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Estimated erosive potential depends on exposure time

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ABSTRACT

Objectives: Evaluate erosive potential of beverages, using exposure times from 3 to 30 min, and to analyse the relationship between erosion and several drink parameters.
Methods: pH, calcium, phosphate and fluoride concentration, saturation, titratable-acidity to pH 5.5 and the viscosity of sixteen beverages were measured or calculated. Enamel samples ($N = 90$) were serially exposed to 1 ml of the beverages for 3, 6, 9, 15 and 30 min and erosion was measured as the loss of calcium to the beverage. Rate of erosion per min was calculated by linear curve fitting using all exposure times. Linear regression analysis was performed to determine the correlation between erosion and the drink parameters. A limited multivariate analysis was performed for the outcome parameter with the highest univariate correlations (erosion per minute) and 4 drink variables.
Results: A negative relationship was observed only for pH for all exposure times. Only for erosion per min a significant relationship with pH and saturation was found. In a model for erosion per min using only saturation, fluoride concentration, titratable acidity and viscosity, both saturation and viscosity were shown to have a significant effect ($p = 0.01$ and $p = 0.05$, respectively).
Conclusion: Exposure times between 3 and 30 min result in very different estimates of erosive potential. There is no sound theoretical ground for preferring one or other exposure time/outcome as being more clinically relevant.
Clinical relevance: This study shows that effect of the choice of study methodology on the measurement of erosive potential of beverages is large.

1. Introduction

Dental erosion is defined as an irreversible loss of dental hard tissue due to a chemical process without involvement of micro organisms.[1] Dental erosion may be caused by either extrinsic or intrinsic factors. One of the extrinsic causes of dental erosion is excessive consumption of acidic beverages.[2] The consumption of acidic beverages has risen over recent years. In the USA a 300% increase in soft drink consumption in 20 years is reported.[3]

Research into drink erosive potential has concentrated on a number of drink parameters such as pH, titratable acidity, concentrations of calcium, phosphate and fluoride and the degree of saturation with respect to hydroxyapatite or fluorapatite.[4–7] There are more beverage characteristics, such as viscosity, that might be expected to influence the erosive potential of a drink.[8] Multivariate modelling has been

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proposed to predict erosive potential of beverages based on their chemical properties.[5,6] This would be an attractive option, but may prove elusive, due to the number of factors that may be involved and their complex interactions.[9]

Study methodology for erosive potential of beverages varies widely.[9] Not only does this hamper comparison but also the validity of different methods is not established. Most studies used single exposures but with different exposure times, usually to fit the measurement technique used, from 15 s to 2 min[10,11] up to more than 24 h.[12] When multiple exposure times were used these were analysed separately.[10] It can be suggested that clinical exposures are short, up to a few minutes, after which oral conditions have returned to normal. However, drinking a normal volume of beverage (e.g., a can of 300 ml) is likely to involve a longer time. There is no current knowledge of the clinically most relevant exposure. Because erosive wear is clinically the result of cumulative exposures to acids times of up to 30 min exposure could well be interesting.

It was the aim of this study to evaluate erosive potential of beverages, using both short and longer exposure times, and to analyse the relationship between erosion and several drink parameters, including viscosity, if possible using a multivariate approach.

2. Materials and methods

2.1. *Preparation of samples*

A total of 90 buccal surfaces of extracted bovine incisors, stored in water, were ground flat with water-cooled silicium-oxide 220 grit grinding discs (SIA siawat P220, Frauenfeld, Switzerland) and cut into blocks of approximally 5 mm × 3 mm using a vertical sawing machine with a diamond saw blade (11-4243, Buehler, Düsseldorf, Germany). The blocks were embedded in acrylic resin (Autoplast polymer, Candulor AG, Wangen, Switzerland) leaving the enamel surface uncovered and subsequently the samples were polished flat (800–1200 grit grinding paper) and thoroughly rinsed with tap water.

