SD | Statistics (df) | *p*-value |
|---|---|---|---|---|---|---|
| Saliva buffering capacity | | | | | | |
| Poor | 5 | 3.6 | 7.60 | 5.51 | T(1,137) = 1.81 | 0.073[a] |
| Inadequate | 70 | 50.4 | 6.83 | 5.78 | | |
| Good | 64 | 46.0 | 8.55 | 5.02 | | |
| *Streptococcus mutans* (CFU/ml) | | | | | | |
| No (0) | 68 | 48.9 | 6.04 | 4.97 | F(2,136) = 7.69 | 0.001[c] |
| Low (<$10^5$) | 43 | 30.9 | 8.35 | 5.45 | | |
| Medium ($10^5$-$10^6$) | 25 | 18.0 | 10.9 | 5.51 | | |
| High (>$10^6$) | 3 | 2.2 | 6.33 | 1.53 | | |
| Lactobacillus (CFU/ml) | | | | | | |
| No (0 ) | 55 | 39.6 | 5.56 | 4.85 | F(2,136) = 9.56 | <0.001[c] |
| Low (<$10^3$) | 43 | 30.9 | 8.19 | 5.84 | | |
| Medium ($10^3$-$10^4$) | 17 | 12.2 | 8.53 | 5.29 | | |
| Medium high ($10^4$-$10^5$) | 18 | 12.9 | 11.3 | 4.97 | | |
| High ($10^5$-$10^6$) | 6 | 4.3 | 9.33 | 1.97 | | |
| Snack before bedtime | | | | | | |
| No | 106 | 76.3 | 7.51 | 5.44 | T(137) =- 0.53 | 0.595[a] |
| Yes | 33 | 23.7 | 8.09 | 5.59 | | |
| Brushed after lunch | | | | | | |
| No | 134 | 96.4 | 7.81 | 5.49 | T(137) = 1.79 | 0.076[a] |
| Yes | 5 | 3.6 | 3.4 | 1.67 | | |
| Brushed before bedtime | | | | | | |
| No | 22 | 15.8 | 5.18 | 5.03 | T(137) = -2.35 | 0.020[a] |
| Yes | 117 | 84.2 | 8.11 | 5.43 | | |
| Used fluoride toothpaste in the previous 6 months | | | | | | |
| No | 34 | 24.5 | 9.47 | 5.64 | T (137) =2.27 | 0.024[a] |
| Yes | 105 | 75.5 | 7.06 | 5.29 | | |
| Frequency snacking per dayMean (SD) =1.63 (2.32); min = 0, max = 11; *r* = 0.039 | | | | | | 0.650[b] |

[a]Independent *t*-test; [b]Pearson correlation coefficient; [c]Analysis of variance (ANOVA).
DMFS, decayed, missing and filled surface index score; SD, standard deviation.

tion between DMFS score and parental occupation ($p$ = 0.923), family monthly income ($p$ = 0.513), frequency of visiting the dentist ($p$ = 0.947) and last dental visit ($p$ = 0.169) (Table 1). We found no statistically significant difference in DMFS score between male and female participants ($p$ = 0.209) and no association between age and DMFS score.

We found a significant association between higher DMFS scores and higher SM levels ($p$ = 0.001) and higher LB levels ($p$ < 0.001) (Table 2). The mean (± SD) DMFS score among participants who brushed before bed [8.11 (± 5.43)] was significantly higher ($p$ = 0.020) than those who did not [5.18 (± 5.03)]. Participants who used fluoride toothpaste had significantly lower

