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Change of erosive potential of apple and orange juice at different dilutions

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SUMMARY

The aim of this in vitro study was to investigate the change of erosive properties of apple and orange juice after dilution with tap water.
Apple juice, orange juice and citric acid were assessed for pH and titratable acidity at different aqueous dilutions (100% – pure liquid, 60% – 3:2 mixing ratio, and 40% – 2:3 mixing ratio respectively). Thus, 72 bovine enamel specimens were distributed to 9 groups ($n = 8$ specimens per group), followed by 25 minutes of erosion by superfusion with the described test liquids according to group allocation. Erosive substance loss (µm) was determined profilometrically. The different substance losses within a dilution series were tested using the Wilcoxon rank sum test. The significance level was set to $p \leq 0.05$.
Erosive substance loss [µm] for 100% concentrations (median ± interquartile range) was highest for apple juice (5.7 ± 0.8), followed by citric acid (4.6 ± 0.4) and orange juice (1.5 ± 0.5). The dilution of apple juice (60%: 4.2 ± 0.7; 40%: 3.1 ± 0.5) and citric acid (60%: 3.7 ± 0.9; 40%: 2.8 ± 0.7) with tap water lead to a significant ($p < 0.05$) reduction of erosive potential in comparison to 100% concentrations. This effect was not consistent for orange juice, where significantly more substance loss was found for pure juice (100%) than for 60% diluted juice (60%: 1.1 ± 0.3, $p < 0.05$), but no significant difference was found between 100% and 40% (40%: 0.9 ± 0.6, $p > 0.05$), and 60% and 40%, respectively.
In conclusion, dilution of apple juice with tap water led to a significant reduction of its erosive potential. For orange juice, the effect of dilution on the erosive substance loss was only limited.

Introduction

Erosion is a chemically induced, irreversible loss of tooth structure caused by exogenous or endogenous acids without the involvement of microorganisms (Zipkin & McClure 1949; Ten Cate & Imfeld 1996). Erosive tooth softening by endogenous acids is caused by any condition in which gastric i.e. hydrochloric acid enters the oral cavity, such as chronic gastrointestinal disturbances, reflux, anorexia nervosa or bulimia. The major source of acidic tooth softening, however, is exogenously caused by excessive consumption of acidic foods, soft drinks and fruit juices (Lussi et al. 1993). Erosive tooth wear presents the clinical manifestation of erosion and is the outcome of the combination of chemical and mechanical forces, i.e. removal of softened tooth substance by abrasive forces such as tooth brushing or chewing (Lussi & Ganss 2014).

The consumption of acidic drinks, i.e. fruit juices, soft or energy drinks is rising (Lussi & Carvalho 2014), which is reflected in a growing prevalence of dental erosion (Schlueter & Luka 2018), and in the necessity of taking preventive countermeasures. Increasing attention was given to ways in which potentially erosive products might be modified, and various recommendations to reduce the erosive potential of low-pH drinks have been postulated. Since the erosive potential of acidic beverages is determined by chemical factors such as pH, buffer capacity, and mineral content, it seems evident that specific alterations of these factors will modify their erosive potential (Larsen & Nyvad 1999; Attin et al. 2003; Wegehaupt et al. 2011). Consumption temperature (Steiger-Ronay et al. 2018) as well as liquid viscosity (Aykut-Yetkiner et al. 2014) or individual drinking habits (Edwards et al. 1998; Moazzez et al. 2000) can also modify the erosive effect. It has further been suggested to dilute acidic drinks with water in order to lower their erosive potential. Systematic studies on the effectiveness of beverage dilution are, however, not available. There was a report on the effect of dilution on the erosive potential of dilutable fruit drinks, a concentrate that requires dilution to taste by consumers, which was based solely on measurement of pH and titratable acidity (Cairns et al. 2002). In this investigation beverage dilution produced only little effect on the measured pH values but a reduction of titratable acidity could be observed. Another in vitro study tested the effect of dilution (1:3, 1:6 and 1:15) of five dilutable fruit drinks on the erosive substance loss following immersion of enamel specimens for one hour (Hunter et al. 2008). For the majority of products an increase of dilution from 1:3 to 1:15 led to a statistically significant rise in pH (though products remained acidic), as well as a decrease in titratable acidity. However, a statistically significant reduction of enamel erosion was observed in only three of the five tested products (Hunter et al. 2008).