2.2. *Beverages*

16 beverages, all available in The Netherlands, were included in this study. Six soft drinks: Sprite, Fanta Orange, Coca Cola, Coca Cola light lemon (all Coca-Cola Enterprises Nederland B.V., Dongen, The Netherlands), Lipton ice tea (Unilever, Rotterdam, The Netherlands), Schweppes Tonic (Riedel Beverages, Ede, The Netherlands). Four fruit based beverages: Appelsientje Apple Juice, Spa & Fruit Forest Fruit, Dubbelfriss orange/pink grapefruit and Vitamientje mixed fruit juice (all Riedel Beverages). Two sport beverages: AA-drink high energy (United Soft Drinks B.V., Utrecht, The Netherlands) and Isostar Lemon (Isostar BVBA, Erpe-Mere, Belgium). Also four alcoholic beverages: Breezer Lime (Bacardi Martini NV, Gouda, Nederlands), Smirnoff Ice (Diageo, London, UK), Grolsch lemon beer (SABMiller, London, UK), and Bavaria beer (Bavaria NV, Lieshout, The Netherlands).

The pH of the beverages was measured 5 times using a calibrated glass pH electrode (Radiometer, PHM 84 Research meter G202C, Copenhagen, Denmark) in 100 ml of the degassed beverages. The temperature in the laboratory was 21 °C (±2 °C is expected). Standard buffers, pH 7.01 and 4.00 were used (measurement uncertainty: ±0.015 units, Merck KGaA, Darmstadt, Germany). Calibration was performed with these buffers at the beginning of every experimental day.

The titratable acidity of the beverages was determined by monitoring the pH changes after serial additions of 1 ml of 0.5 M NaOH recording the volume necessary to increase the pH of the beverage up to pH 5.5 and pH 7.0 in 100 ml of each beverage.

All beverages were analysed for phosphate concentration by a modified acid-molybdate method[13] and for calcium concentration by atomic absorption spectroscopy.[14] Calcium and phosphate concentration were expressed in mmol/l and fluoride concentration in ppm. The beverage's baseline degree of saturation with regard to hydroxyapatite (DS_{HA}) was calculated by means of a computer program,[15] using the baseline pH and calcium and phosphate concentrations of the beverages after degassing.

Fluoride concentration was measured using a fluoride ion-specific electrode in combination with a digital mV metre (fluoride electrode cat. no. 940900, Orion Research Inc., Cambridge, MA, USA) in 5 ml of the beverage after addition of 0.5 ml TISAB III (Orion Research Inc., Cambridge, MA, USA).

Viscosity was determined with 0.5 ml of beverage (21 °C (±2 °C is expected)). In a cone-plate viscometer (Brookfield DV-II + Pro Wells Brookfield cone/plate Middleboro, MA, USA) and expressed in mPas.

2.3. *Erosive exposures*

In order to remove the smear layer and any loosely attached material remaining after polishing from the enamel surfaces, the samples were cleaned for 3 min under agitation in a standard solution of 50 mM citric acid, 0.4 mM KH_2PO_4, 0.4 mM $CaCl_2$ and 1 mM NaN_3 (pH 3) and subsequently rinsed with tap water before starting the demineralization procedure. The samples were partly covered with PVC tape exposing an area of approximately 3 mm × 3 mm in the centre of the enamel sample. Five enamel samples were individually submersed in 1 ml of each beverage (all degassed) in a test tube for exposure periods of 3, 6, 9, 15 and 30 min under constant agitation (shaking table, 100 rpm). After each exposure period the beverage was analysed for calcium concentration and a new beverage volume was used for the next exposure period.

The loss of calcium as measured by atomic absorption spectroscopy was recalculated as loss of enamel expressed in μm as described in an earlier publication.[16] As erosion is expected to be linearly related to exposure time, linear regression was performed on the 5 exposure time (3, 6, 9, 15 and 30 min) results for each drink, and the slope of the fitted line was used as a measure of surface loss per minute.

2.4. *Statistical analysis*

Linear regression analysis was performed to determine the correlation between the 6 erosion outcome measures (5 exposure times and the surface loss per minute) and the drink parameters. A multivariate analysis was not possible for

Table 1 – Drinks used in this study with their composition variables, results are average of 2–5 (pH, Ca) measurements.

