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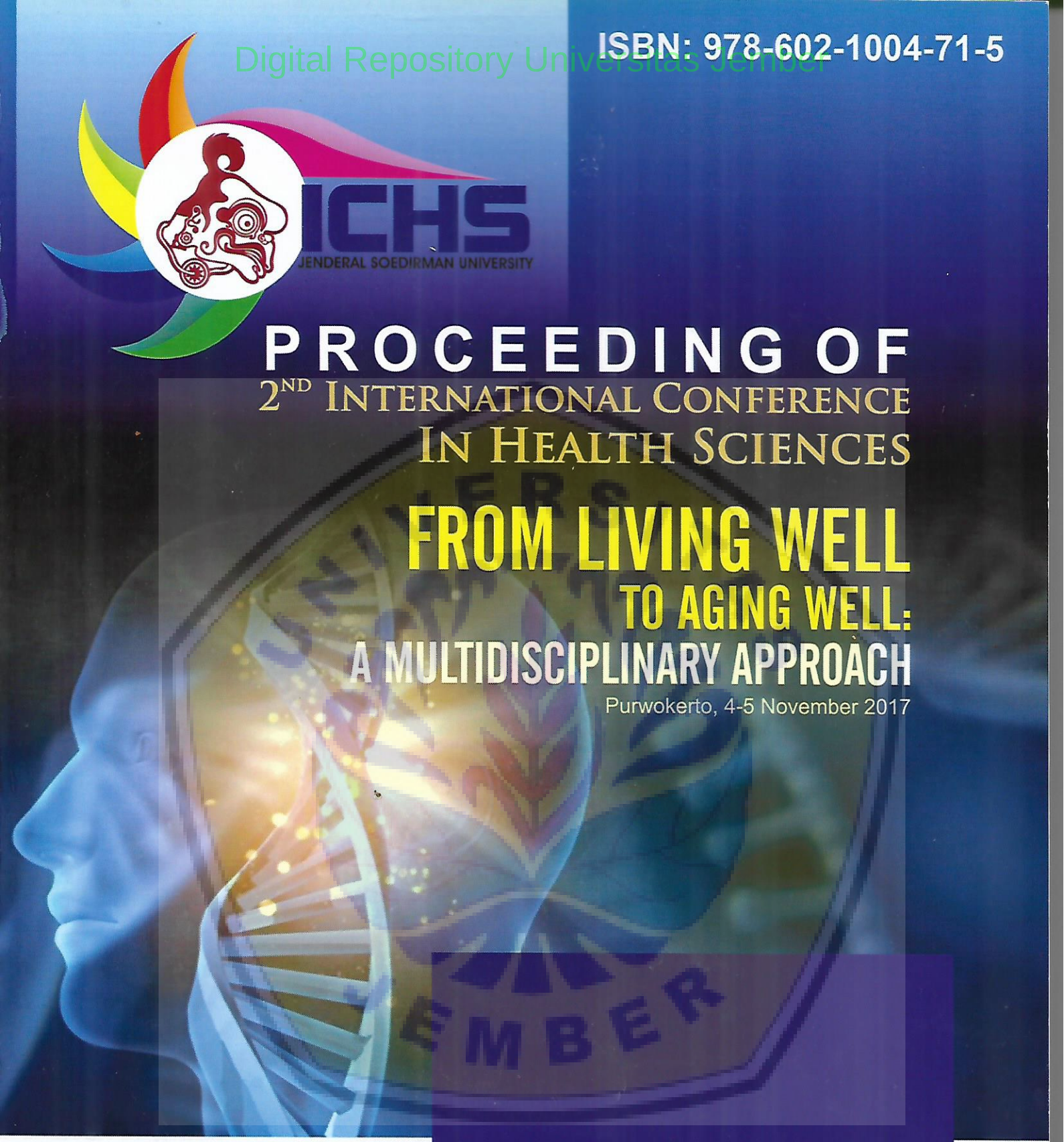
FROM LIVING WELL TO AGING WELL: A MULTIDISCIPLINARY APPROACH

Purwokerto, 4-5 November 2017



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FROM LIVING WELL TO AGING WELL :
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Cetakan Pertama, April 2018