Therefore, the aim of the present study was to systematically investigate the erosive effect of commercially available, ready-to-drink orange and apple juices as well as citric acid at different degrees of dilution with tap water.

Materials and methods

Experimental procedure

For this study, 72 bovine enamel specimens were distributed to 9 groups (n = 8 each). After initial profilometry, specimens were subjected to 25 minutes of erosion. For the erosive challenge, the following test liquids were applied: commercially available orange juice and apple juice, citric acid and dilutions thereof. Erosive enamel loss was assessed by final profilometry.

Enamel specimen preparation

Eighty-eight bovine enamel specimens were obtained from intact bovine incisors, which were cleaned and stored refrigerated in 0.1% thymol solution. A water-cooled diamond core drill (Proxxon, Brütsch-Rüegger, Switzerland) was used to prepare the specimens out of the labial crown surface (3 mm in diameter). Thereafter specimens were embedded in acrylic resin blocks (6 mm in diameter, Paladur, Heraeus Kulzer, Hanau, Germany), which had a notch to ensure exact repositioning of the specimens for profilometry. The specimen's surfaces were ground with silicon carbide paper discs (# 1200, # 2500, # 4000, Gekko-Papier, Struers, Birmensdorf, Switzerland) under water-cooling.

Test liquids

For the erosive challenge, the following test liquids were investigated: apple juice (90% filtered apple juice and 10% filtered pear juice from concentrate, Coop, Basel, Switzerland), orange juice (100% orange juice from concentrate, Sarasay Max Havelaar Orange, Migros, Zurich, Switzerland), and citric acid (Fluka AG, Buchs SG, Switzerland). The orange juice was centrifuged with 3000 rpm for three minutes (Hermle Z320, Hermle Labortechnik GmbH, Germany) before use in order to remove larger suspended particles that could have blocked the tube system. The pH value of citric acid was adjusted based on the pHs of the fruit juices in order to allow for a comparison. The dilutions of orange juice, apple juice and citric acid to 60% and 40% (60% – 3:2 mixing ratio; 40% – 2:3 mixing ratio respectively) were prepared with a precision scale (New Classic HF, Mettler Toledo, Switzerland), a measuring cup and Zurich tap water. The pH values (pH-meter 827; Methrom, Herisau, Switzerland) and the total calcium content (atomic absorption spectroscopy, contrAA® 300, Analytik Jena AG, Jena, Germany) (Wiegand et al. 2008) of all liquids were determined. Furthermore, in order to determine the titratable acid, liquids were titrated (686 Titroprocessor and 665 Dosimat, Metrohm Schweiz AG, Herisau, Switzerland) at room temperature with 0.1 M NaOH solution to pH 5.5 and pH 7.

Erosion

A customized superfusion chamber allowed the continuous erosion of the specimens with the described test liquids (Attin et al. 2013). For each experimental run, eight enamel specimens were simultaneously fixed in the erosion chamber. The system further consisted of an electric pump (IPC, Ismatec; Cole-Parmer GmbH, Wertheim, Germany), which continuously supplied the specimens with test liquids via individual tubes at a flow rate of 1.2 mm/s and an acid volume of 0.675 mm^3. After each experiment, the tube system was thoroughly rinsed with tap water to prevent cross-contamination of liquids. Further, the tubes were regularly checked for air bubbles, which could have resulted in blocking of acid flow.