Drink	pH	TA to pH 5.5	Ca (mmol/l)	Pi (mmol/l)	Fluoride (ppm)	Saturation (HAP)	Viscosity (mPas)
Sprite	2.81	6.80	0.07	0.00	0.16	0.0000	1.32
Fanta orange	3.03	11.80	0.06	0.19	0.11	0.0014	1.55
Coca Cola	2.47	1.60	0.87	4.80	0.00	0.0054	1.49
Coca Cola light lemon	2.73	8.90	0.73	4.90	0.60	0.0085	0.99
Lipton ice tea	3.8	12.40	0.12	0.25	0.46	0.0095	1.19
Schweppes	2.95	4.20	0.00	0.01	0.07	0.0000	1.27
Appelsientje	3.46	14.20	2.61	2.20	0.03	0.0489	1.47
Spa & Fruit	3.19	6.10	0.61	0.70	0.09	0.0101	1.24
Dubbelfriss	3.35	17.10	1.30	0.51	0.05	0.0177	1.29
Vitamientje	3.63	26.00	2.62	3.59	0.16	0.0785	2.32
AA-drink	2.76	10.70	1.12	0.03	0.09	0.0021	1.58
Isostar	3.9	14.50	7.69	5.43	0.07	0.2324	1.20
Breezer Lime	3.87	14.50	0.17	0.02	0.04	0.0056	1.63
Smirnoff Ice	3.43	19.20	0.15	0.00	0.13	0.0000	1.47
Grolsch lemon beer	3.83	6.60	0.96	3.51	0.11	0.0679	1.24
Bavaria beer	4.2	3.60	0.72	5.30	0.09	0.1254	1.44

Table 2 – Enamel loss results for the different drinks, for all exposures/measurements separately ($N = 5$ for each measurement). Loss after 3, 6, 9, 15 and 30 min exposures chemically measured as calcium loss. The slope of a linear curve fitting (and the corresponding R values) is presented as estimated loss per minute. The drinks are arranged in order of decreasing surface loss per minute. All results are presented as μm, calculated from the calcium loss for chemical measurements.

Drink	3 min	6 min	9 min	15 min	30 min	Loss per minute	Loss per minute Linear fit R
Apple Juice	1.06	0.93	1.28	2.04	3.81	0.110	0.99
Coca Cola light lemon	0.37	0.33	0.47	0.76	1.56	0.083	1.00
Dubbelfriss	0.51	0.85	1.10	1.35	2.75	0.080	0.99
Sprite	3.74	3.88	4.04	4.41	5.34	0.060	1.00
Schweppes	0.46	0.39	0.75	1.02	1.81	0.053	0.99
AA-drink Energy	1.53	1.30	1.34	1.74	2.74	0.052	0.94
Spa & Fruit	0.52	0.81	1.07	1.54	2.78	0.047	0.99
Smirnoff Ice	0.80	1.01	1.23	1.09	2.12	0.045	0.95
Breezer Lime	0.43	0.55	0.68	0.89	1.55	0.041	1.00
Fanta Orange	0.40	0.42	0.58	0.80	1.44	0.040	0.99
Coca Cola	0.34	0.46	0.61	0.64	1.18	0.040	0.98
Lipton ice tea	0.38	0.65	0.80	1.04	1.53	0.029	0.98
Grolsch lemon beer	0.29	0.25	0.37	0.39	0.70	0.016	0.97
Vitamientje	1.55	0.86	0.81	1.84	1.24	0.006	0.14
Bavaria beer	0.29	0.28	0.28	0.15	0.31	0.001	0.10
Isostar Lemon	1.59	1.14	1.52	2.13	1.05	−0.011	0.26

all drink parameters, due to the correlation between several parameters and the limited number of beverages. However, a limited multivariate analysis was performed for the outcome parameter with the highest univariate correlations (surface loss per minute) and 4 drink variables.

3. Results

The baseline pH, titratable acidity to pH 5.5, calcium concentration, phosphate concentration, fluoride concentration, saturation with respect to hydroxyapatite (DS_{HA}) and viscosity of the beverages are presented in Table 1. For all outcome measures from the chemical analysis the surface loss in μm as estimated from the measured calcium loss are presented in Table 2: 3, 6, 9, 15, and 30 min exposure and surface loss per minute. The negative control of still mineral water always showed a loss of 0 mm.

