ANNA MARIA CIA DE MAZER PAPA

Efeito *in situ* de formulações infantis à base de leite e soja na desmineralização do esmalte dental decíduo

Dissertação apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas, para obtenção do Título de Mestre em Odontologia, área de concentração em Odontopediatria.

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Co-orientador: Prof Dr Jaime Aparecido Cury

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RESUMO

O efeito de formulações infantis adicionadas de açúcar ou não na desmineralização do esmalte de dentes decíduos e na composição do biofilme dental formado não é conhecido. Desta forma, um estudo *in situ*, cruzado, tipo boca dividida e cego foi conduzido em 3 fases experimentais distintas de 10 dias cada, durante as quais 11 voluntários adultos utilizaram um dispositivo intra-oral palatino, contendo 6 blocos de esmalte decíduo, com dureza de superfície pré-determinada. Os blocos de esmalte foram submetidos extra-oralmente, 8 vezes ao dia, a 6 grupos de tratamentos: água destilada e deionizada, solução de sacarose 10%, formulação à base de leite e formulação à base de soja, com ou sem sacarose 10% adicionada. Após cada fase, a acidogenicidade, composição microbiológica e bioquímica do biofilme formado foram analisadas, bem como a desmineralização do esmalte dental decíduo por meio da microdureza de superfície. Foi realizada uma análise estatística fatorial 3 x 2 para todas as variáveis, tendo como um dos fatores em estudo formulações infantis (3 níveis) e como outro fator em estudo a adição de sacarose (2 níveis). Ambas as formulações induziram uma perda mineral significante, a qual aumentou com a adição de sacarose. Além disso, quando fermentadas, ambas as fórmulas reduziram o pH do biofilme, independentemente da adição de sacarose. Também, a contagem de lactobacilos no biofilme formado foi maior quando ambas as formulações foram usadas comparado ao grupo água. Com relação ao fluido do biofilme, nenhum efeito significativo foi observado para flúor e fósforo inorgânico, enquanto a concentração de cálcio foi maior nos grupos com sacarose e na formulação à base de soja ($p<0,05$). Nenhum efeito significativo foi observado para concentração de polissacarídeos intracelulares ($p>0,05$), enquanto que as concentrações de polissacarídeos extracelulares solúveis e insolúveis foram maiores nos grupos com sacarose, independente da formulação ($p<0,05$). Em conclusão, os resultados sugerem que as formulações à base de leite e à base de soja apresentam potencial para induzir a desmineralização do esmalte de dentes decíduos, a qual é aumentada quando sacarose é adicionada.

Palavras-chave: esmalte dental decíduo, biofilme dental, cárie dental, formulação infantil, sacarose.

ABSTRACT

The effect of infant formulas sweetened or not with sucrose on deciduous enamel demineralization and on dental biofilm formed is not known. Thus, a crossover, split-mouth and blind *in situ* study was conducted during 3 experimental phases of 10 days each, during which 11 adult volunteers wore palatal appliances containing six slabs of human deciduous enamel with pre-determined surface microhardness. The dental slabs were extra-orally subjected 8 times a day to six groups of treatment: distilled and deionized water, 10% sucrose solution, milk-based and soy-based formula, without or with 10% sucrose added. After each phase, the acidogenicity, biochemical and microbiological composition of dental biofilm formed was analyzed, and enamel demineralization was assessed by surface microhardness. A factorial 3 x 2 was considered for the statistical analysis of all variables and the factors under evaluation were formula at 3 levels and sucrose at 2 levels. Both infant formulas induced significant enamel mineral loss, which increased when sucrose was added. In addition, both infant formulas were fermented, decreasing the biofilm pH, irrespective of sucrose addition. Also, lactobacilli counts in the biofilm were higher under the use of both formulas when compared to the water group. With regard to biofilm fluid, there was no statistically significant effect of the factors formula and sucrose for F and Pi, while Ca concentrations in the fluid were higher in the sucrose groups and in the soy-based formula group ($p<0.05$). There was no significant effect of the factors under study for IPS ($p>0.05$), while SEPS and IEPS concentrations were higher in the groups with sucrose, independent of the formula ($p<0.05$). In conclusion, the results suggest that milk and soy-based formulas present potential to induce demineralization in deciduous enamel, which was increased when sweetened with sucrose.

Key words: deciduous dental enamel, dental biofilm, dental caries, infant formula, sucrose.

SUMÁRIO

I - INTRODUÇÃO GERAL

Apesar do declínio da prevalência da cárie dentária em alguns segmentos da população (Marthaler, 2004; Narvai *et al*., 2006), essa doença ainda continua sendo um problema de saúde pública para alguns grupos, tais como crianças na primeira infância (Curzon & Preston, 2004). Estudos realizados em diferentes países, relatam que a prevalência de cárie precoce da infância (CPI) ainda é alta, variando de 7% a 70% das crianças avaliadas (Kaste & Gift, 1995; Tinanoff & O'Sullivan, 1997; Schroth & Cheba, 2007).

Assim como a cárie dentária, a cárie precoce da infância apresenta uma etiologia multifatorial (Peters, 1994). Alguns fatores, como: a presença de bactérias cariogênicas, carboidratos fermentáveis, hospedeiro/superfície dentária susceptível, interagem em um determinado tempo (Harris *et al*., 2004), além dos fatores sociais, ambientais e antropométricos.

Dentre os principais fatores da etiologia da CPI, cita-se o uso de uma dieta cariogênica, tais como leites e sucos açucarados, os quais são oferecidos freqüentemente via mamadeira (Tinanoff & Palmer, 2000). Isto por si só não seria o maior problema se o fluido cariogênico fosse usado de forma restrita. No entanto, esses fluidos são oferecidos principalmente durante a noite em alta freqüência, período em que está ausente a higienização da cavidade oral bem como os efeitos da saliva de limpeza e capacidade tampão, podendo levar ao desenvolvimento da CPI.

Ainda em relação à dieta, uma atenção especial deve ser dada ao uso de formulações infantis na 1ª infância, pois hábitos inapropriados de alimentação nessa fase têm sido identificados como fator associado ao desenvolvimento da doença nesta faixa etária (Sheikh & Erickson, 1996). Considerando o uso dessas formulações infantis

pelas crianças na primeira infância, alguns estudos foram conduzidos para avaliar a relação do uso das mesmas com o desenvolvimento da cárie dentária.