Profilometry

Erosive enamel loss was investigated by contact profilometry (Perthometer S2; Mahr, Göttingen, Germany). Five profiles with a set distance of 250 µm between each profile were recorded before (baseline profiles) and after (final profiles) the experiments. A custom-made jig allowed for an exact repositioning of the specimens for reproducible measurements. Custom-designed software was used for the superimposition of

Tab. I Presentation of the test liquids with their main active acid as well as their pH values, calcium content and titratable acid [mmol/l]

Test liquid	Main active acid	pH value			Calcium content [mmol/l]			Titratable acid [mmol/l] (at 100% concentrations)	
		100%	60%	40%	100%	60%	40%	pH 5.5	pH 7.0
Orange juice	Citric acid	3.9	3.9	4.0	2.3	1.8	1.5	8.4	11.5
Apple juice	Malic/citric acid	3.3	3.4	3.4	1.3	1.2	1.1	7.0	7.9
Citric acid	Citric acid	3.8	3.9	4.0	0.0	0.5	0.7	9.3	14.3
Tap water	–	–	–	–	1.2	–	–		

Tab. II Erosive substance loss [µm] as caused by the test liquids at the concentrations of 100%, 60% and 40% presented as median ± interquartile range (IQR) and mean ± standard deviation (SD)

Test liquid	Median ± IQR [µm]			Mean enamel loss ± SD [µm]		
	100%	60%	40%	100%	60%	40%
Orange juice	1.5 ± 0.5	1.1 ± 0.3	0.9 ± 0.6	1.5 ± 0.4	1.2 ± 0.6	1.3 ± 0.7
Apple juice	5.7 ± 0.8	4.2 ± 0.7	3.1 ± 0.5	5.6 ± 0.5	4.2 ± 0.7	3.1 ± 0.3
Citric acid	4.6 ± 0.4	3.7 ± 0.9	2.8 ± 0.7	4.7 ± 0.3	3.5 ± 0.8	2.8 ± 0.6

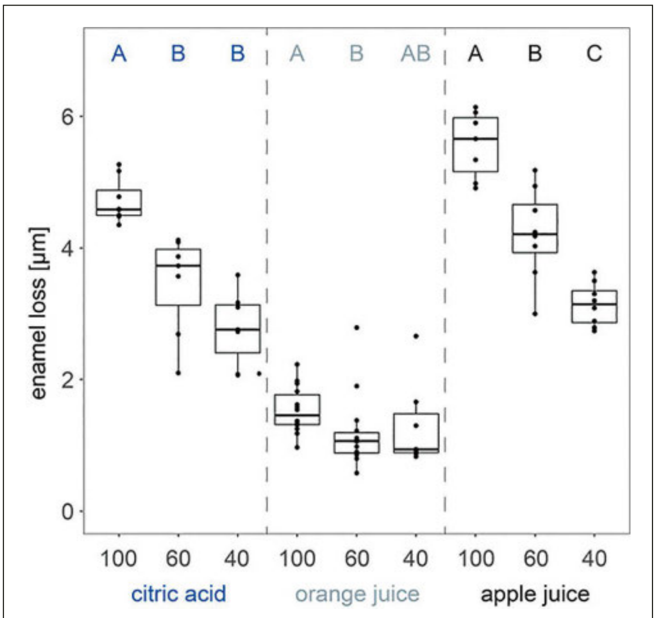

Fig. 1 Boxplot of the enamel loss [µm] after erosion with citric acid, orange juice and apple juice at concentrations of 100%, 60%, and 40%. The boxes represent the interquartile range (IQR). The whiskers indicate 1.5*IQR or minima and maxima of the distribution if below 1.5*IQR. Individual points beyond the whiskers show statistical outliers. Identical capital letters indicate that there was no significant difference within the liquid group at different dilutions (as tested by Wilcoxon rank sum test, significance level: $p \leq 0.05$).

baseline and corresponding final profiles to calculate the average loss of substance per profile (Attin et al. 2009).

Statistical analysis
For the statistical analysis, the mean values of the five profiles per specimen were averaged. The different substance losses within a dilution series were tested using the Wilcoxon rank sum test. The significance level was set to $p \leq 0.05$.

Results
The presentation of the test liquids with their main active acid as well as their pH values, calcium content and titratable acid can be found in Table I. Centrifugation of the orange juice did not have an effect on the described values (data not shown). Erosive substance loss [µm] as caused by the test liquids at the concentrations of 100%, 60% and 40% can be found in Table II, which presents the medians ± interquartile range (IQR) as well as means ± standard deviation (SD). A boxplot presentation of erosive enamel loss [µm] can be found in Figure 1.