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Dr.sc hum Budi Aji, SKM., MSc
Dr. Saryono, S.Kp., M.Kes
Mekar Dwi A, S.Kep, Ners., MKep., PhD
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Hendry Wasito, Sfarm., MSc., Apt
Yunita Sari, S.Kep., M.Kes., PhD
Dr. Ngadiman, M.Kes

Perancang Sampul:
Panitia

Penata Letak:
Panitia

Diterbitkan oleh:
UNIVERSITAS JENDERAL SOEDIRMAN
Jalan Prof. Dr. H.R. Boenyamin 708 Purwokerto
Kode Pos 53122 Kotak Pos 115
Telepon 635292 (Hunting) 638337, 638795
Faksimile 631802
www.unsoed.ac.id

Dicetak oleh:
BPU Percetakan dan Penerbitan
Universitas Jenderal Soedirman
Telepon: (0281) 626070
Email: unsoedpress@yahoo.com

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The Difference of tooth Demineralization after Soaking in Human Milk and Infant Formula Milk using Scanning Electron Microscope

Berlian Prihatiningrum[1], Sulistiyani[2], and Eni Ilmiati[3]
[1, 2, 3] Pediatric Dentristry Department of Dentristry Faculty, Jember University, [3] Jember University
drg_berlian@yahoo.co.id, sulistiyani_fkg@unej.ac.id, ilmiaeni@yahoo.com
Jl. Kalimantan I / 37, Jember, 62100, Indonesia

Abstract

Milk is considered as a good source of Calsium. Comparison studies of tooth demineralization after soaking with milk from human and infant formula feeding in Indonesia are rare, so we compare the effect of soaking tooth with milk from human and formula to deminaralization of enamel. Demineralization of enamel is influenced by pH, buffering capacity, fluoride, calsium and phosphorus contents. The aim of this study was to determine the level of tooth demineralization after soaked with human milk and infant formula Feeding. This was an experimental laboratory study with post-test design only with control group design. Then samples were soaked in artificial saliva pH 7, human milk and infant formula milk for 24 hours. The depth of microporosity that occurs in each sample observed using Scanning Electron Microscope (SEM). The infant formula feeding result the highest level of tooth demineralization followed by human milk and artificial saliva. There is a significant difference in level of tooth demineralization after soaked in human milk dan infant formula feeding. Human milk has a longer pH stability than infant formula feeding, so it is slower to cause enamel solubility than infant formula feeding. In human milk also containing a right amount of protein and non sucrose that can cause enamel solubilty. Level of teeth demineralization was highest from infant formula feeding followed by human milk and artificial saliva.

Keywords : tooth demineralization, human milk, infant formula milk, artificial saliva

Introduction

Human milk is uniquely suited to the human infant, both in its nutritional composition and in the non-nutritive bioactive factors that promote survival and healthy development [1]. Breast milk contains over 200 nutritional, as well as functional, components. The fundamental composition of breast milk includes, protein, salt, and sugar, which are all contained in a fat suspension. In addition to those nutrients breast milk also provides the infant with immune factors, growth and hormone factors, and enzymes [2]. The milk produced during the first month following parturition by mothers delivering between 28-36 weeks gestation contained significantly higher concentration of nitrogen and lower concentration of lactose than milk produced by mothers delivering at term [3]. Human milk is a complex matrix with a general composition of 87% water, 3.8% fat, 1.0% protein, and 7% lactose [4]. In contrast to protein and fat, lactose content is fairly constant in mature milk (after 21 days postpartum). The stable concentration of lactose is important in maintaining a constant osmotic pressure in human milk. Lactose also aids the absorption of minerals and calcium. In human milk, many carbohydrate-based bioactive compounds, such oligosaccharides, are attached to lactose [5]. Human milk, in contrast to formula, contains breast-specific Lactobacilli and substances,

including human casein and secretory IgA, which inhibit the growth and adhesion of cariogenic bacteria, particularly oral Streptococci [6].