Estudos em animais (Bowen *et al*., 1997; Peres *et al*., 2002) avaliaram o efeito cariogênico de formulações infantis à base de leite e soja e observaram que algumas fórmulas apresentaram potencial cariogênico significante, embora sempre menor que o controle positivo, o qual foi sacarose. Outros estudos *in vivo* (Sheikh & Erickson, 1996; Erickson *et al*., 1998, Danchaivijitr *et al*., 2006) investigaram o efeito de diferentes formulações infantis na acidogenicidade do biofilme dentário, e observaram que as mesmas mostraram habilidade em reduzir o pH do biofilme abaixo do inicial, sugerindo que as formulações infantis são capazes de serem fermentadas e por isso podem levar à perda mineral. Além disso, sabe-se que a sacarose pode estar presente como parte da dieta ou mesmo adicionada à mamadeira. Mesmo que não recomendada, ainda é uma prática comum em 70-80% das crianças (Dini *et al*. 2000; Rosenblatt & Zarzar, 2004), podendo aumentar o potencial de cárie da formulação infantil. Assim, considerando que a adição de sacarose em mamadeiras contendo formulações infantis é uma prática comum na primeira infância e o seu potencial cariogênico não é conhecido, o objetivo do presente estudo foi avaliar o efeito *in situ* da combinação de formulações infantis e sacarose na acidogenicidade, composição bioquímica e microbiológica do biofilme dentário formado e sua relação com a desmineralização do esmalte decíduo.

II - CAPÍTULO

Esta dissertação está baseada na Resolução CCPG/002/06/UNICAMP, que regulamenta o formato alternativo para dissertações de Mestrado e permite a inserção de artigos científicos de autoria do candidato (Anexo 3).

Por se tratar de pesquisa envolvendo seres humanos, o projeto de pesquisa deste trabalho foi submetido à apreciação do Comitê de Ética em Pesquisa da Faculdade de Odontologia de Piracicaba, tendo sido aprovado (Anexo 2).

Effect of milk and soy-based infant formulas on in situ demineralization of human deciduous enamel.

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Effect of milk and soy-based infant formulas on *in situ* demineralization of human deciduous enamel

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Short title: Infant formula on human deciduous demineralization

Abstract

Purpose: The effect of infant formulas sweetened or not with sucrose on deciduous enamel demineralization and on dental biofilm formed is not known. **Methods:** A crossover, split-mouth and blind *in situ* study was conducted during 3 phases of 10 days each, during which 11 volunteers wore palatal appliances containing slabs of human deciduous enamel. The dental slabs were extra-orally subjected 8 times a day to six groups of treatment: distilled and deionized water, 10% sucrose solution, milk-based and soy-based formula, without or with 10% sucrose added. After each phase, the acidogenicity, biochemical and microbiological composition of dental biofilm formed was analyzed, and enamel demineralization was assessed by microhardness. **Results:** Both formulas induced significant enamel mineral loss, which increased when sucrose was added. Both infant formulas were fermented, decreasing the biofilm pH, irrespective of sucrose addition. Also, lactobacilli counts in the biofilm were higher under the use of both formulas when compared to the water group. **Conclusions:** The results suggest that milk and soy-based formulas present potential to induce demineralization in deciduous enamel, which was increased when sweetened with sucrose.

Key words: deciduous dental enamel: dental biofilm: dental caries: infant formula: sucrose

Introduction

Even though dental caries prevalence and incidence in some segments of the population has declined in the past decades[1,2], it still continues to be a public health problem in some groups, such as the very young and the elderly.[3] Infants and children may suffer from early childhood caries (ECC), which affect from as low as 7% up to 70% of the children evaluated.[4-6]

One of the main factors in the etiology of ECC is the use of a cariogenic diet, such as sweetened or flavored milk, fruit juices and soft drinks, which are given via a bottle[7], particularly in a high frequency and during the night, when salivary flow is reduced. Considering the widespread concern about the content of baby bottles, infant formulas may be implicated in the development of ECC.

In vitro and *in vivo* studies observed that most infant formulas are acidogenic[8-10], with exposure to soy-based formulas resulting in higher pH drops than the milk-based ones. In addition, studies conducted in animals have shown that milk and soy-based infant formulas present some cariogenic potential.[11,12] Moreover, sucrose may be present as part of the diet or added to the baby bottle, which is not recommended, but is a current practice still given to 70-80% of the children[13,14], and may increase the caries potential of the infant formula.

Therefore, considering the lack of information on the cariogenicity of infant formulas on deciduous teeth and in the presence of sucrose, the present *in situ* study aimed to evaluate the effect of the infant formula and sucrose association on demineralization of deciduous enamel as well as on the acidogenicity, biochemical and microbiological composition of the dental biofilm formed.

Methods

Experimental design

This *in situ* study, approved by the Research and Ethics Committee of the Piracicaba Dental School, had a cross-over and split-mouth design and was carried out in 3 experimental phases of 10 days each, during which 11 volunteers (18-31 years old) wore acrylic intra-oral palatal appliances, containing 2 sets of 3 slabs of sound human deciduous dental enamel. Informed consent was obtained from all participating adult subjects prior to the beginning of the study. During the experimental phases, each set of 3 slabs was extra-orally treated with one of the following treatments: distilled and deionized water; milk-based formula (Nestogeno 2, Nestlé®); soy-based formula (Nan Soy, Nestlé®); 10% sucrose solution; milk-based formula + 10% sucrose; and soy-based formula + 10% sucrose. Treatments without and with sucrose added, for example, distilled and deionized water and sucrose solution, were combined in the same phase, and the absence of any crossed-effect between the treatments is supported by previous studies.[15-16] All volunteers were randomized into 3 different groups, in such a way that one third of volunteers were using each combination of treatment at each experimental phase, as previously designed.[16] This study was blind only with respect to the examiner, since the volunteers were able to partially identify the treatments by the flavor and consistency of the solutions. On the 10th day of the experiment, the acidogenicity of the dental biofilm was determined and the biofilm formed onto dental slabs was collected for microbiological counts and biochemical analyses in fluid biofilm. In addition, mineral loss was determined in dental enamel. For statistical analysis, the volunteer was considered as an experimental block.

Enamel slabs and palatal appliance preparation

Deciduous dental enamel slabs (3 X 3 X 2 mm) were obtained from the middle third of the buccal face of sound human deciduous incisors and molars and were prepared as previously described.[17] Then, 198 dental slabs with a known surface microhardness (346.4 ± 17.3 Kg/mm^2) were randomly divided into 6 different groups, according to the treatments. An acrylic resin palatal intra-oral appliance, containing 3 slabs of enamel on each side, was made for each volunteer. The 2 anterior slabs were kept together and the 3rd was placed separated from them. A cavity was created in the acrylic appliance, leaving a 1.0 mm space for biofilm accumulation. Plastics meshes were fixed over the cavities to protect the enamel slab surface from mechanical attrition. Colourless or red acrylic resin was used to fix the meshes, indicating where each treatment should be made.[15,16] Further details of appliance preparation are given in previous publications.[17-19]