Orange juice
The dilution series of orange juice led to inconsistent results. Significantly more substance loss was found for pure orange juice (100%) than for 60% diluted juice ($p < 0.05$), but no significant difference was found between 100% and 40%, and 60% and 40%, respectively.

Apple juice
Pure apple juice (100%) caused the highest substance loss. With rising dilution, significantly less enamel was lost ($p < 0.05$).

Citric acid
A significant difference could be found between pure (100%) citric acid and its dilutions to 60% and 40% ($p < 0.05$). Dilutions of 60% and 40% concentration did not differ significantly.

Discussion
In this investigation erosive substance loss was highest for apple juice, followed by citric acid and orange juice. While the dilution of apple juice with tap water led to a significant reduction of erosive potential, this effect was not as pronounced for orange juice and citric acid. Erosion with orange juice actually led to inconsistent results. Only the dilution of 100% to 60% concentration led to significantly less erosive substance loss. The titratable acid and the large proportion of undissociated

acid could be responsible for the small change of erosivity despite dilution (Azadi-Schossig et al. 2016). Titratable acidity is the amount of standard base necessary to titrate the pH of an acid from its original value to a predetermined value such as 5.5 or 7.0 (Shellis et al. 2013). It serves as an indication of the buffering capacity of a liquid, i.e. the amount of H^+ ions available which maintain an undersaturated environment. In this investigation only titratable acidity of 100% liquid concentrations was tested, which was highest for citric acid, followed by orange juice and apple juice (Tab. I). A study by Cairns et al. (Cairns et al. 2002), however, investigated the change of titratable acidity after dilution of drinks. The authors found that liquid dilution had very little effect on the measured pH values, which is reflected by the results of our study, while the titratable acidity fell considerably with rising dilution. Both the malic acid contained in apple juice as well as the citric acid contained in orange juice are only partially dissociated acid. This means that in addition to the current proton activity, their undissociated erosive potential is still latent. Titratable acid decreases with the degree of dilution. A moderate dilution (60% or 40%) will lead to protons dissociating and the pH value changing only slightly.

The results of the present investigation confirm again that the pH value per se does not provide sufficient information about the erosive potential of a liquid. Other factors, such as type and concentration of acid, amount of titratable acid and buffer capacity, are important determinants as well (Lussi et al. 1993, 1995; Larsen & Nyvad 1999; Hannig et al. 2005). The calcium content of a liquid influences its erosive action too (Hara & Zero 2008). This could partly explain the reduced erosive potential of the diluted citric acid due to preparation with tap water, which presents a natural source of calcium. Zurich tap water, which was used in this investigation, is characterized by relatively high calcium content (1.16 mmol/l). Dilution of ready-to-drink products or juice concentrates with tap water may therefore result in changed erosivity, which is also, however, dependent on regional differences in water mineral composition. Furthermore, the dilution of a liquid can lead to a change in viscosity, another factor that can modify the erosive potential of an acidic solution (Aykut-Yetkiner et al. 2013).

Following rationale was applied for the choice of test liquids. Apple and orange juice present popular drink choices with high consumption rates. Citric acid is the constituent of most fruits, as well as fruit juices, including apple and orange juice, and is characterized by a high erosive potential (Lussi & Carvalho 2014). Since a dilution of a beverage leads to a change in taste, liquids were diluted to only 60% and 40%, since this dilution range seems realistic in a real-life scenario with an acceptable change of taste.

The present study was an in vitro investigation. Its results therefore cannot be translated into the clinical situation. Many influencing factors are involved in the oral cavity, not only individual drinking habits and beverage composition. This reproducible experimental setup, however, allowed for the investigation of relative differences in erosivity between pure and diluted juices.