Infant formula is intended as an effective substitute for infant feeding and is formulated to mimic the nutritional composition of breast milk for normal infant growth and development [7,8]. Cow milk or soymilk are most commonly used as the base, with supplemental ingredients added to better approximate the composition to human breast milk and to attain health benefits, including iron, nucleotides and compositions of fat blends. The fatty acids of arachidonic acid (AA) and docosahexenoic acid (DHA) are added. Probiotics and compounds, produced by genetic engineering, are either added or currently being considered for addition to formula. The most common infant formulas consumed by infants are made from modified cow's milk with added carbohydrate (usually lactose), vegetable oils, and vitamins and minerals. Casein is the predominant protein in cow's milk [9]. Both human milk and infant formula important to stimulate the bone and teeth modelling, especially to help the strength of dentine and enamel.

Dental Enamel consists of densely packed mineral crystal mainly hydroxyapatite (HA) and it can become demineralized due to exposure to plaque acids. Besides HA, dental enamel consists another minerals such as Ca, Na, Cl, Zn and P. Tooth enamel can undergo a process called demineralisation if the pH of the mouth falls to lower than normal levels [10]. Demineralization of the enamel is damaged hydroxy apatite tooth enamel which is the main component of enamel due to chemical processes [11]. Its occurs through a diffusion process, ie the process of moving molecules or ions dissolved in water to or from the enamel to saliva because there are differences in the concentration of acidic water on the surface of the tooth enamel [12]. In the development of dental caries, the relationship between demineralization and remineralization is influenced by the presence of saliva, which facilitates the transportation of ions, oral bacteria, and fermentable carbohydrates to the exposed surfaces of teeth [13]. Sucrose has the highest cariogenity, followed by sorbitol and lactose. Lactose in milk can be synthesized into lactic acid with an acidity degree (pH) of 5.5. If foods and beverages containing lactose are consumed too often, the oral cavity will be in acidic conditions, which eventually leads to demineralization of the tooth enamel [14].

The relationship between breastfeeding both human milk or infant formula and tooth demineralization has been systematically and narratively reviewed with conflicting results between studies. There is controversy about what constitutes the best form of infant feeding to prevent enamel demineralization and promote optimal dental health. Consequently no definitive optimal breastfeeding practices have been determined to specifically address the risk of enamel demineralization that can lead dental caries. The aim of this study is to determine the level of tooth demineralization after soaked with human milk and infant formula Feeding.

Research Methods

Type of research was an experimental laboratory with post test only control group design. The research performed on Desember 2016 – Januari 2017 in Bioscience Laboratorium at Dental Hospital of Jember University.

Samples were three piece of first upper premolar with healthy surfaces and no visible defects, from each tooth, four fragments containing a superficial enamel layer and a subjacent dentine portion were obtained by cutting with a water-cooled carborundum disk mounted on a low speed handpiece. Two bucco-lingual, combined with two mesio-distal incisions containing occlusal surface enamel were discarded, resulting in a total of 12 dental fragments.

The fragments were covered with a 20mm wide strip of adhesive tape (Scotch, 3M, Sumar´e, SP, Brazil) containing an orifice ranging from 2.5 to 3.5mm in diameter, according to the width of the enamel surface to allow the exposure of a controlled area to the demineralization solution.

Two experimental protocols were designed. In the first protocol, applied to four fragments teeth, first quadrant (labeled A) was used as a control, which is soaking in artificial saliva. Second quadrants (labeled as B) was soaked with human milk at consentration pH 5,5 for 24 hours and changed every 8 hours. The last quadrant (C) was soaked with infant formula milk (Frisian baby, Frisian Flag, Indonesia) at consentration pH 5,5 for 24 hours and changed every 8 hours.

After the demineralization period, the tapes were removed and the fragments were washed with de-ionized water, dried at room temperature and observed with the scanning electrone microscope (SEM, Hitachi TM3030Plus, Japan) examination according to the usual protocols. The samples were examined with a Hitachi TM3030Plus Scanning Microscope (Hitachi Ltd. Tokyo, Japan), at 30 KV acceleration voltage and magnifications of 600×. The images – three for each quadrant of each tooth – were captured with a TM3030Plus (Hitachi Ltd., Tokyo, Japan). The images, viewed on a monitor, were evaluated by three examiners (I–III) and scored as follows: (1) smooth, normal enamel; (2) fissures on the enamel surface; (3) images of mildly increased porosity; (4) images of exposed enamel prisms and dissolution type I–III.

