

Cariogenic Potential of Infant Formulas—An *In Vitro* Study

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Objectives: To compare the *in vitro* cariogenic potential of two infant formulas (IF, for ages 6-12 months) processed with sterile distilled water with or without 1 ppm fluoride. **Study design:** Nine specimens in each water type were inoculated with *Streptococcus mutans* (SM) suspension. The specimens were then divided into one group of samples cultured immediately on *Mitis salivarius* (MS) agar plates (T_0) and another group of specimens cultured on MS agar plates after incubation at 37°C for 4 hours in anaerobic conditions (T_4). Six-fold dilutions of each sample were incubated for 48 hours, and colony-forming units (CFUs) of SM were enumerated. The pH changes associated with bacterial fermentation of each of the suspensions were measured at T_0 and at T_4 following incubation. **Results:** The pH was lower at T_4 than at T_0 in both IF. The SM colonies increased significantly at T_4 compared to T_0 in both IF ($P < 0.001$). There was no significant difference between the CFUs of the mediums at T_0 and T_4 or relation between SM colonies of IF based on fluoridated and non-fluoridated distilled water. **Conclusion:** The cariogenic potential was not significantly different between two IF prepared with fluoridated or non-fluoridated distilled water.

Keywords: Infant formula, cariogenicity

INTRODUCTION

Early childhood caries (ECC) is one of the most prevalent chronic diseases among children¹. The behavioral risk factors that are directly involved in ECC are well documented. In the majority of cases, the child is given a nursing bottle containing sweet fluids and offered frequent snacks, but not provided sufficient tooth cleaning². Severe ECC is associated with a background of social disadvantages and health inequalities, low socioeconomic status, minority ethnic or immigrant status, and a low educational level of the mothers³.

The decay pattern of ECC is characteristic and pathognomonic of the condition. The maxillary incisors are most affected, while the mandibular incisors usually remain sound. The other primary teeth (i.e., the canines, first molars, and second molars) may also exhibit involvement in the decay process. Their involvement depends upon the duration of the active carious process, but the extensiveness of the lesions usually is not as severe as those of the maxillary incisors⁴. The reason for the unique distribution of caries between the maxillary and mandibular incisors, and the unequal severity of the lesions between the incisors and the other teeth, is related to the chronology of primary tooth eruption, the duration of the deleterious habit, and the muscular pattern of infant sucking⁴.

The American Academy of Pediatrics recommends exclusive breastfeeding for about 6 months, with continuation of breastfeeding for 1 year or longer as mutually desired by mother and infant, a recommendation supported by the WHO and the Institute of Medicine⁵.

However, infant formulas (IF) are vital primary sources of nutrition for non-breastfed infants and an important alternative source of nutrition supplementation in cases of early cessation of breastfeeding⁶.

With the aim of attaining high caloric content, IF frequently contain complex synthetic combinations of nutrients, many of which include high concentrations of fermentable carbohydrates (e.g., sucrose, corn syrup, lactose, glucose polymers and maltodextrin), elements which make them highly cariogenic and have been implicated in the development of early childhood caries^{7,8}. The

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effect of fluoride on caries prevention is well documented. It has been noted that the improvement in oral health seen over the past 30 years is attributed mainly to the introduction of fluoridation on a mass scale^{9,10}. The oldest method of applying fluoride is via the water supply at a concentration of 1 part per million (1 ppm)¹⁰. The efficacy of communal water fluoridation in reducing dental caries is greatest for the deciduous dentition, with a range of 30-60% fewer caries in fluoridated communities¹¹. Since IF are processed with water which may be fluoridated, they may well be a significant source of fluoride in infants¹².

Although the effect of fluoride on caries prevention is well known, there is a lack of knowledge regarding the cariogenicity of different types of IF in humans. The purpose of this study was to compare the in vitro cariogenic potential of different kinds of IF and to test the *in vitro* cariogenic potential of IF based on the fluoride content of the water with which they are processed.