In contrast to many erosion studies, where enamel specimens were just immersed into stirred test liquids for a certain time (Amaechi et al. 1999; West et al. 2000), a customized superfusion chamber was applied. By using this concept (Attin et al. 2013) a continuous and controlled erosion of the specimens was ensured, which also presents a more realistic simulation of the development of intraoral erosion.

With a chosen duration of 25 minutes the erosive action was rather long, but with time this exposure can be reached within weeks with high-risk behavior. This time, however, was mainly chosen as a result of the experimental setup, to ensure that sufficient measurable erosive substance loss is reached. More importantly no saliva was included in the experiment. Saliva has a preventive and remineralizing effect on erosions due to its calcium, phosphate (Arends & Ten Cate 1981) and fluoride content (Buzalaf et al. 2012). Furthermore the salivary pellicle is a crucial patient-related determinant against erosive tooth loss (Vukosavljevic et al. 2014). Addressing these features did not fall into the scope of the present investigation. Future research, however, should include this important factor in the assessment of effective preventive measures against tooth erosion.

In summary, the dilution of erosive fruit juices with tap water may lead to a significant reduction of their erosive potential depending on the type of juice and the degree of dilution.

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Zusammenfassung
Einleitung
Der steigende Konsum von säurehaltigen Getränken wie Fruchtsäften, Erfrischungsgetränken und Limonaden spiegelt sich in einer zunehmenden Verbreitung von Erosionen wider. Daraus ergibt sich die Notwendigkeit der Entwicklung von vorbeugenden Gegenmassnahmen. Da die Modifikation von pH-Wert, Pufferkapazität und Mineraliengehalt bei trinkfertigen Getränken in den meisten Fällen kaum möglich ist, besteht auch die Überlegung, saure Getränke mit Leitungswasser zu verdünnen, um deren erosives Potenzial zu senken. Ziel der vorliegenden Studie war es daher, die Auswirkung einer Verdünnung von Orangen- und Apfelsaft mit Leitungswasser auf deren erosive Wirkung zu untersuchen.

Material und Methoden
Es wurden die pH-Werte und die titrierbare Säure folgender unverdünnter (100%) sowie mit Leitungswasser auf 60% (Mischungsverhältnis 3:2) und 40% (Mischungsverhältnis 2:3) verdünnter Flüssigkeiten gemessen: Apfelsaft, Orangensaft und Zitronensäure. Mit diesen Flüssigkeiten wurden 25 Minuten lang je 8 Rinderschmelzproben durch Superfusion mit einer Fliessrate von 1,2 mm/s erodiert. Der resultierende erosive Zahnsubstanzverlust (µm) wurde profilometrisch festgestellt.

Resultate
Der erosive Zahnsubstanzverlust (Median ± Interquartilabstand in µm) der unverdünnten Flüssigkeiten (100%) war für Apfelsaft am höchsten (5,7 ± 0,8), gefolgt von Zitronensäure (4,6 ± 0,4) und Orangensaft (1,5 ± 0,5). Während eine Verdünnung von Apfelsaft mit Leitungswasser zu einer im Vergleich zum unverdünnten Saft signifikant verringerten erosiven Wirkung und damit zu geringerem Schmelzverlust führte (60%: 4,2 ± 0,7; 40%: 3,1 ± 0,5), war dieser Effekt für Orangensaft (60%: 1,1 ± 0,3; 40%: 0,9 ± 0,6) und Zitronensäure (60%: 3,7 ± 0,9; 40%: 2,8 ± 0,7) etwas geringer ausgeprägt.

Diskussion

Die Ergebnisse der vorliegenden Untersuchung bestätigen, dass nicht nur der pH-Wert eines Getränks, sondern auch Faktoren wie Art und Konzentration der Säure, Menge an titrierbarer Säure und Pufferkapazität wesentliche Determinanten des erosiven Potenzials einer Flüssigkeit darstellen. Auch der Kalziumgehalt einer Flüssigkeit beeinflusst deren erosive Wirkung. Da Leitungswasser eine natürliche Kalziumquelle darstellt, kann sich eine Verdünnung mit diesem in einer geringeren Erosivität des Getränks spiegeln, die jedoch auch von regionalen Unterschieden im Mineraliengehalt des verwendeten Wassers abhängig ist. Das in dieser Untersuchung verwendete Zürcher Leitungswasser zeichnet sich durch einen relativ hohen Kalziumgehalt (1,16 mmol/l) aus.

