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The potential acidogenicity of liquid breakfasts

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Running title: Acidogenicity and liquid breakfasts

Key words: cariogenicity; buffering capacity; milk; *Streptococcus mutans*

The potential acidogenicity of liquid breakfasts

Abstract

Objectives: To determine the potential acidogenicity of liquid breakfasts.

Methods: *In vitro* acid production by *Streptococcus mutans* was measured in the beverages at a pH of 5.5, as was the fall in pH over 10 min. The buffering capacity was determined, as well as the calcium, inorganic phosphate and fluoride concentrations (total and soluble) of the beverages. Bovine milk (UHT) was used for comparison.

Results: The rate of acid production by *S. mutans*, and pH fall over 10 min was greater in liquid breakfasts compared to bovine milk. All beverages except one demonstrated a significantly lower buffering capacity than bovine milk. All beverages contained significantly greater concentrations of soluble calcium than bovine milk, and all except two contained significantly more soluble inorganic phosphate.

Conclusions: *S. mutans* was able to generate significantly more acid in the liquid breakfasts than in bovine milk, indicating these drinks may contribute to a cariogenic diet. In general, the liquid breakfasts required significantly less acid than bovine milk to reduce their pH to the approximate critical pH for enamel demineralisation. However, the liquid breakfasts also tended to contain significantly more soluble calcium and inorganic phosphate than bovine milk.

Clinical significance: The substantial amounts and various types of sugars found within liquid breakfast beverages may result in a significant pH drop in dental plaque following consumption of these products.

Introduction

Dental caries is the dissolution of tooth structure by the acid produced through bacterial fermentation of dietary carbohydrates in the oral environment [1]. Many microorganisms are able to contribute to acid production within the supragingival plaque biofilm and as a result, the caries-associated microbiota is complex and may include *Streptococcus*, *Bifidobacterium*, *Propionibacterium*, *Lactobacillus*, *Actinomyces* and *Granulicatella* species [2-6]. Dental caries is a multifactorial disease; a combination of microbiological shifts within the complex plaque biofilm as well as salivary flow and composition, fluoride exposure, preventive behaviours such as tooth cleaning, and dietary sugar determines the likelihood of development and progression of disease [7].

Recent figures indicate a rise in the incidence of dental caries in Australian children and young adults [8-10]. One possible explanation is the increased consumption of sugar-sweetened beverages [11-13]. An example of such a beverage is the increasingly popular category of liquid breakfasts. As widely available pre-packaged milk-based drinks, liquid breakfast products are marketed as a liquid version of cereal with milk, for people wanting to consume breakfast on the go. In a recent investigation into liquid breakfast products, 10 of 23 liquid breakfasts tested were found to contain double the amount of sugar present in full cream milk, at more than 23 g of sugar per serve [14] indicating the addition of substantial amounts of sugar.

There has long been an association between the consumption of dietary sugars and the development of dental caries [15]. Studies have found high sugar consumption, particularly in beverage form to be associated with increased caries rates in children [16, 17], teenagers [12, 17] and adults [11]. A recent systematic review undertaken on behalf of the World Health Organisation reaffirmed the relationship between the amount of sugar consumed and dental caries development [18]. Following this review it was recommended that in order to reduce

the caries burden in both adults and children sugar intake should ideally be less than 3% of energy intake [19].

Whilst dietary sugars have been demonstrated to be cariogenic, cow's milk is considered to be non-cariogenic [20] and may indeed exhibit caries protective effects [21]. A high intake of dairy products appears to be associated with less future caries development [22] and a recent review of dairy intake and health outcomes found an inverse relationship between the consumption of milk and dairy products and dental caries in children and adolescents [23]. The consumption of cheddar cheese immediately after a sweet meal has been shown to significantly reduce the amount of lactic acid produced in the oral cavity, when compared with the amount of acid obtained from the sweet food alone [24]. The consumption of milk after a sugary cereal challenge has also been found to significantly reduce the plaque pH drop following this challenge [25]. Despite the presence of the naturally occurring fermentable disaccharide lactose, the lack of cariogenicity associated with dairy products is thought to be related to their high buffering capacity, and high casein, calcium and phosphate content [26], however the addition of sucrose to bovine milk may affect this property [27].

Therefore, the aim of this study was to determine the acid production following *Streptococcus mutans* fermentation, as well as the acid buffering capacity and fluoride, calcium and inorganic phosphate contents of liquid breakfasts. Together, these parameters may indicate the potential cariogenicity of these products.

Materials and methods

Beverages

Six liquid breakfast beverages were chosen for analysis. Up & Go™ products (Sanitarium, NSW, Australia) were selected due to their popularity and availability. Oats Express (Dairy Farmers, NSW, Australia), Sustagen® Ready to Drink (Nestlé, NSW, Australia) and Musashi®P30® (Nestlé, Victoria, Australia) were also selected. All beverages chosen were vanilla flavoured to reduce possible experimental variation. A long shelf-life regular bovine milk product, Pura® Milk Long Life/UHT (National Foods, Victoria, Australia) was chosen to compare with the similarly UHT-treated liquid breakfast products. Experiments were conducted prior to the stated expiry dates on the packaging. The commercial products used were; Pura Milk (Long life/UHT), Up & Go, Up & Go Energize, Up & Go Vive, Oats Express, Sustagen Ready to Drink and Musashi P30.