Treatments

The treatment solutions were prepared daily and milk and soy-based formulas suspensions were prepared according to the information on the label. The infant formulas tested in this study are special formulas for babies (from 6 months of age): the milk-based formula (Nestogeno 2) contains 3.2% of maltodextrin and 4.06% of lactose and the soy-based formula (Nan Soy) contains 7.31% of maltodextrin as the only added carbohydrate, according to the manufacturer. The concentration of 10% sucrose used in the present experiment simulates the addition of 1 soupspoon of sugar to a 150-ml baby bottle. During the 10 days of each experimental phase, 8 times per day, at predetermined times (08.00, 09.30, 11.30, 14.00, 15.30, 17.00, 19.00 and 21.00 hours)

the volunteers were instructed to remove the appliances from the oral cavity, drip one drop of the treatment solutions onto each enamel slab according to the treatment protocol and wait 5 min before replacing the appliance in the mouth. The labels of the dropping bottles containing treatments with sucrose were marked in red, indicating to the volunteers that these solutions should be dripped on the side of the appliance where the mesh was fixed with red resin. The excess fluid was removed with gauze in an attempt to avoid carry-across effect of the treatments. The volunteers were instructed to wear the appliance all time, removing it only during meals, drinking and oral hygiene. Considering the crossover design of this study, no restriction was made with regard to the volunteers' diet. Throughout the entire experiment, the volunteers used a non-fluoridated dentifrice, but drank water optimally fluoridated (0.69 ± 0.03 mg F/l). A wash-out interval of 7 days was established between the experimental phases.

Dental biofilm analysis

Dental plaque collection

On the 10^{th} day of the experiment, approximately 10 hours after the last exposure to treatments, with the volunteers in fasting conditions and without having brushed their teeth, the appliances were removed and all plastics meshes were dislocated. The biofilm formed onto the 2 anterior enamel slabs was firstly collected for biofilm fluid analysis. Then, acidogenicity was assessed in the most posterior slabs and dental biofilm formed onto these slabs was further collected for microbiological and polysaccharides analysis.

Dental biofilm acidogenicity assessment

For the acidogenicity assessment, the plastic mesh that covered the most posterior enamel slab on each side of the appliance was removed to facilitate positioning of the electrode in the biofilm formed. With the intra-oral appliance positioned in the volunteer's oral cavity, the pH of the biofilm was determined after overnight fasting (baseline). Then, the intra-oral appliance was removed from the oral cavity and the respective treatment solutions were dripped onto the slabs. After 1 min, the appliance was replaced in the oral cavity and, after 4 min had elapsed, the pH was determined again after 5 min (pH_{5min}) as a parameter of plaque acidogenicity. A contact micro-electrode (Beetrode MEPH-3L; WPI, Sarasota, FL, USA) connected to a pH meter (720-A; Orion, Boston, MA, USA) in combination with a reference electrode (9002; Orion) was used. A salt bridge was created with a 3 M KCl solution between the reference electrode and the volunteer's finger.[20]

Microbiological analysis

The dental biofilm after pH assessment was collected and a homogeneous aliquot (5-10 mg) was weighed in sterile microcentrifuge tubes, resuspended in 1 ml of sterile saline solution (0.9% NaCl) and sonicated.[21] The suspensions were serially diluted in 0.9% NaCl and inoculated in duplicate by drop technique in the following culture media: blood agar, for total microbiota (TM); mitis salivarius agar plus 0.2 units of bacitracin/ml, for mutans streptococci group (MS)[22]; and Rogosa SL agar (Difco 248020; Becton Dickinson, Sparks, MD, USA), for lactobacillus (LB). The plates were incubated in 10% CO_2 at 37ºC for 48 h. The blood agar plates were additionally incubated for 24 h at 37ºC in aerobiosis. The colony-forming units (CFU) were counted and the results expressed in CFU/mg dental biofilm (wet weight), in percentage of MS

in relation to TM (%MS/TM) and percentage of LB in relation to TM (%LB/TM). The rest of the suspension was used for the extraction of polysaccharides.

Biochemical Analysis

Dental biofilm was collected with a plastic spatula for fluid analysis and the samples were immediately placed inside an oil-filled centrifuge tube[23], weighed (± 10 µg) and centrifuged to separate the fluid from the biofilm solids. The biofilm fluid was recovered with oil-filled capillary micropipettes and deposited on the surface of an oil-covered inverted F electrode (Orion, 94-09) with the use of a microscope. Samples of fluid were diluted with TISAB III (1:10)[23] under microscope using micropipettes and F was measured using a micro-reference electrode, held in a micromanipulator, to close the circuit.[23] For analyses of Ca and P_i in the biofilm fluid, quartz nanopipettes[24] were used to transfer standardized volumes of the fluid samples and standards into colorimetric reagents, i.e. for Ca, Arsenazo III reagent and for P_i, malachite green/molibdate.[25] The absorbance of the mixtures was read at 650 nm, using a micro-cuvette (Hellma, 105.202, Müllheim, Germany) in a Beckman DU-800 spectrophotometer.

The extraction of soluble extracellular polysaccharide (SEPS), insoluble extracellular polysaccharide (IEPS) and intracellular polysaccharide (IP) was performed according to Aires et al[21] and total carbohydrate was estimated by phenol sulphuric method.[26]

Microhardness Analysis

At the end of each experimental phase, enamel surface microhardness of 2 enamel slabs from each treatment, which were placed in the most anterior position, was

measured again, and an average per volunteer was obtained. Five indentations were made at 100 μm from the baseline indentations. The mean values of all 5 measurements were then averaged within a treatment group. These analyses were carried out according to Cury et al[17] and percentage of surface microhardness change (%SMC) was calculated. The microhardness tester, Shimadzu HMV-2000, was used for these analyses and a Knoop indenter was used with a 50 – gram load for 5 s.

Statistical Analysis

A factorial 3 x 2 was considered for the statistical analysis of all variables. The factors under evaluation were: formula at 3 levels (water, milk and soy) and sucrose at 2 levels (without or with). In addition, volunteers were considered as statistical blocks. The assumptions of equality of variances and normal distribution of errors were checked for all the response variables tested, and those that did not satisfy these assumptions were transformed.[27] The values of P_i and total Ca in biofilm fluid, soluble and insoluble EPS, lactobacillus and mutans streptococci counts were transformed to the $\log_{10}(X)$. In addition, the values of F in plaque fluid were transformed by 1/root square, the values of TM were transformed by root square, %LB/TM were transformed by 1/(X), the values of %SMC and pH_{5min} were transformed by power of $(X)^{0.3}$ and $(X)^{-2}$, respectively. For baseline pH and IP, the data were not transformed. Besides, since no transformation of the data of %MS/TM was possible, these data were ranked prior to the statistical analysis. Tukey test was used for post-ANOVA comparisons. SAS software system, v.8.01 was used and the significance level was set at 5%.

Results

A significant interaction (P<0.05) between the factors infant formula and sucrose addition was found for %SMC. Without sucrose, a significantly higher %SMC was found for groups treated with milk and soy-based formulas when compared to the water group (P<0.05, fig.1). With sucrose, %SMC was higher in all groups and no significant difference was found among them (P>0.05, fig.1).