Each examiner scored all the three images of the quadrants of each tooth; the mean score (the mean of the three images scored by each examiner) was included into Tables 1 and 2. In the first experiment, for the calculation of the statistical significance of changes in the enamel surface of samples A, B and C the final mean of scores for all three teeth was used. The statistic analysis was performed using paired samples *t*-test (significance at $p<0.05$).

Results

Typical SEM aspects of the dental enamel surface of teeth used in these experiments are presented in Figures 1–3. Figure 1 depicts unsoaked enamel. The surface is not completely smooth, however the aprismatic surface layer is uniform, but there is no pores and superficial irregularities, such as grooves on the control samples. the mean scores for the control quadrants is 0.00±0,00 (Table 1). For quadrant B, the mean scores is 13,73±1,70 and the difference between the studied group and the control group was statistically significant (p=0.00). For the quadrants C the mean of scores is 20,25±4,99 and the difference between the studied group and the control group was statistically significant (p=0.00).

Table 1. The scores of SEM images for tooth demineralization for each group

Group / Samples	Control (μm)	Quadrant B (Human Milk) (μm)	Quadrant C (Infant Formula Milk) (μm)
1	0,00	12.14	24.22
2	0,00	15.26	15.52
3	0,00	12.38	16.35
4	0,00	15.14	24.89
mean (μm) ± Standard Deviation	0,00±0.00	13.73±1.70	20.25±4,99

Morphological changes were observed in some of the samples treated with human milk, for 24 hours soaking, consisting in areas of depressions which seem sometimes deeper, generating a more variable aspect of the enamel surface (Figures 2 and 3). This aspect suggests an increase in the enamel porosity, as compared to the control samples. However, areas of depression were observed deeper in the samples treated with infant formula milk, for 24 hours soaking, as compared to the control samples and quadrant B.

Table 2. Statistically Difference between Groups

Group	mean (µm) ± Standard Deviation	Control	Quadrant B	Quadrant C
Control	0,00±0.00	N/A	0.001*	0,004*
Quadrant B	13.73±1.70	0.001*	N/A	0.049*
Quadrant C	20.25±4,99	0,004*	0.049*	N/A

The statistical significance of the difference of means compared between quadrant B and C group was high (p=0.049, respectively) (table 2). In this study, the pattern of demineralization followed either a version in which the central part of the prism was involved, or an irregular meshwork.

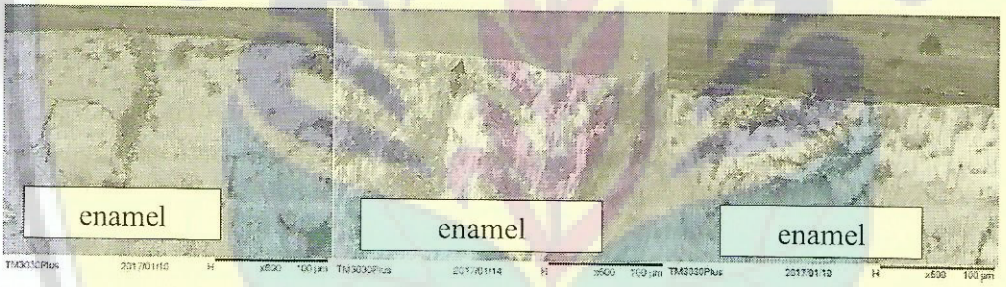

Fig 1. The SEM Micrograph of enamel surface of a control tooth sample (quadrant A)

Fig 2. The SEM Micrograph of enamel surface of a tooth sample soaked with human milk for 24 hours (quadrant B)

Fig 3. The SEM Micrograph of enamel surface of a tooth sample soaked with infant formula for 24 hours

Discussion

Demineralization is the process of removing minerals ions from HA crystals of hard tissues, for example, enamel, dentin, cementum, and bone. Demineralization is a reversible process; hence, the partially demineralized HA crystals in teeth can grow to their original size if they are exposed to oral environments that favor remineralization [12]. Similar to bone, teeth are composites comprised of the phosphate-based mineral HA in the enamel, collagen in the dentine, and living tissues. However, it is the anatomical arrangement and location of teeth that sets them apart from bones. Exposed to food, drink, and the microbiota of the mouth, teeth have developed a high resistance to localized demineralization unmatched by other mineralized tissues. This resistance is chiefly due to the enamel layer that covers the crown of the teeth.