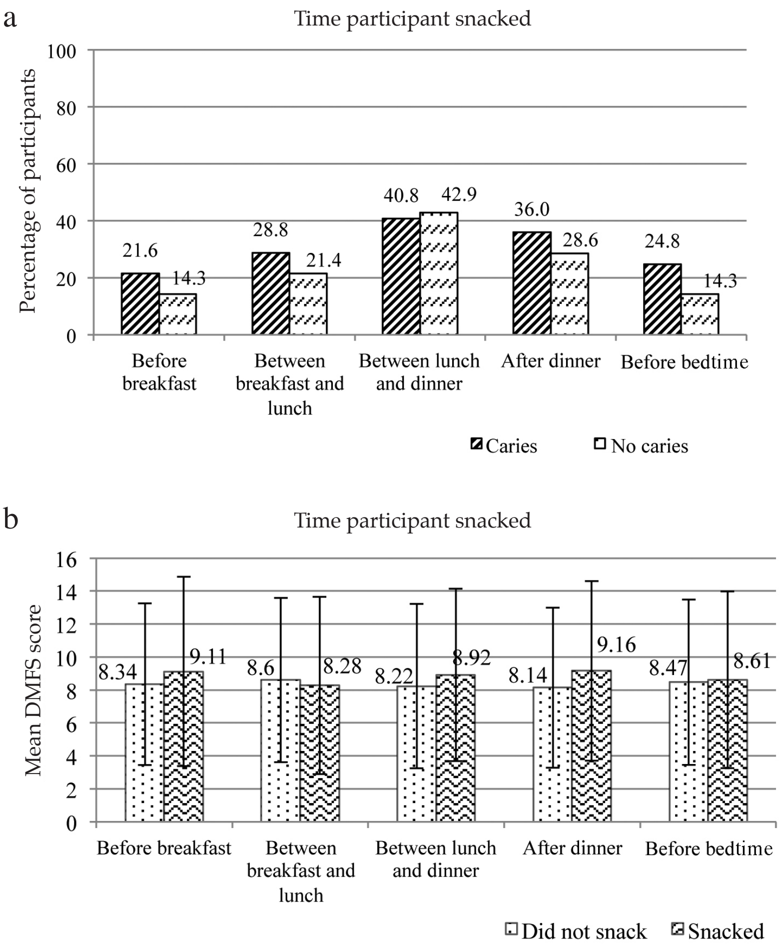


Fig 3–Comparison between time of snacking with: a) Participants with ($n$=125) and without ($n$=14) caries. b) The mean DMFS (±SD) scores of participants with caries.

($p$ = 0.024) mean (± SD) DMFS score [7.06 (± 5.29)] than the one who did not [9.47 (± 5.64)].

**Multiple regression analyses**

On multiple regression analysis, the factors significantly associates with DMFS score were buffering capacity of saliva (GBF) and LB level (LLB and HLB) ($p$ <0.001) (Table 3).

## DISCUSSION

The goal of this study was to identify microbial and behavioral determinants of dental caries in permanent dentition among healthy children aged 11-17 years. The prevalence of dental caries and DMFS score were used to indicate the magnitude and severity of dental caries, respectively. The participants in our study had a higher prevalence of dental caries (89.9%) than participants in the Thai National Oral Health Survey (Bureau of Dental Health, 2013) (52.3% and 62.4% among those aged 12 and 15 years, respectively). In our study, we investigated both cavitated and non-cavitated dental caries; most participants had cavitated dental caries. The mean DMFS and DMFT scores among our participants were 7.65 and 5.53, respectively, much higher than the Thai National average (DMFT=1.3 and 1.9 among those aged 12 and 15 years, respectively) (Bureau of Dental Health, 2013).

In our study, the mean DMFS score was higher among the older participant, similar to previous studies (Attin *et al*, 1999; Baelum *et al*, 2009; Bureau of Dental Health, 2013). Female participants had higher DMFS scores (8.32) than males (7.14) because female students snacked more in the day, similar to other study findings (Molina-Frechero *et al*, 2009; Sgan-Cohen *et al*, 2015). However another study reported females had lower DMFS