Bacteria and growth conditions

Streptococcus mutans was chosen as the model cariogenic microorganism for this study as it is able to rapidly catabolise simple carbohydrates including sucrose, lactose, glucose and fructose to generate organic acids, and has the ability to metabolise and grow at a low environmental pH [28]. *Streptococcus mutans* strain Ingbritt was obtained from the culture collection of The Melbourne Dental School, The University of Melbourne. *S. mutans* was stored and grown in batch culture using Todd Hewitt-Yeast Extract (THYE) broth at 37 °C. Bacterial cells in logarithmic growth phase were harvested by initial centrifugation (1500 x g), washed twice with fermentation minimal medium (FMM – 50 mM KCl, 5 mM NaCl, 2 mM MgSO₄, 2 mM MnCl₂, and 8 mM (NH₄)₂SO₄ at pH 7.0) and resuspended in FMM to attain 2 mg dry weight cells/mL [29, 30].

Acid production by S. mutans

A *S. mutans* cell suspension (8 mL) was mixed with an equal volume of the beverage sample in the fermentation vessel of a TIM856 titration assembly (Radiometer, Copenhagen, Denmark) that was stirred and maintained at a constant temperature of 37 °C. Acid production by *S. mutans* was determined at a constant pH of 5.5. Acid produced by the bacteria was neutralised by the automatic addition of 0.1 M NaOH and the rate of acid production by *S. mutans* calculated as described previously [30, 31]. The decrease in pH over time (10 min) of the *S. mutans* cell suspensions mixed with each beverage was determined using the same equipment but with the titration system disabled.

Titration

The ability of the beverages to resist acidification was determined by titrating beverage samples in the TIM856 titration assembly (Radiometer, Copenhagen, Denmark) at 37 °C with a 0.1 M HCl solution until a stable pH of 5.5 was attained.

Mineral analyses – total calcium, inorganic phosphate and fluoride

Protein was removed from 5 mL of each beverage sample by addition of 20 mL deionised water followed by 25 mL of 24% trichloroacetic acid (TCA) which was then mixed well and allowed to stand for 30 min. This was then filtered through Whatman No. 1 filter paper to remove the precipitate, and the filtrates were stored at 4 °C. Each sample was analysed in triplicate. Prior to determining the concentration of total calcium and inorganic phosphate, the refrigerated samples were warmed to room temperature and shaken well.

Calcium analysis

Samples (80 µL) of the filtrate were made up to 500 µL with deionised water then 500 µL of 1 M HCl and 1.0 mL LaCl₃ (2%, w/v) were added prior to analysis on a Varian® AA2240 atomic absorption spectrophotometer at a wavelength of 422.7 nm.

Inorganic phosphate analysis

After appropriate dilution of the filtrates with deionised water (1:10), 20 µL of each sample was made up to 100 µL with deionised water. Then 500 µL of Malachite Green colour reagent containing ammonium molybdate in HCl was added followed by 20 µL of 1.5% Tween and the tubes were vigorously vortexed. These were analysed after 30 min using a Varian 50 Bio® UV-Visible light spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) at a wavelength of 660 nm.

Fluoride analysis

Fluoride content was determined using a Dionex ICS-300 ion chromatography system (Dionex Corporation, CA, USA) equipped with an Ion Pac AS18 anion column and a ICS3000 conductivity detector. Samples were diluted with deionised water and filtered through a 0.2 µm filter (Millex-FG, Millipore, MA, USA) before analysis.

Soluble calcium and inorganic phosphate

Frozen samples were warmed to room temperature and shaken well. A volume of 15 mL of each sample was centrifuged (1000 x g, 20 min, 25 °C) which resulted in the sample

separating into three zones: an upper solid zone consisting of fat, a much larger intermediate fluid zone comprising the bulk of the volume and the solid sediment or pellet in the lowest zone. The intermediate fluid zone was then further centrifuged (78,000 x g, 90 min, 25 °C) and the supernatant removed carefully and filtered through a 0.22 µm filter. These filtrates were then stored at 4 °C until analysis. Each sample was analysed in triplicate. Prior to determining the concentration of soluble calcium and inorganic phosphate, the refrigerated samples were warmed to room temperature and shaken well.

Soluble calcium

The sample filtrates were diluted (1:10) with deionised water. A 250 µL aliquot of each sample was made up to 500 µL with deionised water, followed by the addition of 500 µL of 1 M HCl and 1.0 mL LaCl₃ (2%, w/v). Samples were then analysed on a Varian® AA2240 atomic absorption spectrophotometer at a wavelength of 422.7 nm.