MATERIALS AND METHOD

We designed a model that enabled us to check the cariogenic potential of two IF brands and to extrapolate the findings to generalize about the cariogenic potential of other IF that are available worldwide. The experimental groups contained two locally leading brands of IF suitable for infants aged 6-12 months: Formula I (IF I) was the Materna Extra Care, Stage 2 (Materna, Osem group, Nestlé), and Formula II (IF II) was the Similac Advance Plus, Stage 2 (Similac, Abbot). They represent over 90% of the local market of IF, and their main ingredients are shared by the prominent products that are commercially available globally (Table 1). The IF were prepared according to the manufacturer’s instructions and based on sterile water with and without fluoride (1 ppm). The results of the experimental groups were compared to a control group, which included distilled water with and without fluoride, and a positive control group, which included brain heart infusion broth and 10% sucrose solution in brain heart infusion broth.

Bacterial growth

Nine 15 ml specimens from each group of water were inoculated with 0.5 ml *Streptococcus mutans* (SM) suspension. Those specimens were then divided into two groups, one consisting of the samples that were cultured immediately on *Mitis salivarius* (MS) agar plates (T₀), and the other consisting of samples that were cultured on MS agar plates after incubation at 37°C for 4 hours in anaerobic conditions (T₄). Samples (0.1 ml) were taken from each specimen, and underwent serial 6-fold dilutions, and 0.01 ml from each dilution was spread onto individual MS agar plates in triplicates. The plates were incubated under anaerobic conditions at 37°C for 48 hours, after which colony forming units (CFUs) of SM were counted.

pH changes

The pH changes associated with bacterial fermentation were assessed with a pH meter by sampling each of the experimental and control suspensions (5 ml at each time point) for pH measurement at T₀ and at T₄ following incubation.

Statistical analysis.

Comparisons between ΔT=number of CFUs/ml at T₀ and at T₄ and pH measurements at T₀ and T₄ of each medium with and without fluoridated water were evaluated using a 2-way ANOVA test.

RESULTS

Table 2 summarizes both the CFU and pH measurements of the original mediums and after they were processed with distilled or fluoridated water at T₀ and T₄. Although the initial pH of the medium of IF II was lower than that of IF I (7.13 vs 7.34, respectively), there was no significant difference between the CFUs of the two mediums at T₀ and T₄ of both IF.

Table 1. Eight of the largest commercially available infant formula brands and their ingredients

Product	Nutrilon Standard 2 Follow-up Milk	Combiotic® Follow-on Milk 2	Good Start® 2 Baby Formula	Enfamil® 2 Infant Formula	Nurture® Gold Follow-On Formula 2	Beingmate Stage 2 Follow-on Milk	Similac® Advance Plus® Baby Formula	Materna Extra Care, Stage 2
Brand	Nutrilon, Danone	Combiotic®, Hipp	Good Start® Nestlé®	Enfamil®, MJN™	Nurture® Heinz	Beingmate	Similac®, Abbott	Materna, Osem Group, Nestlé®
Glucose	0.3g							
Lactose	6.1g	7.6g			7.9g	7.2g		
Maltose	0.2g							
Polysaccharides	2g							
Total Carbohydrate	8.6g	7.9g	7.5g	7.4g	7.9g	7.5g	7.59g	6.5g
Minerals								
Iron	1 mg	1 mg	1.3 mg	1.2 mg	1 mg	0.86 mg	1.19 mg	1.22 mg
Calcium	65 mg	73 mg	81 mg	80 mg	78 mg	43 mg	115 mg	80 mg
Phosphorus	36 mg	43 mg	45 mg	52 mg	55 mg	28.2 mg	66 mg	

Table 2. CFUs and pH measurements of the original mediums and after being processed with distilled water with or without fluoride at T₀ and T₄

		IF I	IF I +F*	IF II	IF II +F*
Log 10 CFU	T ₀	4.81±0.18	4.85±0.22	4.8±0.2	4.79±0.21
	T ₄	5.33±0.17	5.21±0.24	5.34±0.26	5.21±0.31
pH	Original	7.34±0.41	7.21±0.35	7.13±0.72	7.04±0.39
	T ₀	7.32±0.26	7.21±0.28	7.04±0.26	7.14±0.29
	T ₄	7.03±0.13	7.15±0.34	6.86±0.31	6.88±0.3

*Prepared with water containing fluoride

IF I: Materna Extra Care, Stage 2 (Materna, Osem group, Nestlé); IF II: Similac Advance Plus, Stage 2 (Similac, Abbot); T₀: cultured immediately; T₄: cultured after 4 hours.