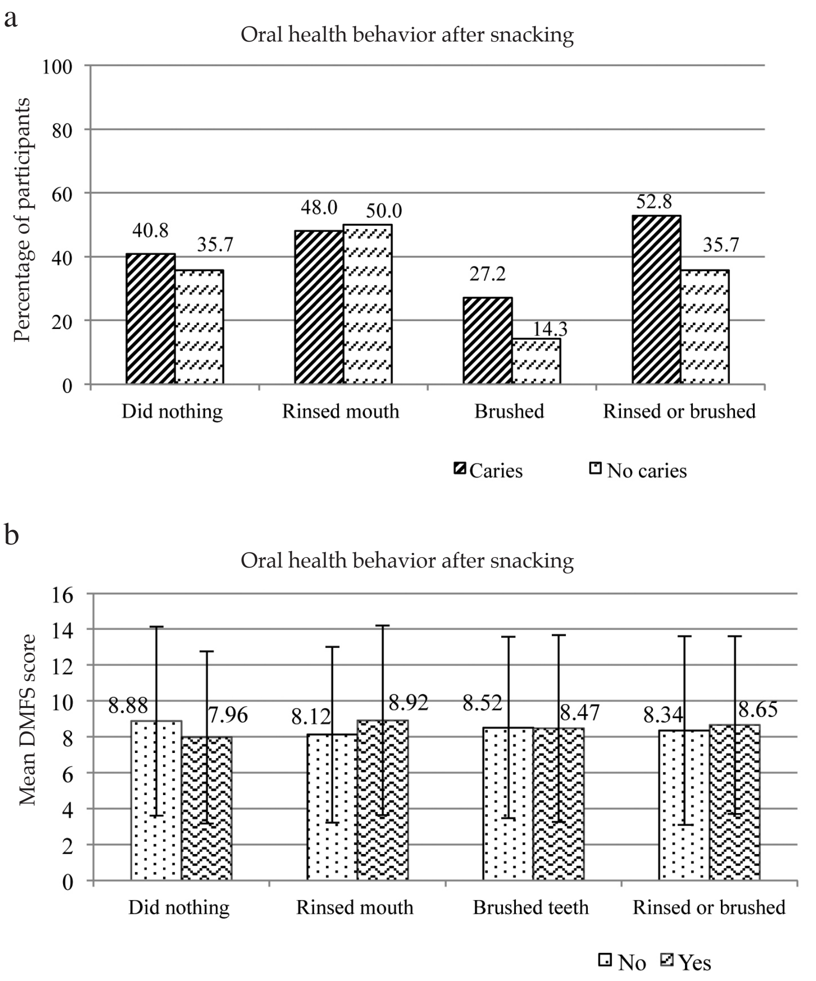


Fig 4–Comparison of behavior after snacking with: a) participants with (*n*=125) and without (*n*=14) caries; b) the mean DMFS (±SD) scores of participants with caries.

scores than males (Crossner and Unell, 2007).

Most of our participants were from a lower socio-economic level as they were privately employed with monthly family income less than 15,000 Baht. Moreover, dental problems were common in our study participants as they stated they only went to see a dentist when there was problem and they had visited a dentist within the previous 6 months. Participants in our study with a privately employed father had a lower mean DMFS score, similar to a previous study (Kumar *et al*, 2016), but in contradiction to other studies (Källestål and Wall, 2002; Al Agili and Alaki, 2014). This might be associated to their appropriate microbial factors (low SM and LB levels with inadequate or good buffering capacity) and behavioral factor (fluoride toothpaste use and did not snack during the daytime or before bed) which might be the consequence of their low income (<15,000 Baht/month). Other potential factors influencing DMFS scores could include psychosocial factors of the children and their parents (Alm, 2008).

Fluoride toothpaste use, frequency and time of brushing, time of snacking and behaving after snacking did not differ significantly between those with and without caries. The effect of using fluoride toothpaste and frequency of brushing in a day of this study revealed as the protective factor for dental caries development which is similar to the study of Aranibar Quiroz *et al* (2014).

In our study, DMFS scores were positively associated with SM and LB levels, similar to previous studies (Crossner *et al*, 1989; Alaluusua, 1993; Vitorino *et al*, 2006;

Table 3
Association between DMFS score and selected variables on multiple regression analyses.

| Model | Best fit | | Full | | Microbial | | Snacking | |
|---|---|---|---|---|---|---|---|---|
| | B | *p*-value[a] | B | *p*-value[a] | B | *p*-value[a] | B | *p*-value[a] |
| (Constant) | 1.62 | 0.379 | -8.16 | 0.239 | 1.57 | 0.307 | -1.84 | 0.789 |
| Good buffer capacity | 2.19 | 0.011 | 1.92 | 0.031 | 2.06 | 0.018 | | |
| LLB (LB ≤$10^4$) | 2.22 | 0.022 | 2.13 | 0.029 | 2.65 | 0.007 | | |
| HLB (LB >$10^4$) | 4.14 | 0.002 | 4.56 | 0.001 | 4.47 | 0.001 | | |
| LSM (SM <$10^5$) | 1.69 | 0.086 | 1.67 | 0.09 | 1.97 | 0.045 | | |
| HSM (SM ≥$10^5$) | 2.11 | 0.088 | 2.12 | 0.091 | 2.68 | 0.029 | | |
| Brushed after lunch | -2.66 | 0.244 | -1.57 | 0.519 | | | | |
| Brushed before bedtime | 2.03 | 0.083 | 2.04 | 0.083 | | | | |
| Used fluoride toothpaste in the past 6 months | -1.87 | 0.066 | -1.86 | 0.075 | | | | |
| Snacking before bedtime | | | 0.46 | 0.651 | | | 0.7 | 0.536 |
| Frequency of snacking during the day | | | 0.05 | 0.806 | | | 0.1 | 0.646 |
| Age | | | 0.74 | 0.145 | | | 0.69 | 0.179 |
| Adjusted $R^2$ | 0.204 | | 0.199 | | 0.173 | | -0.006 | |
| F | 5.41 | | 4.12 | | 6.76 | | 0.74 | |
| *p*-value[b] | <.001 | | <.001 | | <.001 | | 0.528 | |