Soluble inorganic phosphate

The sample filtrates were diluted (1:80) with deionised water. A 16 µL aliquot of each sample was made up to 100 µL with deionised water. Then 500 µL of a Malachite Green colour reagent containing ammonium molybdate in HCl was added, followed by 20 µL of 1.5% Tween and the samples vortexed vigorously. Solutions were analysed after 30 min using a Varian 50 Bio® UV-Visible light spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) at a wavelength of 660 nm.

Statistical analysis

Unless otherwise stated, all experiments were conducted in quadruplicate, with the results expressed as the mean. Analysis of variance (ANOVA) models were fitted means of the various measurements, using IBM SPSS statistical software (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY, USA).

Results

S. mutans acid production

The rate of acid production at pH 5.5 in all liquid breakfast products tested was four to six times higher than that of the Pura Milk (Long life/UHT) (**Table 1**). The highest rate of acid production in a liquid breakfast product was found with Up & Go while the lowest rate of acid production was found with Oats Express.

S. mutans induced pH fall

The initial pHs for all beverages when mixed with an equal volume of an *S. mutans* suspension with a cell density of 2 mg dry weight cells/mL were within the range of 6.29 – 6.54 (**Table 2**) with the initial pH of Sustagen, Musashi and Oats Express all significantly lower than that of Pura Milk (Long life/UHT) ($p < 0.05$). Following a 10 min incubation with *S. mutans*, there was a significant decrease in pH in all liquid breakfasts, when compared with Pura Milk (Long life/UHT), with the pH in three of the liquid breakfasts dropping below pH 5.5. The liquid breakfast product with the greatest pH change was Up & Go, with a pH drop of 1.17 pH units. The liquid breakfast product with the lowest pH change was Sustagen, with a pH drop of 0.71 pH units.

Titration

The amount of HCl required to lower the pH of the Pura Milk (Long life/UHT) to 5.5 was significantly higher than that needed to lower the pH of all the liquid breakfast products with the exception of Up & Go Energize (**Table 3**). Oats Express and Up & Go had significantly less buffering capacity than any of the other beverages tested.

Calcium, phosphate and fluoride content of the beverages

The total calcium concentration of the beverages varied between 48.5 – 167.3 mg/100 mL.

The Pura Milk (Long life/UHT) had a significantly lower soluble calcium concentration than all the liquid breakfasts. With the exception of Up & Go and Oats Express, which had the same soluble calcium concentration, all the beverages exhibited significantly different soluble calcium concentrations ranging between 39.2 – 60.5 mg/100mL.

Regarding total inorganic phosphate concentrations, other than Pura Milk (Long life/UHT) which had a similar concentration to Musashi, Up & Go Energize and Oats Express, the total inorganic phosphate concentrations differed significantly across the products (**Table 4**). With the exception of Oats Express (37.5 mg/100 mL), which did not differ significantly from Pura Milk (Long life/UHT) (34.8 mg/100 mL) or Up & Go (41.5 mg/100 mL), the soluble inorganic phosphate concentration also differed significantly across all beverages (range 28.5 – 76.4 mg/100 mL). In general, the calcium and phosphorous levels given on the product information could be considered a fairly good indication of the total calcium and inorganic phosphate concentration of the liquid breakfasts, however there were some notable exceptions with Up & Go having 2/3rd less calcium than indicated on the nutritional panel (**Tables 4 and 5**). The extra phosphorous indicated on the Up & Go label may have been attributable to organic phosphate which was not measured in this study. All products were found to contain some fluoride with a range of values from 0.65 – 1.09 ppm F (**Table 4**).

Discussion

Since their introduction the popularity of liquid breakfasts has increased, with more than 25% of households purchasing these products in 2013, up from 9% in 2008 [32]. Liquid breakfasts are particularly popular in households with children [32]. To the best of our knowledge, to date there have been no studies investigating the potential acidogenicity or cariogenicity of these particular sweetened, bovine milk based beverages, and limited analyses of sweetened bovine milk in general.

The pH fall that occurs in dental plaque following the consumption of liquid breakfasts will depend upon the interplay between a number of factors, including the rate and amount of acid produced as a result of the bacterial metabolism of fermentable carbohydrates, as well as the ability of the beverage to buffer that acid.

There was a clear difference between the bovine milk and most of the liquid breakfasts in their ability to resist the decrease in pH to 5.5 following the addition of acid (**Table 3**). With the method used in this study, the liquid breakfasts with significantly less ability to resist acidification required between 14 and 36% less acid than the bovine milk to reduce the pH of the beverage to 5.5, the approximate pH required for enamel demineralisation [33]. When comparing the liquid breakfasts to non-dairy beverages, the ability to resist acidification does appear to be higher in the majority of liquid breakfast products. A study of soy beverages found that these required 32 to 57% less acid than bovine milk to reduce their pH to 5.5 [34]. The superior ability of bovine milk to resist acidification can be attributed largely to the combination of casein proteins and inorganic phosphate [35].