For the microbiological analysis (Table 1), none of the factors showed a statistically significant effect for TM counts (P>0.05), while the variables LB counts and %LB/TM showed statistical significance only for the separate factors (P<0.05), but not for the interaction between them (P>0.05). The groups treated with both formulas showed significantly higher LB counts and %LB/TM in relation to the water groups (P<0.05; table 1), either without or with sucrose. In addition, with sucrose, independent of the treatment, LB counts and %LB/TM were statistically higher when compared to the groups without sucrose (P<0.05; table 1). Regarding MS counts and %MS/TM, statistically significant effect was observed only for the factor sucrose (P<0.05); these values were statistically higher in the groups with sucrose (P<0.05), independent of the formula.

The group treated with the soy-based formula presented the lowest baseline pH (P<0.05; table 2), independently of the presence of sucrose. However, a significant interaction between formula and sucrose was found for the pH_{5min}; without sucrose, the lowest pH_{5min} was observed for the soy-based formula, followed by the milk-based and the water group. With sucrose added, the pH_{5min} decreased significantly for the water group and milk-based formula, but not for the soy-based one. Also, the lowest pH_{5min} with sucrose added was found for the soy-based formula.

Regarding the fluid of dental biofilm (table 2), no significant effect was observed for F and P_i (P>0.05), while Ca showed statistical significance only for the separate factors (p<0.05). Independent of the treatment, concentration of Ca in the fluid was statistically higher in the groups with sucrose than in the groups without this sugar (P<0.05).

With regard to polysaccharide analysis, the addition of sucrose increased the concentration of both SEPS and IEPS in all groups (P<0.05; table 2), and no effect was observed for IP (P>0.05). The concentrations of SEPS and IEPS were not affected by the milk and soy-based formulas (P>0.05).

Discussion

In the present study, both milk and soy-based infant formulas caused a significant demineralization on deciduous enamel, even in the absence of sucrose, when used at the frequency of 8 times a day and in the absence of fluoridated dentifrices. These findings are in agreement with results of studies conducted with animals[11,12], which found that infant formulas may have some cariogenic potential, although lower than that of sucrose. These results may be explained by the acidogenicity of the biofilm formed. The *in situ* biofilm pH assessment confirmed *in vitro/in vivo* findings of Sheikh & Erickson[8], Erickson et al[9] and Danchaivijitr et al[10], and suggests that the carbohydrates present in these formulas (lactose and maltodextrin for the milk formula and only maltodextrin for the soy-based formula) were fermented, resulting in acid production that decreased the biofilm pH. Different types of sugars can produce different amounts of organic acid, among which, lactose is one of the least acidogenic.[28,29] Soy-based formula is lactose free, but do contain other non-milk

extrinsic sugars, such as maltodextrins, which may be considered potentially cariogenic[30,31], and in our study even lowered the plaque pH more than the milk-based one (P<0.05), which is in agreement with *in vitro/in vivo* findings.[8,9,32]

In addition, when sucrose was added, the milk and soy-based formula groups caused higher demineralization on deciduous human dental enamel than in the absence of this carbohydrate, and similar to that of the 10% sucrose solution group. Even though both formulas tested do not present sucrose in their composition, which is supported by the data of SEPS and IEPS, the remainder of the children's diet may contain this cariogenic carbohydrate and some studies[13,14] show that feeding bottles with added sugars are still being given to a great number of children, making this a relevant issue. The higher demineralization found for both formulas in the presence of sucrose may be explained by the changes provoked by sucrose in the biofilm matrix, such as the synthesis of extracellular polysaccharides. In the absence of sucrose, the formulas did not affect SEPS and IEPS concentrations (Table 2), which is in agreement with the literature[17,33], as sucrose is the only substrate for extracellular polysaccharide synthesis. In the presence of sucrose, there was a significant increase in the concentration of these polysaccharides (Table 2), which may have changed the biofilm matrix, improving its cariogenicity, as can be seen in the mineral loss of all treatment groups with sucrose. Extracellular polysaccharides are considered important virulence factors of the dental biofilm formed in the presence of sucrose[34] and the relation between these polysaccharides and enamel demineralization has been well supported by in situ studies.[17,35] Moreover, these changes in biofilm matrix may also have affected the shape of the pH curve, which could not be evaluated in this study since only the baseline and pH_{5min} values were measured. Further studies should be conducted evaluating more

closely the effect of the formulas on the pH curve of the biofilm formed in the presence and absence of sucrose.

The present study showed that both infant formulas, even when no sucrose was added, increased the counts of *Lactobacillus* ($P<0.05$; table 1). This effect may be related to the biofilm pH fall caused by these treatments (Table 2), selecting these microorganisms, which are highly aciduric[36], and confirms previous data showing an increase in LB counts in biofilms treated with highly fermentable sugars.[37] With regard to MS populations in the biofilm formed, data are in agreement with Ribeiro et al[16] and Vale et al[38], showing increased numbers in the presence of sucrose, but no effect of the formulations could be observed. This observation may suggest the important role of sucrose in increasing the adherence of mutans streptococci, mediated by extracellular polysaccharides produced in the presence of this carbohydrate.[39]

Also, the results of mineral availability in the biofilm fluid confirm that the higher Ca concentration in the fluid, as found by Tenuta et al[40] and Ccahuana-Vásquez et al[41], can be the result of a low biofilm pH, which may release these ions from enamel or biofilm mineral reservoirs to the fluid.[40] The absence of such an effect for P_i or F concentrations, which is in agreement with Tenuta et al[40] and Ccahuana-Vásquez et al[41], suggests that on fasting these minerals levels in the fluid are not affected by the higher acidogenicity of the biofilm.

Conclusions

In conclusion, the results suggest that both milk and soy-based formula present potential to induce demineralization in deciduous enamel, which was increased when sweetened with sucrose.