Chemical demineralization of teeth is caused by acidic attack through two primary means: dietary acid consumed through food or drink and microbial attack from bacteria

present in the mouth. During an acidic attack, or a typical demineralization regime, chemical dissolution of both the organic and inorganic matrix components takes place. This is brought about by the water content of enamel and dentine, which facilitate acid diffusion in and mineral content out of tooth [14].

One of the best methods to study the enamel surface is SEM. There are conflicting reports regarding the effects of human milk and infant formula containing lactose on the enamel. Some authors reported morphological changes of the enamel surface after soaking of human teeth to both human milk or infant formula, such as focal areas of shallow erosion, loss of the prismatic layer, pitting, and exposure of the enamel prisms; moreover, it was suggested that after a prolonged exposure to increased concentration pH 5,5 of both human milk and infant formula such changes could be the cause of abrasion or cusp fractures, mainly in restored, weakened teeth [12].

In the present study, the SEM micrograph of Control Group (Quadrant A) showed that there is no enamel surface change. In this case, saliva is considered one of the most important biological factors in dictating the intraoral neutralizing effects of acid exposure. in addition to its cleansing and antibacterial action [16], saliva acts as a constant source for calcium and phosphate that helps in maintaining supersaturation with respect to tooth minerals, therefore inhibiting tooth demineralization during periods of low pH, and they promote tooth remineralization when the pH returns to neutral state. Furthermore, when saliva secretion is stimulated, a rapid rise in pH to above neutrality occurs. Due to its high solubility of calcium phosphate in salivary proteins (eight to ten times higher than calcium phosphate in tooth), it serves as a sacrificial mineral that dissolves preferentially before tooth mineral, ie, reducing demineralization. It also acts as a source of calcium and phosphate ions that are required for remineralization of decalcified tooth [12].

In this case, treated tooth using human milk with consentration pH 5,5 for 24 hours showed an irregular porosity on the enamel surfaces, and also showed a statistically significan than control group. An assessment of the composition of human milk is important to understand the reduced buffer capacity in comparison to bovine milk. Human milk has significantly less phosphate (15 mg/ dL), especially inorganic phosphate (5 mg/dL), when compared to bovine milk (100 mg/dL total phosphate, 75 mg/dL inorganic phosphate). Human milk also has less protein with approximately one-fifth the amount of amino acids when compared to bovine milk [14]. Human milk containing carbohydrate and sugar (lactose). This component can dissolve become lactic acid. This acid will reduce the pH of saliva.

Sample quadrant C showed the deeper and shallower porosity of tooth enamel. In this case, infant formula containing of a higher carbohydrate and sugar (lactose and sucrose) than human milk. The composition of lactose between 6,8 – 23 g/100 ccal and sucrose between 0 – 7g/ 100 ccal. In this study, we use infant formula with 6,8g lactose and 0,5g sucrose. Sucrose is the higher cariogenic ingredient, followed by sorbitol and lactose. Sucrose and lactose will dissolve in to glucose and lactic acid. This structure of acid can influence the pH saliva, to he critical condition that can lead a demineralization of enamel. The condition occurs when the pH of the solution surrounding the enamel surface is lower than 5.5, (generally ranging from 2.3 to 3.6 pH) and the concentration of the acid that does not dissociate higher on the surface enamel, rather than in the enamel [13]. The sucrose control solution did dissolve calcium and phosphate from the powdered enamel, whereas the water control did not.

Human milk showed a shorter porosity on enamel surface than infant human milk. In this case, some authors reported that *S.mutans* may not be able to use lactose, the sugar found in breast milk, as readily as sucrose, found in food or artificial milk, and some breast-milk antibodies may help impede bacterial growth [14]. Phosphate and protein present within

human milk is capable of buffering the free hydrogen ions associated with these organic acids and thereby maintaining the pH near neutral when unchallenged by other acid sources. However, when additional acid is present, the buffering capacity is exceeded.