Table 3 summarizes all the correlation coefficients of enamel loss with the drink parameters. Only the relationship with pH is consistently negative, and it shows a monotonic relationship with erosive challenge time. For all single chemical measurement outcomes the correlations are quite low and variable. Only when they are combined into the loss per minute outcome variable do correlations become substantial. Although still only the relation with pH and saturation are significant.

Although most beverages show a linear relationship between erosion and exposure time (Fig. 1), two beverages show no relationship of erosion with exposure time at all: Vitamientje and Isostar. This is reflected in Table 4, where they rank among the highest eroders in the 3 min exposure, but among the lowest in the 30 min exposure. Also, the regression lines of several beverages do not cross the Y-axis at or near the 0-level, indicating relatively high erosion during the first few minutes, with Sprite as the most extreme example.

Table 3 – Pearson's correlation of measured loss with drink parameters for all outcome measures. A star indicates a significant correlation.

	3 min	6 min	9 min	15 min	30 min	Loss per minute
pH	−0.23	−0.34	−0.35	−0.36	−0.54*	−0.53*
TA to pH 5.5	0.15	0.03	0.02	0.16	0.03	−0.03
Calcium	0.23	0.04	0.09	0.24	−0.14	−0.35
Phosphate	−0.13	−0.21	−0.21	−0.12	−0.38	−0.39
Fluoride	−0.04	0.03	0.03	0.02	0.07	−0.26
Saturation	0.09	−0.09	−0.07	0.01	−0.40	−0.62*
Viscosity	0.17	−0.01	−0.09	0.06	−0.13	−0.26

Multivariate analysis was not possible using all drink parameters, as there was substantial correlation between many of them and the data set was limited. However, in a model for erosion per minute and using only saturation (assuming that pH, calcium and phosphate were represented in this variable), fluoride concentration, titratable acidity and viscosity, both saturation and viscosity were shown to have a significant effect ($p = 0.01$ and $p = 0.05$, respectively). However,