Acknowledgments

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References

1. Marthaler TM. Changes in dental caries 1953-2003. Caries Res 2004;38:173-81.
2. Narvai PC, Frazão P, Roncalli AG, Antunes JL. Dental caries in Brazil: decline, polarization, inequality and social exclusion. Panam Salud Publica 2006;19:385-93.
3. Curzon MEJ, Preston AJ. Risk groups: nursing bottle caries/caries in the elderly. Caries Res 2004;38:24-33.
4. Kaste LM, Gift HC. Inappropriate infant bottle feeding. Status of the Healthy People 2000 objective. Arch Pediatr Adolesc Med 1995;149:786-91.
5. Tinanoff N, O'Sullivan DM. Early childhood caries: overview and recent findings. Pediatr Dent 1997;19:12-6.
6. Schroth RJ, Cheba V. Determining the prevalence and risk factors for early childhood caries in a community dental health clinic. Pediatr Dent 2007;29:387-96.
7. Tinanoff N, Palmer CA. Dietary determinants of dental caries and dietary recommendations for preschool children. J Public Health Dent 2000;60:197-206.
8. Sheikh C, Erickson PR. Evaluation of plaque pH changes following oral rinse with eight infant formulas. Pediatr Dent 1996;18,200-4.
9. Erickson PR, McClintock KL, Green N, LaFleur J. Estimation of the caries-related risk associated with infant formulas. Pediatr Dent 1998;20:395-403.
10. Danchaivijitr A, Nakornchai S, Thaweeboon B, et al. The effect of different milk formulas on dental plaque pH. Int J Paediatr Dent 2006;16:192-8.
11. Bowen WH, Pearson SK, Rosalen PL, Miguel JC, Shih AY. Assessing the cariogenic potential of some infant formulas, milk and sugar solutions. J Am Dent Assoc 1997;128:865-71.

12. Peres RC, Coppi LC, Franco EM, Volpato MC, Groppo FC, Rosalen PL. Cariogenicity of different types of milk: an experimental study using animal model. Braz Dent J 2002;13:27-32.
13. Dini EL, Holt RD, Bedi R. Caries and its association with infant feeding and oral health-related behaviours in 3-4-year-old Brazilian children. Community Dent Oral Epidemiol 2000;28:241-8.
14. Rosenblatt A, Zarzar P. Breast-feeding and early childhood caries: an assessment among Brazilian infants. Int J Paediatr Dent 2004;14:439-45.
15. Pecharki GD, Cury JA, Paes Leme AF, Tabchoury CPM, Del Bel Cury AA, Rosalen PL, Bowen WH. Effect of sucrose containing iron (II) on dental biofilm and enamel demineralization in situ. Caries Res 2005;39:123-9.
16. Ribeiro CCC, Tabchoury CPM, Del Bel Cury AA, Tenuta LMA, Rosalen PL, Cury JA. Effect of starch on the cariogenic potential of sucrose. Br J Nutr 2005;94:44-50.
17. Cury JA, Rebelo MA, Del Bel Cury AA, Derbyshire MT, Tabchoury CPM. Biochemical composition and cariogenicity of dental plaque formed in the presence of sucrose or glucose and fructose. Caries Res 2000;34:491-7.
18. Cury JA, Rebello MA, Del Bel Cury AA. In situ relationship between sucrose exposure and the composition of dental plaque. Caries Res 1997;31:356-60.
19. Hara AT, Queiroz CS, Paes Leme AF, Serra MC, Cury JA. Caries progression and inhibition in human and bovine root dentine in situ. Caries Res 2003;37:339-44.
20. Lingström P, Birkhed D, Ruben J, Arends J. Effect of frequent consumption of starchy food items on enamel and dentin demineralization and on plaque pH in situ. J Dent Res 1994;73:652-60.

21. Aires CP, Del Bel Cury AA, Tenuta LMA, Klein MI, Koo H, Cury JA. Effect of starch and sucrose on dental biofilm formed and on root dentine demineralization. Caries Res 2008 (accepted).
22. Gold OG, Jordan HV, Van Houte J. A selective medium for *Streptococcus mutans*. Arch Oral Biol 1973;18:1357-64.
23. Vogel GL, Mao Y, Carey CM, Chow LC. Increased overnight fluoride concentrations in saliva, plaque, and plaque fluid after a novel two-solution rinse. J Dent Res 1997;76:761-7.
24. Vogel GL, Carey CM, Chow LC, Tatevossian A. Micro-analysis of plaque fluid from single-site fasted plaque. J Dent Res 1990;69:1316-23.
25. Vogel GL, Chow LC, Brown WE. A microanalytical procedure for the determination of calcium, phosphate and fluoride in enamel biopsy samples. Caries Res 1983;17:23-31.
26. Dubois M, Gillis KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem 1956;28:350-6.
27. Box GEP, Hunter WG, Hunter JS. *Statistics for Experimenters*. New York: John Wiley & Sons Inc; 1978.
28. Neff D. Acid production from different carbohydrate sources in human plaque in situ. Caries Res 1967;1:78-87.
29. Edgar WM, Dodds MW. The effect of sweeteners on acid production in plaque. Int Dent J 1985;35:18-22.
30. Levine RS. Briefing paper: maltodextrins and caries. Br J Nutr 1998;185:392.
31. Moynihan PJ. Update on the nomenclature of carbohydrates and their dental effects. J Dent 1998;26:209-18.

32. Bhat SS, Dubey A. Acidogenic potential of Soya infant formula in comparison with regular infant formula and bovine milk: a plaque pH study. J Indian Soc Pedod Prev Dent 2006;21:30-4.

33. Paes Leme AF, Koo H, Bellato C, Bedi G, Cury JA. The role of sucrose in cariogenic dental biofilm formation – New insight. J Dent Res 2006;85:878-87.

34. Bowen WH. Do we need to be concerned about dental caries in the coming millennium? Crit Rev Oral Biol Med 2002;13:126-31.

35. Aires CP, Tabchoury CPM, Del Bel Cury AA, Koo H, Cury JA. Effect of sucrose concentration on dental biofilm formed in situ and on enamel demineralization. Caries Res 2006;10:28-32.

36. Marsh PD. Are dental diseases examples of ecological catastrophes? Microbiology 2003;149:279-94.

37. Tenuta LMA, Ricomini Filho AP, Del Bel Cury AA, Cury JA. Effect of sucrose on the selection of mutans streptococci and lactobacilli in dental biofilm formed in situ. Caries Res 2006;40:546-9.

38. Vale GC, Tabchoury CPM, Arthur RA, Del Bel Cury AA, Paes Leme AF, Cury JA. Temporal relationship between sucrose-associated changes in dental biofilm composition and enamel demineralization. Caries Res 2007;41:406-12.

39. Schilling KM, Bowen WH. Glucans synthesized in situ in experimental salivary pellicle function as specific binding sites for *Streptococcus mutans*. Infect Immun 1992;60:284-95.

40. Tenuta LMA, Del Bel Cury AA, Vogel GL, Cury JA. Ca, P_i and F in the fluid of biofilm formed under sucrose. J Dent Res 2006;85:834-8.

41. Ccahuana-Vásquez RA, Tabchoury CPM, Tenuta LM, Del Bel Cury AA, Vale GC, Cury JA. Effect of frequency of sucrose exposure on dental biofilm composition and enamel demineralization in the presence of fluoride. Caries Res 2007;41:9-15.