In this study, beverages with a similar protein concentration to that of Pura Milk (Long life/UHT) demonstrated significantly poorer buffering capacity (**Tables 3 and 5**). While the liquid breakfasts are milk based drinks, all containing skim milk, skim milk powder or non-fat milk solids among their ingredients, there is no indication as to the

concentration of caseins present. So while the overall protein concentration may be similar to that in Pura Milk (Long life/UHT), we speculate that the casein concentration may be lower, affecting the ability of these drinks to resist acidification. The only liquid breakfast which demonstrated a comparable ability to resist acidification was Up & Go Energize (**Table 3**) which contained double the protein found naturally in Pura Milk (Long life/UHT) (**Table 5**). This may explain the significantly greater volume of HCl required to reduce the pH of Up & Go Energize to 5.5 compared with the other liquid breakfasts, as the other non-casein proteins such as soy or whey proteins would contribute to the buffering [36]. However, the oral health benefits of casein go beyond their ability to buffer acids. Caseins can also inhibit enamel demineralisation by binding the enamel crystals to form a surface barrier [37], increasing the bioavailability of calcium and phosphate ions in plaque to maintain a supersaturation with respect to enamel mineral [38], and may alter the local environment to one which does not favour colonisation by acidogenic species [39]. Furthermore, caseins have also been demonstrated to provide remineralisation capabilities through the increase of bioavailable calcium and inorganic phosphate ion concentrations at the tooth surface via different mechanisms, thus aiding in enamel crystal formation [40, 41].

Inorganic phosphate ion concentration in these products is also of interest as it has a major role in buffering acid in the oral environment. While it is expected that a high inorganic phosphate concentration should improve the buffering capacity of these beverages, this study found no such correlation. Sustagen, which had the highest total and soluble inorganic phosphate concentration of 133.1 mg/100 mL and 76.4 mg/100 mL respectively, had lower buffering capacity than Pura Milk (Long life/UHT), which had less than half this total and soluble inorganic phosphate concentration with 61.5mg/100mL and 34.8mg/100mL respectively (**Tables 3 and 4**).

With regard to all the beverages tested, the total calcium and inorganic phosphate concentrations were not indicative of their soluble calcium and inorganic phosphate concentrations; the form in which they are considered available for remineralisation. While most liquid breakfasts had a greater soluble calcium and inorganic phosphate concentration than Pura Milk (Long life/UHT), further investigation is required to determine if this factor provides any improved anti-cariogenic benefits.

Whilst the lactose found naturally in bovine milk is able to be fermented by some oral bacteria, with its combination of proteins, calcium and phosphate ions, bovine milk is generally considered to be non-cariogenic [21, 37, 42]. However, addition of 2% sucrose to milk has been demonstrated to enhance its cariogenicity [43], with the addition of 10% doing so exponentially [27].

In this study, the rate of acid production by *S. mutans* was four to six times higher in the liquid breakfast beverages than in the Pura Milk Long Life (UHT) (**Table 1**), reflective of the addition of substantial amounts of fermentable sugars to most of these beverages (**Table 5**). *S. mutans* is able to metabolise not only any naturally occurring lactose, but also the sucrose/glucose and other sugars added to these drinks, generating lactic acid [31, 44]. These results demonstrate clearly that liquid breakfasts are able to provide fermentable carbohydrates to oral microorganisms, with the subsequent production of significant amounts of acid.

The added sucrose and glucose as well as the inferior ability to withstand acidification (**Table 3**) resulted in the liquid breakfast beverages exhibiting a significantly greater pH fall over 10 min compared with Pura Milk (Long life/UHT) when *S. mutans* was suspended (**Table 2**). It is therefore possible that consumption of these drinks may result in plaque pH to drop to the critical pH for enamel demineralisation.

Despite the fermentable lactose in the bovine milk, the *S. mutans* induced pH fall over 10 min was very small, with the pH in Pura Milk (Long life/UHT) not dropping below 6.5. However, the pH fall observed in all the liquid breakfasts was significantly greater (**Table 2**) with the pH in at least three of the liquid breakfasts falling below the approximate pH required for enamel mineral dissolution [33].

It should be noted that the rate of acid production by *S. mutans* and the change in pH over 10 min were not directly proportional to the concentration of sugar in the liquid breakfasts tested. For example, Oats Express, which had the highest concentration of sugars at 10.1 g/100 mL (**Table 5**), had one of the lowest rates of acid production and change in pH (**Tables 1 and 2**). This is consistent with glycolysis by *S. mutans* being saturated at relatively low levels of sugar [45] and other factors present in the beverages affecting acid production. Furthermore the rate of acid production by *S. mutans* has been demonstrated to be higher from monosaccharides glucose and fructose than from disaccharides lactose and sucrose [46, 47], indicating that the available carbohydrate source in the tested beverages may differentially affect their cariogenicity. The sugar present in Oats Express is maltodextrin, a glucose polymer of 3-20 subunits. While *S. mutans* is able to take up maltodextrins with as many as 7 glucose units [48], larger oligosaccharides need to be enzymatically cleaved prior to transport into the cell, with further cleavage required prior to fermentation. Therefore the rate of acid production from maltodextrin may be low relative to glucose or sucrose. And interestingly, while proteins can act to aid in buffering pH change, the increased protein levels found in the majority of liquid breakfasts may also be promoting acid production by *S. mutans*, as nitrogenous compounds have recently been found to stimulate bacterial carbohydrate metabolism and increase acid production from glucose by *S. mutans* [49]. As a result, the combination of glucose as the main fermentable carbohydrate, with the high non-

casein protein content, may help explain the high rate of acid production in Musashi despite it having a very similar total sugar content to Pura Milk (Long life/UHT) (**Table 5**).