Die Verdünnung von Apfelsaft mit Leitungswasser führte zu einer erheblichen Verringerung von dessen erosiver Wirkung. Bei Orangensaft fiel dieser Verdünnungseffekt geringer aus. Man kann daher schlussfolgern, dass durch Verdünnung von Fruchtsäften mit Leitungswasser eine erhebliche Verringerung des erosiven Potenzials erreicht werden kann. Das Ausmass dieser Reduktion ist jedoch abhängig von der Art des Fruchtsaftes und dem Verdünnungsgrad.

Résumé

Introduction

La consommation croissante de boissons acides telles que les jus de fruits, les boissons rafraîchissantes et les sodas se traduit par une prévalence croissante des érosions dentaires. D'où la nécessité de développer des contre-mesures préventives. Étant donné que dans la plupart des cas, il est très difficile de modifier le pH, la capacité tampon et le contenu en minéraux des boissons prêtes à boire, on peut aussi recourir à la possibilité de diluer les boissons acides avec de l'eau du robinet afin de réduire leur potentiel érosif. L'objectif de la présente étude était donc d'investiguer les effets de la dilution du jus d'orange et du jus de pomme avec de l'eau du robinet sur le potentiel érosif de ces boissons.

Matériel et méthodes

Les valeurs du pH et l'acidité titrable des boissons suivantes ont été mesurées : jus de pomme, jus d'orange et acide citrique, non dilués (100 %), ou dilués avec de l'eau du robinet, à 60 % (rapport de mélange 3:2), ou à 40 % (rapport de mélange 2:3). Huit échantillons d'émail bovin ont été érodés avec ces liquides appliqués en surfusion à un débit de 1,2 mm/s pendant 25 minutes. La perte érosive de substance dentaire (µm) résultant de ce processus a été déterminée par profilométrie.

Résultats

La perte érosive de substance dentaire (médiane ± intervalle interquartile en µm) des liquides non dilués (100 %) a été la plus élevée pour le jus de pomme (5,7 ± 0,8), suivi par l'acide citrique (4,6 ± 0,4) et le jus d'orange (1,5 ± 0,5). Alors que la dilution du jus de pomme avec l'eau du robinet a entraîné un effet érosif considérablement réduit par rapport au jus de pomme non dilué, et donc une perte d'émail réduite (60 % : 4,2 ± 0,7 ; 40 % : 3,1 ± 0,5), cet effet a été légèrement moins prononcé en ce qui concerne le jus d'orange (60 % : 1,1 ± 0,3 ; 40 % : 0,9 ± 0,6) et l'acide citrique (60 % : 3,7 ± 0,9 ; 40 % : 2,8 ± 0,7).

Discussion

Les résultats de la présente étude confirment que les facteurs déterminants du potentiel érosif d'une boisson comprennent non seulement le pH du liquide, mais aussi des facteurs tels que le type d'acide et sa concentration, la quantité d'acide titrable et la capacité tampon. La teneur en calcium d'un fluide influence également son effet érosif. L'eau du robinet est une source naturelle de calcium ; la dilution avec cette eau peut ainsi se traduire par une érosivité plus faible de la boisson, qui dépend cependant aussi des différences régionales quant à la teneur en minéraux de l'eau utilisée. L'eau du robinet utilisée dans cette étude, de provenance zurichoise, se caractérise par une teneur relativement élevée en calcium (1,16 mmol/l).

La dilution du jus de pomme avec de l'eau du robinet a entraîné une réduction significative de l'effet érosif de cette boisson. Pour le jus d'orange, cet effet de la dilution a été plus faible. On peut donc en conclure que la dilution des jus de fruits avec de l'eau du robinet permet de réduire considérablement leur potentiel érosif. L'ampleur de cette réduction dépend du type de jus de fruits et du degré de dilution.

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