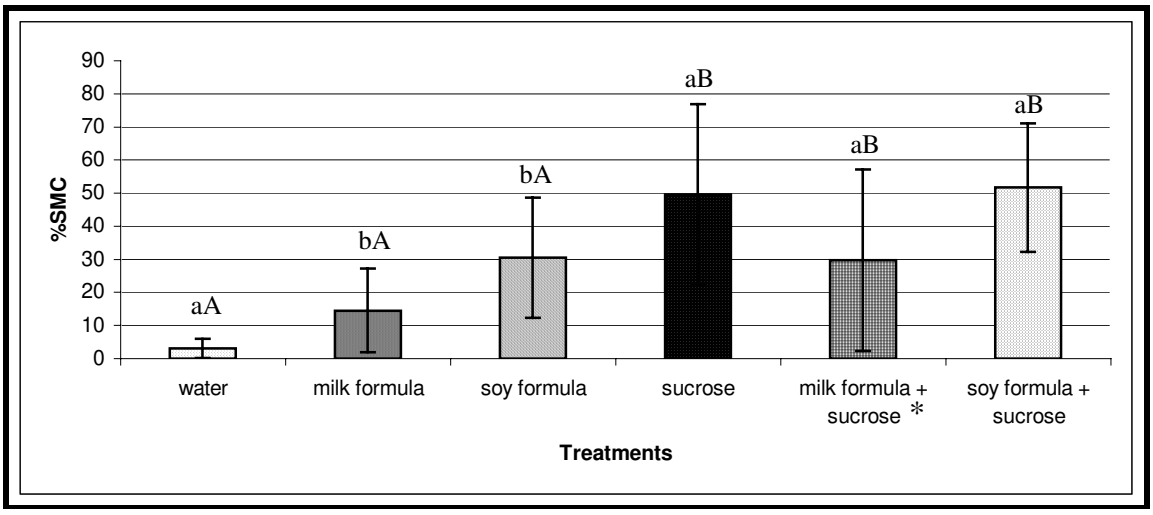

Fig. 1. Mean percentage of surface microhardness change (%SMC) according to the treatment groups (n=11; *n=10). Vertical bars denote standard deviations. Lower case-letters compare the effect of formulas and capital letters compare the effect of sucrose.

Table 1. Microbiological data of dental biofilm according to the treatments (mean ± SD; n=11).

Variables	Treatments Group					
	water	**milk formula**	**soy formula**	**sucrose**	**milk formula + sucrose**	**soy formula + sucrose**
TM (CFU/mg x 10^7)	2.14 ± 1.23	2.33 ± 1.58	1.63 ± 0.68*	1.71 ± 1.22	1.87 ± 0.77*	1.71 ± 1.79
LB (CFU/mg x 10^5)	0.01 ± 0.03	4.11 ± 12.43	13.93 ± 39.07*	3.88 ± 9.99	11.79 ± 17.29	11.74 ± 22.46
%LB/TM	0.01 ± 0.02	4.56 ± 13.45	9.24 ± 22.68*	2.92 ± 7.03	5.57 ± 8.76*	10.07 ± 13.73
MS (CFU/mg x 10^3)	0.38 ± 0.98	2.24 ± 3.61	0.69 ± 1.70*	3.78 ± 5.54	4.63 ± 7.23	1.49 ± 2.96
%MS/TM	0.003 ± 0.006	0.02 ± 0.041	0.004 ± 0.011*	0.053 ± 0.089	0.026 ± 0.037*	0.007 ± 0.008

*n = 10

CFU=colony-forming units; LB=lactobacilli; MS=mutans streptococci; TM=total micro-organisms.

No statistically significant effect of the factors formula and sucrose was observed for TM counts. LB increased in the groups treated with both formulas and with sucrose. MS increased in the treatment groups with sucrose, independent of the formula.

Table 2. Mean (± SD; n=11) of pH analysis and biochemical composition of biofilm according to the treatments.

Variables	Treatments Group					
	water	**milk formula**	**soy formula**	**sucrose**	**milk formula + sucrose**	**soy formula + sucrose**
baseline pH	7.4 ± 0.5	7.3 ± 0.4	7.0 ± 0.5	6.8 ± 0.6	7.1 ± 0.5	6.7 ± 0.6
pH_{5min}	7.3 ± 0.6 aA	6.2 ± 0.6 bA	5.6 ± 0.4 cA	5.9 ± 0.5* aB	5.8 ± 0.5 aB	5.4 ± 0.4 bA
F in fluid (µM)	4.0 ± 2.2*	3.4 ± 1.6	5.8 ± 7.5*	3.3 ± 1.3	3.5 ± 1.7	3.4 ± 3.3
Ca in fluid (mM)	0.8 ± 0.6*	1.5 ± 0.8*	1.9 ± 1.1*	1.7 ± 0.5*	2.0 ± 1.4**	2.7 ± 2.0
P_i in fluid (mM)	9.1 ± 3.3*	10.8 ± 5.1	9.7 ± 3.1*	9.3 ± 3.1*	10.1 ± 4.1	9.3 ± 2.8
SEPS (µg/mg)	0.9 ± 0.4**	1.1 ± 1.2	0.9 ±0.5*	1.9 ± 1.1	1.4 ± 0.9	2.1 ± 3.1
IEPS (µg/mg)	2.7 ± 1.5	3.1 ± 3.8	3.6 ± 3.2*	12.0 ± 10.2	6.1 ± 3.2	6.0 ± 3.6
IPS (µg/mg)	2.0 ± 1.4	3.6 ± 1.0*	3.8 ± 1.5*	3.6 ±1.2*	3.3 ± 2.0	3.5 ± 1.8

* n=10; **n=9

Lower case-letters compare the effect of formulas and capital letters compare the effect of sucrose.

SEPS=soluble extracellular polysaccharide; IEPS=insoluble extracellular polysaccharide; IPS=intracellular polysaccharide

Baseline pH was lower in soy-based formula group and also in the groups with sucrose ($P<0.05$). No statistically significant effect of the factors formula and sucrose was observed for F and P_i in the fluid ($P>0.05$). Ca concentration in the fluid was higher in soy-based formula group and in the groups with sucrose ($P<0.05$). There was no significant effect of the factors under study for IPS ($P>0.05$), while SEPS and IEPS concentrations were higher in the groups with sucrose, independent of the formula ($P<0.05$).

III- CONCLUSÃO GERAL

Os resultados sugerem que ambas formulações, à base de leite e à base de soja, apresentaram potencial para induzir a desmineralização no esmalte dental decíduo, a qual foi aumentanda quando adoçadas com sacarose, sendo que, em relação ao biofilme, ambas formulações levaram à queda de pH e aumentaram a contagem de lactobacilos.