Due to their high added sugar content, liquid breakfast products could be broadly categorised as a sugar-sweetened beverage (SSB), a group that also includes fruit juices, soft drinks and even cordial [17]. These drinks possess a large quantity of fermentable carbohydrate, and their consumption has been associated with dental caries [11, 17]. However, in the case of liquid breakfasts, their potential cariogenicity due to increased acidogenicity may be alleviated to some extent by the protective benefits provided by the presence of the minerals and some proteins in their milk base, components which are lacking in other SSBs, and milk alternatives such as some soy beverages [34].

Conclusions

Dental caries is a preventable disease, and whether net demineralisation will result depends upon the interplay between a variety of pathological and protective factors, both of which exist to various extents in both liquid breakfasts and bovine milk. Results from this study provide further evidence that bovine milk may be considered to be non-cariogenic, as the critical pH range was not reached following the metabolism of lactose by *S. mutans*. Despite having high concentrations of soluble calcium and inorganic phosphate the substantial amounts and various types of sugars found within liquid breakfast beverages may result in a significant pH drop in dental plaque following consumption of these products.

References

- [1] W.G. Wade, The oral microbiome in health and disease, *Pharmacol. Res.* 69 (2013) 137-43.
- [2] D. Beighton, M. Al-Haboubi, M. Mantzourani, S.C. Gilbert, D. Clark, L. Zoitopoulos, et al., Oral Bifidobacteria: caries-associated bacteria in older adults, *J. Dent. Res.* 89 (2010) 970-4.
- [3] W. Jiang, J. Zhang, H. Chen, Pyrosequencing analysis of oral microbiota in children with severe early childhood dental caries, *Curr. Microbiol.* 67 (2013) 537-42.
- [4] I. Johansson, E. Witkowska, B. Kaveh, P. Lif Holgerson, A.C. Tanner, The microbiome in populations with a low and high prevalence of caries, *J. Dent. Res.* 95 (2016) 80-6.
- [5] L. Chen, B. Qin, M. Du, H. Zhong, Q. Xu, Y. Li, et al., Extensive description and comparison of human supra-gingival microbiome in root caries and health, *PLoS One* 10 (2015) e0117064.
- [6] A. Simon-Soro, A. Mira, Solving the etiology of dental caries, *Trends Microbiol.* 23 (2015) 76-82.
- [7] R.H. Selwitz, A.I. Ismail, N.B. Pitts, Dental caries, *Lancet* 369 (2007) 51-9.
- [8] J.M. Armfield, A.J. Spencer, Quarter of a century of change: caries experience in Australian children, 1977-2002, *Aust. Dent. J.* 53 (2008) 151-9.
- [9] M.S. Hopcraft, K.E. Yapp, G. Mahoney, M.V. Morgan, Dental caries experience in young Australian Army recruits 2008, *Aust. Dent. J.* 54 (2009) 316-22.

- [10] AIHW. *Oral Health and dental care in Australia: key facts and figures trends 2014*: Australian Institute of Health and Welfare; 2014.
- [11] E. Bernabe, M.M. Vehkalahti, A. Sheiham, A. Aromaa, A.L. Suominen, Sugar-sweetened beverages and dental caries in adults: a 4-year prospective study, *J. Dent.* 42 (2014) 952-8.
- [12] J. Skinner, R. Byun, A. Blinkhorn, G. Johnson, Sugary drink consumption and dental caries in New South Wales teenagers, *Aust. Dent. J.* 60 (2015) 169-75.
- [13] J.G. Lee, L.J. Brearley Messer, Contemporary fluid intake and dental caries in Australian children, *Aust. Dent. J.* 56 (2011) 122-31.
- [14] E. Dalley, Liquid breakfasts, <https://www.choice.com.au/food-and-drink/drinks/juices-and-smoothies/articles/liquid-breakfasts> (2013).
- [15] B.E. Gustafsson, C.E. Quensel, L.S. Lanke, C. Lundqvist, H. Grahnen, B.E. Bonow, et al., The Vipeholm dental caries study; the effect of different levels of carbohydrate intake on caries activity in 436 individuals observed for five years, *Acta Odontol. Scand.* 11 (1954) 232-64.
- [16] S. Karjalainen, M. Tolvanen, K. Pienihakkinen, E. Soderling, H. Lagstrom, O. Simell, et al., High sucrose intake at 3 years of age is associated with increased salivary counts of mutans streptococci and lactobacilli, and with increased caries rate from 3 to 16 years of age, *Caries Res.* 49 (2015) 125-32.
- [17] J.M. Armfield, A.J. Spencer, K.F. Roberts-Thomson, K. Plastow, Water fluoridation and the association of sugar-sweetened beverage consumption and dental caries in Australian children, *Am. J. Public Health* 103 (2013) 494-500.
- [18] P.J. Moynihan, S.A. Kelly, Effect on caries of restricting sugars intake: systematic review to inform WHO guidelines, *J. Dent. Res.* 93 (2014) 8-18.