[a] t-test; [b]Analysis of variance (ANOVA).
DMFS, decayed, missing and filled surface index score; B, unstandardized coefficient;
LLB, low Lactobacilli (LB) level (≤$10^4$ CFU/ml); HLB, high LB level (>$10^4$ CFU/ml);
LSM, low *Streptococcus mutans* (SM) level (<$10^5$ CFU/ml); HSM, high SM level (≥$10^5$ CFU/ml);
Adjusted $R^2$, amount of variance explained by only the independent variables that actually affect the DMFS.

Hong and Hu, 2010; Relvas *et al*, 2014; Arino *et al*, 2015). Seventeen point three percent of the DMFS variability could be explained by the level of SM and LB by regression (Table 3) which is similar to the previous study (Russel *et al*, 1991; Almushayt *et al*, 2010). The result indicated a dose-dependent relationship between DMFS and level of SM and LB. Participants with a higher SM or LB level had a higher mean DMFS scores except for the highest SM and LB levels. This might be depend on the location and carious stage of dental caries. SM has the pits and fissures as their harbor (Adetunji *et al*, 1996) while LB were numerous in the advance carious lesion (Byun *et al*, 2004).

In addition to cariogenic bacteria, buffer capacity is associated with dental caries. In our study, most caries-free children had low SM and LB levels and did not have poor saliva buffering capacity. Other factors are also associated with dental caries development. Sugar consumption frequency, oral hygiene, low socio-economic status and SM levels are significantly associated with dental caries (Beighton *et al*, 1996; Akpata *et al*, 2009; Relvas *et al*, 2014; Sgan-Cohen *et al*, 2015). However, the presence of SM or LB in saliva cannot be an accurate predictor for dental caries prediction (Thenisch *et al*, 2006; Twetman and Fontana, 2009) as participants in our study with no SM and no LB with good buffering capacity had high DMFS. Moreover, it might be the weakness of using DMFS score which indicates past dental caries experience. The progression of new caries over a period of time might be the better indicator for caries risk assessment.

Bacterial, dietary, socioeconomic, physiological and behavioral factors are potential determinants of dental caries (Caufield *et al*, 2005). In our study, 20.4% of the variation in DMFS score could be explained by those microbial, salivary and brushing behavior factors (Table 3). The strongest determinants of DMFS scores in our participants were microbial and salivary factors, which explained 17.3% of variability in DMFS scores (Table 3). Hence, the minimization of microbial factors would be the significant strategy for dental caries prevention. Children should be encourage to effectively brush their teeth. Dental education on effective tooth brushing technique might be an alternative approach for dental caries prevention in schoolchildren. Moreover, the participants who indicated that they brushed before bedtime had higher mean DMFS than those who did not. This information suggests that additional professional preventive strategies to reduce the cariogenic bacteria are required along with the good quality of tooth brushing, and such study are interesting to carry out. Our result indicated snacking behaviors and age factors did not affect the variability in DMFS scores at all (adjusted $R^2$ = -0.006). However, it is interesting to include other variables such as oral hygiene, perceptions to dental caries or other psychological variables in further studies for identifying determinants of DMFS.

Our study has some limitations. First, the participants had a narrow age range (grade 6-8) which might affect the significant of age on dental caries. Second, the results of the saliva and microbial levels were reported on the ordinal rather than the ratio scale. Third, data regarding brushing / snacking behaviors was self-reported and may have recall bias. Direct observation of brushing behavior and / or dietary diary would be more accurate but is not practical. Some caries-free children did not indicate that they brushed before bedtime. In our study, the presence of certain types of bacteria and brushing behavior were associated with dental caries among study subjects.

## ACKNOWLEDGEMENTS

This study would not have been possible without the support of Science Tech. Co, Ltd. for giving a special price on the saliva and microbial test kits, which is much appreciatied. We also thank the principal, teachers and schoolchildren of Wat Patumwanaram School for participating in this study. We would also like to express our gratitude to Ms Wanida Chuasuwan for helping with data collection. We are grateful for the statistical suggestions from Assoc Prof Patita Bhuridej and Prof Sirichai Kanjanawasee. This paper could not have been written without the valuable suggestions of Professor Martin Tyas. We also thank the Faculty of Dentistry, Chulalongkorn University for financial support.

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