The IF that were prepared with sterile distilled fluoridated water showed a slight and non-significant increase of the pH of both IF at T₄. Furthermore, there was no significant relation between the SM colonies in IF processed by fluoridated and non-fluoridated water.

The pH measurements of both IF processed with and without fluoridated water at T₀ and T₄ are described in Fig.1: Preliminary measurements of the pH of both IF II and IF I were taken in order to determine their original pH values. There was a significant interaction ($P < 0.001$) between them and the time of the pH measurements, and the pH values were lower at T₄ than T₀ in both IF.

The numbers of SM CFUs of both IF processed with either fluoridated or non-fluoridated water at T₀ and T₄ are described in Fig. 2. The increase in SM colonies at T₄ compared to T₀ in both IF reached a level of significance ($P < 0.001$).

DISCUSSION

The cariogenic potential of IF is of considerable concern since mothers often put their babies to sleep with a bottle of milk or IF that may remain in the mouth for several hours. This can result in decreased salivary flow and prolonged exposure of dental plaque to potentially fermentable carbohydrates in dentulous infants¹³. Both IF tested in this study contain carbohydrates that may be fermentable. IF I contains lactose, glucose syrup solids and GOS, and IF II contains lactose, maltodextrin and glico-oligosachrides (GOS). In spite of the carbohydrates in both IF and the significantly related reduction of pH between T₀ and T₄, the pH measurement did not go under 6.86 at any time. Notably, non-carbohydrate ingredients in IF, such as calcium, phosphorus, iron and peptides, could also influence cariogenic potential¹⁴.

Since both IF contained probiotic bacteria, the two original mediums with no SM suspension were first spread on SM agar plates in order to check for any bacterial growth. The lack of any such growth confirmed that the CFUs seen on plates inoculated with mediums and SM suspension solely represented the SM growth.

The use of fluoridated water when reconstituting a powdered IF influences cariogenic properties of the formula¹⁵. In this study, all test groups had higher SM CFU counts in T₄ than in T₀, but the use of fluoridated water did not significantly influence the CFU counts. The fluoride supplementation did increase the pH measurements of the Similac and Materna formulas but not to a level of significance. While the findings of this study did not show any beneficial effect of fluoride supplementation on the cariogenic potential of IF, the use of fluoridated water to process IF is nevertheless recommended¹³ given the proven efficacy of communal water fluoridation in reducing caries in deciduous teeth¹¹.

Figure 1. pH measurements of both infant formulas processed with distilled water with or without fluoride at T₀ and T₄. IF I: Materna Extra Care, Stage 2 (Materna, Osem Group, Nestlé); IF II: Similac Advance Plus, Stage 2 (Similac, Abbot); T₀: cultured immediately; T₄: cultured after 4 hours.