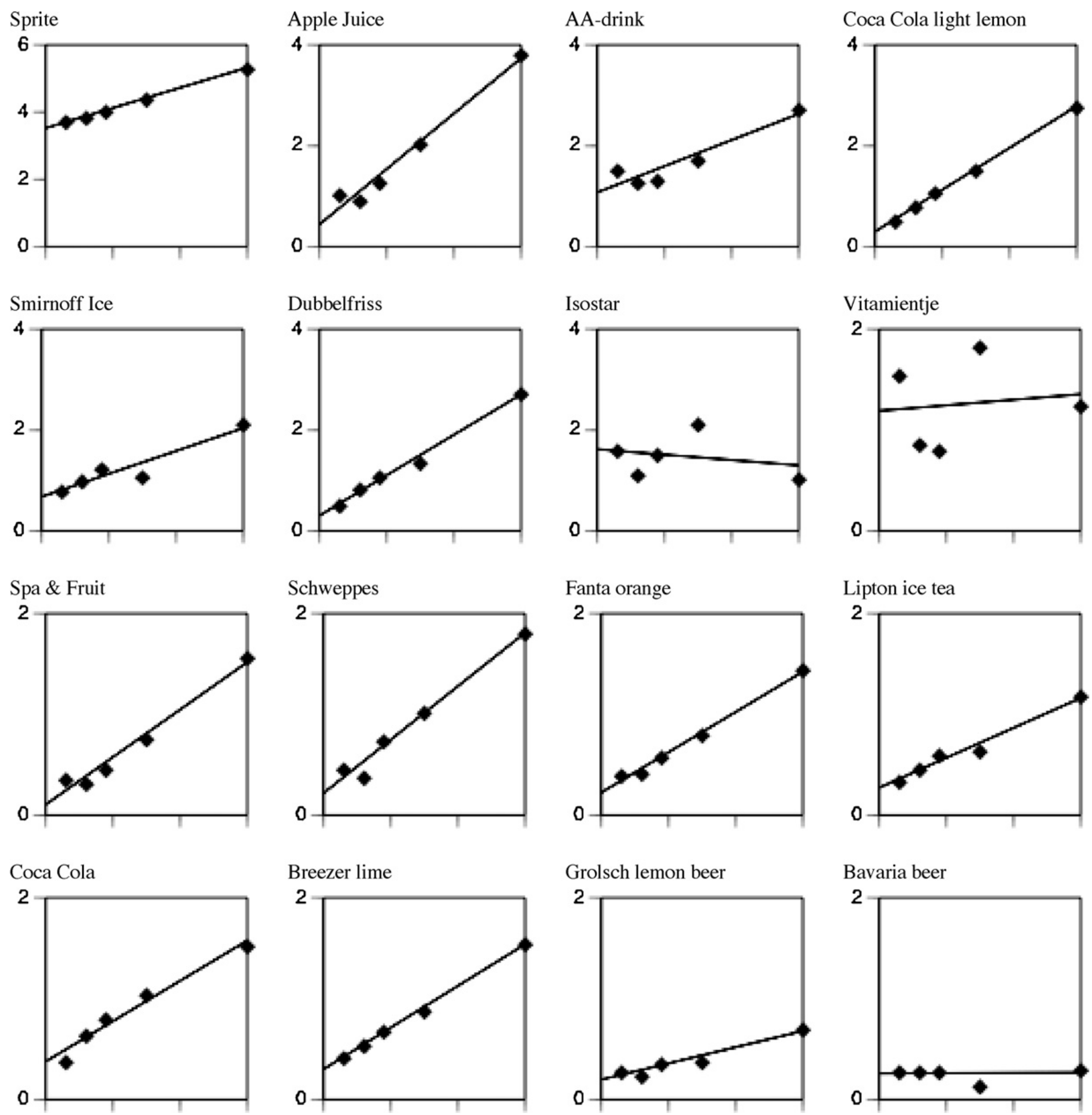

Fig. 1 – Results of the chemical measurement of erosion at the 5 different exposure times for all beverages, with linear curve fitting. On the *Y*-axis surface loss (in μm) is shown, on the *X*-axis exposure time (always up to 30 min).

Table 4 – Ranking of the beverages in erosive potential, using selected outcome measures. While some drinks have a fairly stable position (for example, Sprite and Apple Juice in the high range, and the beers and Lipton ice tea in the low range), for some drinks, notably Vitamientje and Isostar, their ranking is highly dependent on the selected outcome measure.

3 min	30 min	Loss per minute
Sprite	Sprite	Apple Juice
Isostar	Apple Juice	Coca Cola light lemon
Vitamientje	Coca Cola light lemon	Dubbelfriss
AA-drink	Dubbelfriss	Sprite
Apple Juice	AA-drink	AA-drink
Smirnoff Ice	Smirnoff Ice	Spa & Fruit
Coca Cola light lemon	Schweppes	Smirnoff Ice
Dubbelfriss	Spa & Fruit	Schweppes
Schweppes	Breezer Lime	Fanta Orange
Breezer Lime	Coca Cola	Coca Cola
Fanta Orange	Fanta Orange	Breezer Lime
Coca Cola	Vitamientje	Lipton ice tea
Spa & Fruit	Lipton ice tea	Grolsch lemon beer
Lipton ice tea	Isostar	Vitamientje
Grolsch lemon beer	Grolsch lemon beer	Bavaria beer
Bavaria beer	Bavaria beer	Isostar

the strength of the model was limited (adjusted $R^2 = 0.37$), and the plot of erosion per minute by saturation (Fig. 2) shows that the assumption of a linear relationship does not hold.

4. Discussion

In this study it was confirmed that the main parameters involved in erosive potential are pH and saturation. The only consistent parameter across the different outcomes, even if only significant for 3 of them was pH, confirming previous reports.[7,17,18] In our study the enamel loss decreased linearly with a rise in pH between pH 2 and 4, again in accordance with previous reports.[7,19] Also the apparent limitation of erosion at about pH 5.0 fits with other publications.[9]

It is well recognized that degree of saturation is the basic thermodynamic driving force for dissolution. However, the value of this parameter in predicting levels of erosive potential has been questioned, especially below levels of about 0.005.[9] It was expected that most beverages would show lower saturations levels. However, in our study only 5 out of 16 beverages fell below this level. Overall, the relationship between saturation and one of the outcome measures, loss per minute, was strong if not linear (Fig. 2).