- [19] A. Sheiham, W.P. James, A reappraisal of the quantitative relationship between sugar intake and dental caries: the need for new criteria for developing goals for sugar intake, *BMC Public Health* 14 (2014) 863.
- [20] R.C. Peres, L.C. Coppi, M.C. Volpato, F.C. Groppo, J.A. Cury, P.L. Rosalen, Cariogenic potential of cows', human and infant formula milks and effect of fluoride supplementation, *Brit. J. Nutr.* 101 (2009) 376-82.
- [21] E.C. Reynolds, I.H. Johnson, Effect of milk on caries incidence and bacterial composition of dental plaque in the rat, *Arch. Oral Biol.* 26 (1981) 445-51.
- [22] S.M. Lempert, L.B. Christensen, K. Froberg, K. Raymond, B.L. Heitmann, Association between dairy intake and caries among children and adolescents. Results from the Danish EYHS follow-up study, *Caries Res.* 49 (2015) 251-8.
- [23] D.K. Dror, L.H. Allen, Dairy product intake in children and adolescents in developed countries: trends, nutritional contribution, and a review of association with health outcomes, *Nutr. Rev.* 72 (2014) 68-81.
- [24] H.A. Linke, H.K. Riba, Oral clearance and acid production of dairy products during interaction with sweet foods, *Ann. Nutr. Metab.* 45 (2001) 202-8.
- [25] S. Naval, A. Koerber, L. Salzmann, I. Punwani, B.R. Johnson, C.D. Wu, The effects of beverages on plaque acidogenicity after a sugary challenge, *J. Am. Dent. Assoc.* 144 (2013) 815-22.
- [26] E.C. Reynolds, Dairy components in oral health, *Aust. J. Dairy Technol.* 58 (2003) 79-81.
- [27] A.R. Prabhakar, A.J. Kurthukoti, P. Gupta, Cariogenicity and acidogenicity of human milk, plain and sweetened bovine milk: an *in vitro* study, *J. Clin. Pediatr. Dent.* 34 (2010) 239-47.

- [28] W.J. Loesche, Role of *Streptococcus mutans* in human dental decay, *Microbiol Rev* 50 (1986) 353-80.
- [29] S.G. Dashper, E.C. Reynolds, pH regulation by *Streptococcus mutans*, *J. Dent. Res.* 71 (1992) 1159-65.
- [30] S.G. Dashper, E.C. Reynolds, Effects of organic acid anions on growth, glycolysis, and intracellular pH of oral streptococci, *J. Dent. Res.* 79 (2000) 90-6.
- [31] S.G. Dashper, E.C. Reynolds, Lactic acid excretion by *Streptococcus mutans*, *Microbiology-UK* 142 (1996) 33-39.
- [32] Nielsen, From breakfast to 'breakfaster': liquid breakfast buyers double over past five years, <http://www.nielsen.com/au/en/insights/news/2014/from-breakfast-to-breakfaster-liquid-breakfast-buyers-double-over-past-five-years.html> (2014).
- [33] C. Dawes, What is the critical pH and why does a tooth dissolve in acid?, *J. Can. Dent. Assoc.* 69 (2003) 722-4.
- [34] S.G. Dashper, B.N. Saion, M.A. Stacey, D.J. Manton, N.J. Cochrane, D.P. Stanton, et al., Acidogenic potential of soy and bovine milk beverages, *J. Dent.* 40 (2012) 736-41.
- [35] F. Salaun, B. Mietton, F. Gaucheron, Buffering capacity of dairy products, *Int. Dairy J.* 15 (2005) 95-109.
- [36] Z. Cheaib, A. Lussi, Role of amylase, mucin, IgA and albumin on salivary protein buffering capacity: a pilot study, *J. Biosci.* 38 (2013) 259-65.
- [37] E.C. Reynolds, P.F. Riley, E. Storey, Phosphoprotein inhibition of hydroxyapatite dissolution, *Calcif. Tiss. Int* 34 Suppl 2 (1982) S52-6.

