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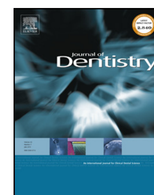

The potential acidogenicity of liquid breakfasts

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ABSTRACT

Objectives: To determine the potential acidogenicy 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.
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1. 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

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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.

2. Materials and methods

2.1. 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.

2.2. Bacteria and growth conditions

S. 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]. *S. 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 $MgSO_4$, 2 mM $MnCl_2$, and 8 mM $(NH_4)_2SO_4$ at pH 7.0) and resuspended in FMM to attain 2 mg dry weight cells/mL [29,30].

2.3. 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.

2.4. Titration

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

2.5. 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.

2.6. 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 $LaCl_3$ (2%, w/v) were added prior to analysis on a Varian® AA2240 atomic absorption spectrophotometer at a wavelength of 422.7 nm.

2.7. 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–vis light spectrophotometer (Agilent Technologies, Santa Clara, CA, USA) at a wavelength of 660 nm.

2.8. 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.

2.9. 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*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*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

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