Calcium and phosphate have been identified as factors in erosive potential many times with calcium being the more important factor.[9] This was not confirmed in our study. Possibly the range of concentrations represented in the study was not high enough. In a study with beverages with added calcium, a significant effect of calcium was found, but for generally higher concentration (≥3.2 mmol/l[10]). However, when calcium and phosphate are added the pH also usually rises and the effects are hard to separate.[9]

The limitations of the above mentioned variables to predict erosive potential could be seen when two beverages are compared: Apple Juice and Vitamientje fruit drink. Quite similar in pH, calcium, phosphate concentration and degree of saturation, they still have completely different erosive behaviour (Fig. 1). It must be concluded that there are important variables yet unknown and unmeasured, which influence this behaviour.

Titratable acidity did not emerge as an important parameter. In our model we only included titratable acidity to pH 5.5 and not to pH 7 as has been used before.[5] In many studies, as well as in this study, erosion is minimal from a pH of about 5.0 (in our study even pH 4) or higher.[9] It could therefore be assumed that a titratable acidity above pH 5.0 is not relevant anymore.

Fluoride concentration was not confirmed as a significant factor in this study. Earlier, Lussi et al.[5,6] found a significant effect using 20 min exposures, whereas others found no effect using 48–72 h exposures.[7,18] Overall it is unlikely that the fluoride levels in the beverages, all well below 1 ppm, would have an erosion reducing effect.[20]

The factor that was not studied before, viscosity, was only found to be significant in a multivariate model using loss per minute as the outcome variable. It was hypothesized that viscosity would contribute to the effect of a so-called Nernst layer, a thin layer of solution closest to the enamel surface, which is relatively stable. By slowing down replacement of the solution at the surface, viscosity could slow down erosion. This phenomenon could also be related to the penetration

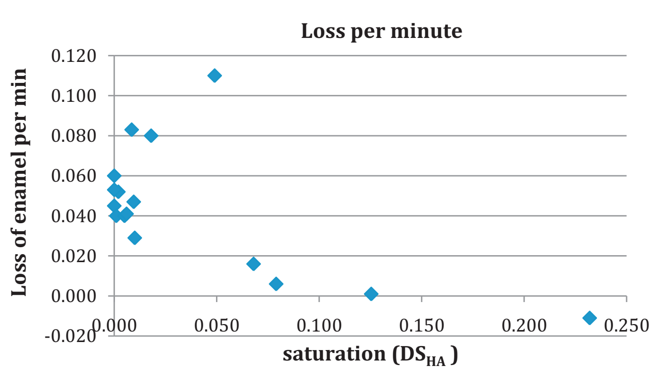

Fig. 2 – Relationship between saturation and enamel loss per minute.

coefficient of liquids. The viscosity of a drink, together with contact angle and surface tension, determines its penetration coefficient,[21] a measure of the ability of a liquid to penetrate into a capillary space such as pores. According to this theory a beverage with a low viscosity will have a high penetration coefficient and this results in a higher erosive potential. This phenomenon would depend on the formation of a porous, softened layer. The direction of the effect found agreed with this hypothesis, however, the evidence is for now too weak to conclude that drink viscosity is a relevant factor.

Our study used both of short and long exposure times, in order to evaluate whether this aspect of study methodology would have a large effect on results regarding erosive potential. The results show that this effect is very large, and for some beverages the estimated erosive potential is relatively high for short exposures and low for long exposure (Table 4). The lack of linear relationship between exposure time and erosion (Fig. 1, Vitamientje and Isostar) and the relatively high erosion values for some beverages at the shortest exposure time (Fig. 1; Sprite, AA-drink and Apple Juice) are two features, which hamper conclusions about relative erosive potential of beverages from a single exposure measurement. Table 4 shows how different conclusions about some beverages may be, depending on the chosen outcome variable.

This study showed that the choice of exposure time between 3 and 30 min resulted in very different estimates of erosive potential. There is no sound theoretical ground for preferring one or other outcome variable as being more clinically relevant and clinical studies comparing the erosive effect of different beverages are needed to be able to determine the validity of in vitro experiments. For ethical reasons, such studies will be difficult to perform.

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