- [38] E.C. Reynolds, The prevention of sub-surface demineralization of bovine enamel and change in plaque composition by casein in an intra-oral model, *J. Dent. Res.* 66 (1987) 1120-7.
- [39] E.C. Reynolds, A. Wong, Effect of adsorbed protein on hydroxyapatite zeta potential and *Streptococcus mutans* adherence, *Infect. Immun.* 39 (1983) 1285-90.
- [40] E.C. Reynolds, Remineralization of enamel subsurface lesions by casein phosphopeptide-stabilized calcium phosphate solutions, *J. Dent. Res.* 76 (1997) 1587-95.
- [41] N.J. Cochrane, S. Saranathan, F. Cai, K.J. Cross, E.C. Reynolds, Enamel subsurface lesion remineralisation with casein phosphopeptide stabilised solutions of calcium, phosphate and fluoride, *Caries Res.* 42 (2008) 88-97.
- [42] T.H. Grenby, A.T. Andrews, M. Mistry, R.J. Williams, Dental caries-protective agents in milk and milk products: investigations *in vitro*, *J. Dent.* 29 (2001) 83-92.
- [43] W.H. Bowen, S.K. Pearson, Effect of milk on cariogenesis, *Caries Res.* 27 (1993) 461-6.
- [44] J.D. Hillman, A. Chen, J.L. Snoep, Genetic and physiological analysis of the lethal effect of L-(+)-lactate dehydrogenase deficiency in *Streptococcus mutans*: complementation by alcohol dehydrogenase from *Zymomonas mobilis*, *Infect. Immun.* 64 (1996) 4319-23.
- [45] E.C. Reynolds, P.F. Riley, Kinetics of hydroxyapatite dissolution during incubation with sucrose and *Streptococcus mutans* and its application to cariogenicity testing, *Caries Res.* 15 (1981) 501-7.

- [46] F.S. Botha, S.J. Botha, pH and acid production of *Streptococcus mutans* and *Lactobacillus paracasei* in glucose, fructose and sucrose with and without added fluoride, J. Dent. Res. 2000 (2000) 464-64.
- [47] S. Takahashi-Abbe, K. Abbe, N. Takahashi, Y. Tamazawa, T. Yamada, Inhibitory effect of sorbitol on sugar metabolism of *Streptococcus mutans in vitro* and on acid production in dental plaque *in vivo*, Oral Microbiol. Immunol. 16 (2001) 94-9.
- [48] A.J. Webb, K.A. Homer, A.H. Hosie, Two closely related ABC transporters in *Streptococcus mutans* are involved in disaccharide and/or oligosaccharide uptake, J. Bacteriol. 190 (2008) 168-78.
- [49] Y. Norimatsu, J. Kawashima, T. Takano-Yamamoto, N. Takahashi, Nitrogenous compounds stimulate glucose-derived acid production by oral Streptococcus and Actinomyces, Microbiol. Immunol. 59 (2015) 501-6.

Table 1. *S. mutans* acid production at a constant pH of 5.5. The beverages were mixed with an equal volume of a *S. mutans* cell suspension in a pH stat at 37 °C. The experiment was conducted in quadruplicate and the mean and standard deviation of the four replicates is shown.

Beverage	Acid production (nmol H ⁺ /mg dry wt/min) at pH 5.5 (mean ± SD)
Pura Milk (Long life/UHT)	67 ± 11 ^a
Up & Go	420 ± 8 ^b
Up & Go Energize	364 ± 21 ^c
Up & Go Vive	338 ± 24 ^c
Sustagen	322 ± 25 ^c
Musashi	269 ± 29 ^d
Oats Express	251 ± 19 ^d

^{a,b,c,d} Different lower case letters indicate statistically significant differences within the same column as determined by ANOVA and Tukey multiple comparison adjustments (p<0.05)

Table 2. Initial pH, final pH and change in pH of milk and liquid breakfast beverages during incubation with *S. mutans*.

Beverage	Initial pH	Final pH	Δ pH
	t = 0 min	t = 10 min	
Pura Milk (Long life/UHT)	6.54 \pm 0.04 ^a	6.50 \pm 0.05 ^a	0.04 ^a
Up & Go	6.36 \pm 0.10 ^{a,b}	5.19 \pm 0.20 ^b	1.17 ^{b,c}
Up & Go Energize	6.45 \pm 0.06 ^{a,c}	5.69 \pm 0.28 ^c	0.76 ^{d,e,f,g}
Up & Go Vive	6.38 \pm 0.07 ^{a,d}	5.28 \pm 0.15 ^{b,c}	1.10 ^{b,d,h}
Sustagen	6.30 \pm 0.11 ^{b,c,d}	5.59 \pm 0.29 ^{b,c}	0.71 ^{e,i}
Musashi	6.30 \pm 0.16 ^{b,c,d}	5.55 \pm 0.20 ^{b,c}	0.75 ^{f,h,i}
Oats Express	6.29 \pm 0.10 ^{b,c,d}	5.48 \pm 0.24 ^{b,c}	0.81 ^{c,g,h,i}

^{a,b,c,d,e,f,g,h,i} Significantly different from other values not similarly marked in the same column as determined by ANOVA and Tukey multiple comparison adjustments (p<0.05)

Table 3. Buffering capacity of milk and liquid breakfast beverages determined by the volume (mL) of 0.1M hydrochloric acid necessary to lower the pH of 20 mL of beverage to 5.5 at 37 °C.

Beverage	Volume of 0.1 M HCl (mL)
Pura Milk (Long life/UHT)	3.59 ± 0.13 ^a
Up & Go	2.39 ± 0.05 ^b
Up & Go Energize	3.41 ± 0.04 ^a
Up & Go Vive	2.74 ± 0.07 ^c
Sustagen	2.97 ± 0.14 ^d
Musashi	3.10 ± 0.05 ^d
Oats Express	2.31 ± 0.03 ^b

^{a,b,c,d} Different lower case letters indicate statistically significant differences within the same column.