On the other hand, these irregularities are difficult to be considered as secondary effects of the treatment. When the enamel surfaces were examined in the control groups, sometimes pores, shallow depressions and superficial irregularities are observed, but this situation was reported by other authors, as well. On the surface of normal, sound teeth, circumferentially horizontal lines, known as perikimata, may be found across the face of the crown; on the other hand, lamellae or cracks are not unusual. Electron microscopy observations showed that the surface of the enamel varies with age [15].

Conclusion

Human milk can not lead a tooth demineralization, adding sugar ingredient such as sucrose will stimulate a rapid demineralization on enamel surfaces.

References

[1] Oftedal OT. The evolution of milk secretion and its ancient origins. Animal : an international journal of animal bioscience. 2012; 6(3):355–368. [PubMed: 22436214]
[2] Pereira, aG.R. and Barbosa, N.M.M. (1986) Controversies in neonatal nutrition. Pediat.Clin. North Amer. 33, 65-89.
[3] Narang, A.P.S., Bains, H.S., Kansal, S., Singh, D. Serial Composition of Human Milk in Preterm and Term Mothers. Indian Journal of Clinical Biochemistry. 2006; 21 (1) : 89-94
[4] Guo, M. Human Milk Biochemistry and Infant Formula. In Manufacturing Technology; Elsevier: Cambrdige, UK, 2014.
[5] Duchén K, Thorell L. Nucleotide and polyamine levels in colostrum and mature milk in relation to maternal atopy and atopic development in the children. *Acta Paediatr* 1999; **88**: 1338–1343.
[6] Institute of Medicine of the National Academics. Infant Formula: Evaluating The Safety of New Ingredients; The National Academics Press: Washington, DC, USA, 2004.
[7] Stevens, E.E.; Patrick, T.E.; Pickler, R. A history of infant feeding. J. Perinat. Educ. **2009**, 18, 32–39. [CrossRef] [PubMed]
[8] Adhani, R.S., Hidayat., Arya, W. 2014. Perbedaan pH Saliva Menggosok Gigi Sebelum dan Sesudah Mengkonsumsi Makanan Manis dan Lengket Pengukuran Menggunakan pH Meter pada Anak Usia 10-12 Tahun di SDN Melayu 2 Banjarmasin. Dentino (Jur. Ked. Gigi). Vol. II (1). p: 39-45
[9] Ollila P, Larmas M. A seven-year survival analysis of caries onset in primary second molars and permanent first molars in different caries risk groups determined at age two years. Acta Odontol Scand 2007; 65: 29–35.
[10] Bahuguna R, Younis Khan S, Jain A. Influence of feeding practices on dental caries. A case–control study. Eur J Paediatr Dent 2013; 14: 55–8.
[11] Y. F. Ren. (April 2014). Dental erosion: etiology, diagnosis and prevention. [Online]. Available: http://www.rdhmag.com
[12] R. Lagocka, J. S. Bochinska, I. Nocen, K. Jakubowska, M. Gora, and J.B. Radlinska, "Influence of the mineral compotition of drinking water taken from surface water intake in enhancing regeneration processes in mineralized human teeth tissue," *Polish J of Environ. Stud*, vol. 20, no. 2, pp. 411-416, 2011.
[13] Nerves, AA., Primo, LG., Ramos, PR. Microscopic Investigation of artificially demineralized surface enamel exposed to controlled intra-oral periods. Australian Dental Journal, 2003; 48 (4): 248-254
[14] Tham, R., Dharmage, SC., Tan, DJ., Lau MXZ., Allen, KJ., Lodge, CI. Breastfeeding and the risk of dental caries : a systematic review and meta analysis, Acta Paediatrica. 2015, 104, pp: 62-84
[15] Dudea, D., Florea, A., Mihu, C., Campeanu, R, Nicola, C., Benga, GH. The use of scanning electron microscopy in evaluating the effect of a bleaching agent on the enamel surface. Romanian Journal of Morphology and Embryology, 2009, 50 (3): 435-440