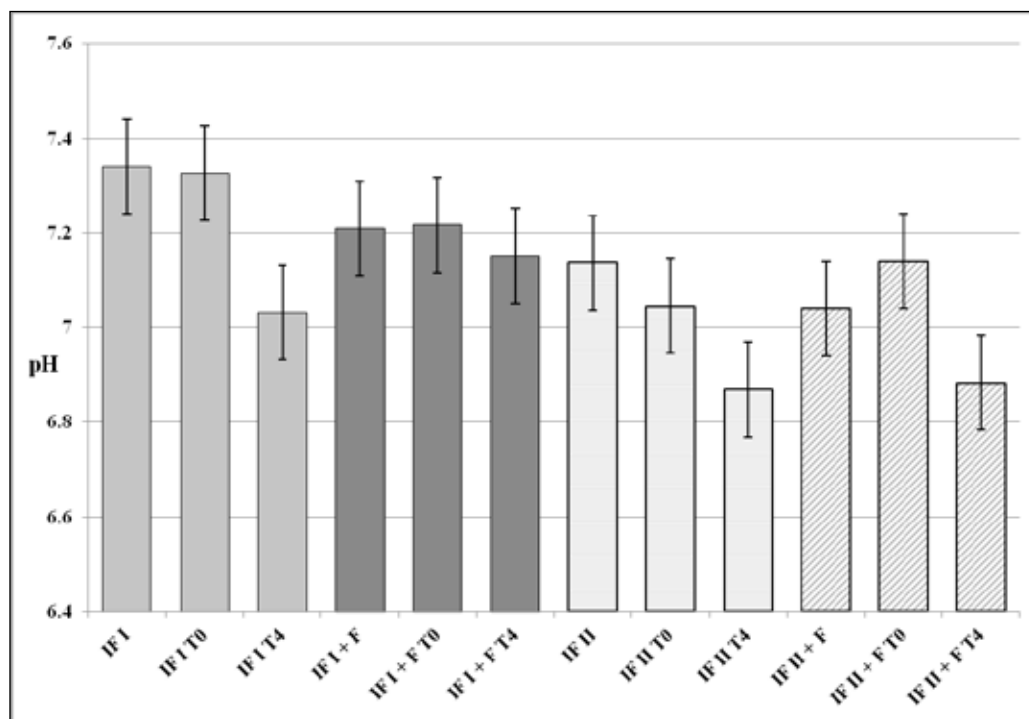
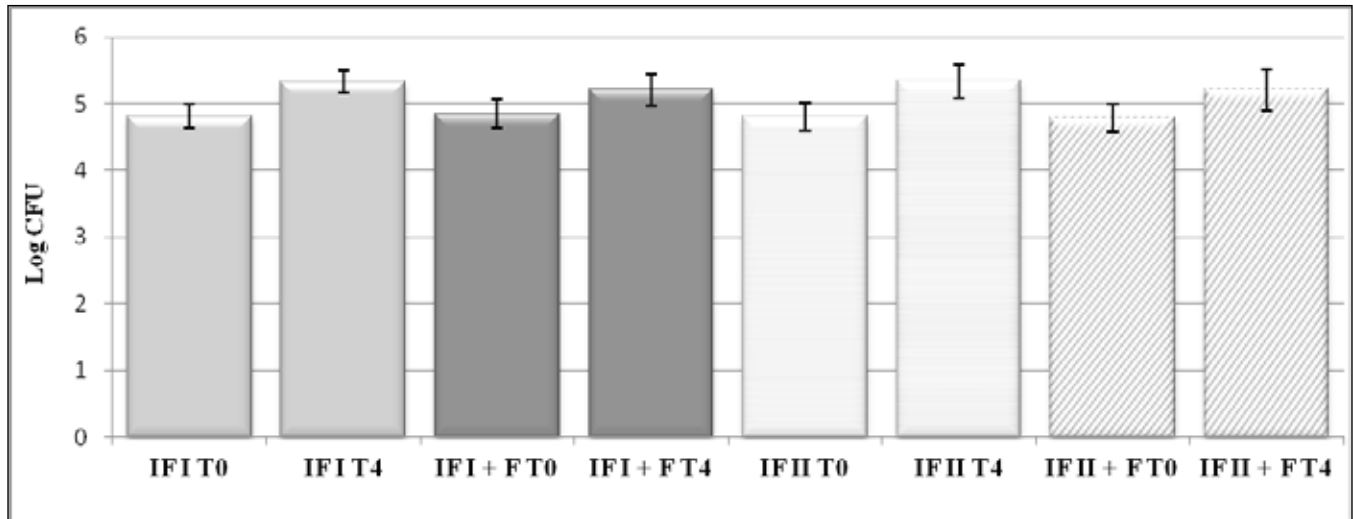


Figure 2. Numbers of SM CFUs of both tested infant formulas processed with distilled water with or without fluoride at T₀ and T₄. IF I: Materna Extra Care, Stage 2 (Materna, Osem Group, Nestlé); IF II: Similac Advance Plus, Stage 2 (Similac, Abbot); T₀: cultured immediately; T₄: cultured after 4 hours.



CONCLUSION

The results of this study did not reveal any significant difference in cariogenic potential between the two tested IF. IF may have a cariogenic effect since they raise SM CFUs and lower the pH levels. The provision of good oral hygiene by parents and caregivers is imperative to prevent infection and subsequent dental caries in infants aged 6-12 months who are consuming IF.

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