Table 4. Mineral content of beverages

Beverage	Total [Ca] (mg/100mL)	Soluble ^a [Ca] (mg/100mL)	Total [Pi] ^b (mg/100mL)	Soluble [Pi] ^b (mg/100mL)	Fluoride content (ppm)
Pura Milk (Long life/UHT)	119.2 ± 1.5 ^d (29.74 ± 0.38) ^c	36.7 ± 0.6 ^d (9.16 ± 0.15)	61.5 ± 1.6 ^d (19.85 ± 0.51)	34.8 ± 0.2 ^d (11.25 ± 0.05)	0.81 ± 0.01 ^{d,e,h,i}
Up & Go	48.5 ± 1.5 ^e (12.09 ± 0.38)	39.2 ± 0.9 ^e (9.79 ± 0.23)	44.2 ± 1.1 ^e (14.27 ± 0.34)	41.5 ± 0.4 ^e (13.38 ± 0.13)	0.97 ± 0.10 ^{d,e,f,g,i}
Up & Go Energize	116.8 ± 1.0 ^d (29.13 ± 0.24)	52.1 ± 0.8 ^f (12.99 ± 0.19)	73.0 ± 2.0 ^f (23.58 ± 0.65)	53.4 ± 1.7 ^f (17.23 ± 0.55)	1.05 ± 0.06 ^{e,f,g,i}
Up & Go Vive	161.4 ± 5.1 ^f (40.28 ± 0 1.27)	41.9 ± 0.3 ^g (10.45 ± 0.08)	103.4 ± 3.8 ^g (33.39 ± 1.22)	46.9 ± 1.0 ^g (15.13 ± 0.32)	1.08 ± 0.12 ^{e,f,g}
Sustagen	167.3 ± 3.6 ^f (41.73 ± 0.89)	60.5 ± 1.2 ^h (15.10 ± 0.29)	133.1 ± 4.9 ^h (42.98 ± 1.59)	76.4 ± 3.4 ^h (24.65 ± 1.11)	1.09 ± 0.02 ^{e,f,g}
Musashi	148.5 ± 1.0 ^g (37.04 ± 0.24)	48.6 ± 1.1 ⁱ (12.13 ± 0.27)	61.3 ± 0.9 ^d (19.80 ± 0.28)	28.5 ± 0.3 ⁱ (9.22 ± 0.10)	0.65 ± 0.07 ^{d,h,i}
Oats Express	115.5 ± 1.4 ^d (28.81 ± 0.36)	39.2 ± 0.6 ^e (9.78 ± 0.15)	69.5 ± 1.3 ^f (22.45 ± 0.41)	37.5 ± 0.7 ^{d,e} (12.10 ± 0.22)	0.85 ± 0.10 ^{d,e,f,h,i}

Results indicate the mean concentration ± standard deviation of three replicates

a - refers to concentration determined in the beverages that was not removed by high speed centrifugation following TCA precipitation

b - expressed as weight of phosphorus

c - values in parentheses are expressed as mM

d, e, f, g, h, i – significantly different to all other values not similar marked in the same column as determined when tested using ANOVA and Tukey HSD multiple comparison adjustment ($p < 0.05$).

Table 5. Composition of the beverages as provided by the manufacturers on their respective websites^a or product labels^b

Beverage	Pura Milk (Longlife/UHT)	Up & Go	Up & Go Energize	Up & Go Vive ^b	Sustagen	Musashi P30	Oats Express ^b
Energy (kJ/100mL)	266	326	351	280	420	275	293
Protein (g/100mL)	3.4	3.3	6.9	4.0	5.0	8.0	4.0
Fat, total (g/100mL)	3.4	1.5	1.5	1.0	1.3	0.3	0.5
Carbohydrate total (g/100mL)	4.8	11.8	9.8	9.2	16.9	7.4	12.0
Sugars (g/100mL)	4.8	7.7	7.4	6.8	9.2	4.6	10.1
Calcium (mg/100mL)	128	160	114	160	160	107	151
Phosphorus (mg/100mL)	not given	110	77	100	153	Not given	Not given
Types of carbohydrate added	N/A	cane sugar, wheat maltodextrin, inulin, hi- maize TM starch, corn syrup solids, fructose, oat flour	cane sugar, wheat maltodextrin, fructose, inulin, hi- maize TM starch, corn syrup solids	cane sugar, fructose, inulin, hi- maize TM starch, maltodextrin, oat flour, barley beta glucan	dried glucose syrup, maltodextrin, sugar	glucose, corn syrup solids, oligofructose	tapioca maltodextrin

a - www.pura.com.au/images/elements/PURA-Original.pdf, www.sanitarium.com.au/products/up-and-go/up-and-go/up-and-go-vanilla-ice,
www.sanitarium.com.au/products/up-and-go/up-and-go-energize/up-and-go-energize-vanilla, www.sustagen.com.au/products/sustagen-ready-to-drink/,
www.musashi.com.au/drinks-bars/musashi-p30-ugt-drink, b - as per label.

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