IV- REFERÊNCIAS*

1) Bowen WH, Pearson SK, Rosalen PL, Miguel JC, Shih AY. Assessing the cariogenic potential of some infant formulas, milk and sugar solutions. J Am Dent Assoc. 1997; 128(7):865-71.
2) Curzon MEJ, Preston AJ. Risk groups: nursing bottle caries/caries in the elderly. Caries Res. 2004; 38:24-33.
3) Danchaivijitr A, Nakornchai S, Thaweeboon B, Leelataweewud P, Phonghanyudh A, Kiatprajak C, Surarit R. The effect of different milk formulas on dental plaque pH. Int J Paediatr Dent. 2006; 16:192-8.
4) Dini EL, Holt RD, Bedi R. Caries and its association with infant feeding and oral health-related behaviours in 3-4-year-old Brazilian children. Community Dent Oral Epidemiol. 2000; 28:241-8.
5) Erickson PR, McClintock KL, Green N, LaFleur J. Estimation of the caries-related risk associated with infant formulas. Pediatr Dent. 1998; 20:395-403.
6) Harris R, Nicoll AD, Adair PM, Pine CM. Risk factors for dental caries in young children: a systematic review of the literature. Community Dent Health. 2004; 21(1 Suppl): 71-85.
7) Kaste LM, Gift HC. Inappropriate infant bottle feeding. Status of the Healthy People 2000 objective. Arch Pediatr Adolesc Med. 1995; 149:786-91.
8) Marthaler TM. Changes in dental caries 1953-2003. Caries Res. 2004; 38:173-81.
9) Narvai PC, Frazão P, Roncalli AG, Antunes JL. Dental caries in Brazil: decline, polarization, inequality and social exclusion. Panam Salud Publica. 2006; 19:385-93.
10) Peres RC, Coppi LC, Franco EM, Volpato MC, Groppo FC, Rosalen PL. Cariogenicity of different types of milk: an experimental study using animal model. Braz Dent J. 2002; 13(1):27-32.
11) Peters R. Risk factors in nursing caries syndrome: A literature survey. J Dent Assoc S Afr. 1994; 49:169-175.

* De acordo com a norma da UNICAMP/FOP, baseadas nas normas do International Committee of Medical Journal Editors – Grupo de Vancouver. Abreviatura dos periódicos de acordo com o Medline.

12) Rosenblatt A, Zarzar P. Breast-feeding and early childhood caries: an assessment among Brazilian infants. Int J Paediatr Dent. 2004; 14(6):439-45.

13) Schroth RJ, Cheba V. Determining the prevalence and risk factors for early childhood caries in a community dental health clinic. Pediatr Dent. 2007; 29:387-96.

14) Sheikh C, Erickson PR. Evaluation of plaque pH changes following oral rinse with eight infant formulas. Pediatr Dent. 1996; 18(3):200-4.

15) Tinanoff N, O'Sullivan DM. Early childhood caries: overview and recent findings. Pediatr Dent. 1997; 19:12-6.

16) Tinanoff, N.; Palmer, C.A. Dietary determinants of dental caries and dietary recommendations for preschool children. J Public Health Dent. 2000; 60(3):197-206.

* De acordo com a norma da UNICAMP/FOP, baseadas nas normas do International Committee of Medical Journal Editors – Grupo de Vancouver. Abreviatura dos periódicos de acordo com o Medline.

ANEXO 1: Instruções aos voluntários.

USO DO DISPOSITIVO INTRABUCAL

1. O dispositivo deve ser usado durante todo o dia e à noite, inclusive para dormir
2. Quando estiver fora da boca, em nenhum momento o dispositivo deve ser deixado à seco. Guarde-o no porta-aparelho, com uma gaze umedecida em água.
3. Procure evitar que o dispositivo fique fora da boca por um período prolongado, restringindo-se ao tempo necessário para alimentação (1 hora).
4. Não utilize produtos para bochecho ou agentes tópicos de qualquer natureza na cavidade bucal durante a fase experimental
5. Não utilize vitaminas ou suplementos sistêmicos que contenham flúor durante a fase experimental.
6. Não utilize alimentos que possam ser fonte de flúor como chá verde
7. Quando o dentifrício ou a gaze estiver acabando, entre em contato com a pesquisadora para que possam ser repostos.

GOTEJAMENTO DAS SOLUÇÕES

1. Gotejar uma gota da solução que lhe for dada sobre cada bloco dental 8 vezes ao dia nos seguintes horários:

08:00	**15:30**
09:30	**17:00**
11:30	**19:00**
14:00	**21:00**

2. Para gotejar as soluções, remova o dispositivo da boca, seque com gaze a região da telinha (com cuidado) e goteje uma gota da solução indicada sobre cada bloco dental, sem tocar a ponta do conta-gotas no dispositivo para evitar a contaminação da solução. Aguarde 5 minutos, para que a solução se difunda pela placa dental e retorne à cavidade bucal.

3. Se o primeiro gotejamento do dia não puder ser realizado às 08:00 horas, atrase os outros gotejamentos de acordo com o horário do primeiro, com um intervalo mínimo de 1h e30 min entre eles, até totalizar 8 x ao dia.

4. Após o gotejamento da solução, o aparelho deve retornar à boca em 5 minutos.

5. As soluções devem ser trocadas todos os dias. Solicitamos que você venha ao laboratório buscar a nova solução nesses dias.

NOS FINAIS DE SEMANA, VOCÊ MESMO DEVE PREPARAR AS SOLUÇÕES, CONFORME INSTRUÇÕES ABAIXO:

- esquentar a água no microondas, até ficar morna;
- colocar a água morna no frasco com o pó;
- misturar até dissolver bem;
- colocar no frasco conta-gotas e está pronto para uso.

ESCOVAÇÃO

1. Utilize somente o dentifrício fornecido
2. Realize a escovação dos dentes 3 vezes ao dia
3. Sempre que escovar os dentes, escove também o dispositivo intrabucal. A área da telinha não deve ser escovada. Porém, a espuma gerada pela escovação do dispositivo deverá ser trazida sobre ela com a escova. Para esse procedimento, tome cuidado para não retirar a placa.
4. Ao enxaguar o dispositivo, tome cuidado para que jatos de água da torneira não atinjam diretamente a telinha, causando perda da placa acumulada.

Qualquer dúvida, entre em contato pelos telefones:

3434 -4703 (casa)
8144 9309 (celular)
2106-5303 (laboratório)

Não deixe de ligar e se necessário, ligue a cobrar.

Obrigada pela colaboração!

ANEXO 2: Certificado do Comitê de Ética.

COMITÊ DE ÉTICA EM PESQUISA
FACULDADE DE ODONTOLOGIA DE PIRACICABA
UNIVERSIDADE ESTADUAL DE CAMPINAS

CERTIFICADO

O Comitê de Ética em Pesquisa da FOP-UNICAMP certifica que o projeto de pesquisa "**Cariogenicidade de formulações infantis contendo amido e sacarose**", protocolo nº **044/2006**, dos pesquisadores **CÍNTHIA PEREIRA MACHADO TABCHOURY, ALTAIR ANTONINHA DEL BEL CURY, ANNA MARIA CIA DE MAZER PAPA, JAIME APARECIDO CURY, LIVIA MARIA ANDALÓ TENUTA e RODRIGO ALEX ARTHUR**, satisfaz as exigências do Conselho Nacional de Saúde – Ministério da Saúde para as pesquisas em seres humanos e foi aprovado por este comitê em 04/05/2006.

Piracicaba, 19 de dezembro de 2007

The Ethics Committee in Research of the School of Dentistry of Piracicaba - State University of Campinas, certify that the project "**Cariogenicity of infant formulas containing starch and sucrose**", register number **044/2006**, of **CÍNTHIA PEREIRA MACHADO TABCHOURY, ALTAIR ANTONINHA DEL BEL CURY, ANNA MARIA CIA DE MAZER PAPA, JAIME APARECIDO CURY, LIVIA MARIA ANDALÓ TENUTA and RODRIGO ALEX ARTHUR**, comply with the recommendations of the National Health Council – Ministry of Health of Brazil for research in human subjects and therefore was approved by this committee at 04/05/2006.

Piracicaba, SP, Brazil, December 19 2007

Profa. Cinthia Pereira Machado Tabchoury
Secretária
CEP/FOP/UNICAMP

Prof. Jacks Jorge Júnior
Coordenador
CEP/FOP/UNICAMP

Nota: O título do protocolo aparece como fornecido pelos pesquisadores, sem qualquer edição.
Notice: The title of the project appears as provided by the authors, without editing.

ANEXO 3: Informação CCPG

INFORMAÇÃO CCPG/002/06

Tendo em vista a necessidade de revisão da regulamentação das normas sobre o formato e a impressão das dissertações de mestrado e teses de doutorado e com base no entendimento exarado no Parecer PG nº 1985/96, que trata da possibilidade do formato alternativo ao já estabelecido, a CCPG resolve:

Artigo 1º - O formato padrão das dissertações e teses de mestrado e doutorado da UNICAMP deverão obrigatoriamente conter:

I. Capa com formato único ou em formato alternativo que deverá conter informações relativas ao nível (mestrado ou doutorado) e à Unidade de defesa, fazendo referência à Universidade Estadual de Campinas, sendo o projeto gráfico das capas definido pela PRPG.
II. Primeira folha interna dando visibilidade à Universidade, a Unidade de defesa, ao nome do autor, ao título do trabalho, ao número de volumes (quando houver mais de um), ao nível (mestrado ou doutorado), a área de concentração, ao nome do orientador e co-orientador, ao local (cidade) e ao ano de depósito. No seu verso deve constar a ficha catalográfica.
III. Folha de aprovação, dando visibilidade à Comissão Julgadora com as respectivas assinaturas.
IV. Resumo em português e em inglês (ambos com no máximo 500 palavras).
V. Sumário.
VI. Corpo da dissertação ou tese dividido em tópicos estruturados de modo característico à área de conhecimento.
VII. Referências, formatadas segundo normas de referenciamento definidas pela CPG da Unidade ou por critério do orientador.
VIII. Todas as páginas deverão, obrigatoriamente, ser numeradas, inclusive páginas iniciais, divisões de capítulos, encartes, anexos, etc... As páginas iniciais poderão ser numeradas utilizando-se algarismos romanos em sua forma minúscula.
IX. Todas as páginas com numeração "impar" serão impressas como "frente" e todas as páginas com numeração "par" serão impressas como "verso".

§ 1º - A critério do autor e do orientador poderão ser incluídos: dedicatória; agradecimento; epígrafe; lista de: ilustrações, tabelas, abreviaturas e siglas, símbolos; glossário; apêndice; anexos.

§ 2º - A dissertação ou tese deverá ser apresentada na língua portuguesa, com exceção da possibilidade permitida no artigo 2º desta Informação.

§ 3º - As dissertações e teses cujo conteúdo versar sobre pesquisa envolvendo seres humanos, animais ou biossegurança, deverão apresentar anexos os respectivos documentos de aprovação.

Artigo 2º - A critério do orientador e com aprovação da CPG da Unidade, os capítulos e os apêndices poderão conter cópias de artigos de autoria ou de co-autoria do candidato, já publicados ou submetidos para publicação em revistas científicas ou anais de congressos sujeitos a arbitragem, escritos no idioma exigido pelo veículo de divulgação.

§ único - O orientador e o candidato deverão verificar junto às editoras a possibilidade de inclusão dos artigos na dissertação ou tese, em atendimento à legislação que rege o direito autoral, obtendo, se necessária, a competente autorização, deverão assinar declaração de que não estão infringindo o direito autoral transferido à editora.

Artigo 3º - Dependendo da área do conhecimento, a critério do orientador e com aprovação da CPG da Unidade, a dissertação ou tese poderá ser apresentada em formato alternativo, desde que observados os incisos I, II, III, IV, V e VII do artigo 1º.

Artigo 4º - Para impressão, na gráfica da Unicamp, dos exemplares definitivos de dissertações e teses defendidas, deverão ser adotados os seguintes procedimentos:

§ 1º - A solicitação para impressão dos exemplares de dissertações e teses poderá ser encaminhada à gráfica da Unicamp pelas Unidades, que se responsabilizarão pelo pagamento correspondente.

§ 2º - Um original da dissertação ou tese, em versão definitiva, impresso em folha tamanho carta, em uma só face, deve ser encaminhado à gráfica da Unicamp acompanhado do formulário "Requisição de Serviços Gráficos", onde conste o número de exemplares solicitados.

§ 3º - A gráfica da Unicamp imprimirá os exemplares solicitados com capa padrão. Os exemplares solicitados serão encaminhados à Unidade em, no máximo, cinco dias úteis.

§ 4º - No formulário "Requisição de Serviços Gráficos" deverão estar indicadas as páginas cuja reprodução deva ser feita no padrão "cores" ou "foto", ficando entendido que as demais páginas devam ser reproduzidas no padrão preto/branco comum.

§ 5º - As dissertações e teses serão reproduzidas no padrão frente e verso, exceção feita às páginas iniciais e divisões de capítulos; dissertações e teses com até 100 páginas serão reproduzidas no padrão apenas frente, exceção feita à página que contém a ficha catalográfica.

§ 6º - As páginas fornecidas para inserção deverão ser impressas em sua forma definitiva, ou seja, apenas frente ou frente/verso.

§ 7º - O custo, em reais, de cada exemplar produzido pela gráfica será definido pela Administração Superior da Universidade.

Artigo 5º - É obrigatória a entrega de dois exemplares para homologação.

Artigo 6º - Esta Informação entrará em vigor na data de sua publicação, ficando revogadas as disposições em contrário, principalmente as Informações CCPG 001 e 002/98 e CCPG/001/00.

Campinas, 13 de setembro de 2006

Profa. Dra. Teresa Dib Zambon Atvars
Presidente
Comissão Central de Pós-Graduação