Reduction of the pH-values of Whole Saliva after the Intake of Apple Juice Containing Beverages in Children and Adults

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Purpose: The aim of the present study was to compare changes in salivary pH after intake of apple juice and its various dilutions with mineral water, in children and adults.

Materials and Methods: 15 children (mean age 4.4 ± 0.9 years), and 15 adults (mean age 30 ± 2.4 years) participated in this study. Carbonated mineral water, apple juice and its various dilutions with carbonated mineral water were tested. The pH-value of unstimulated whole saliva was measured at the same time of day. The pH-value was measured again immediately after intake of a beverage, and 5, 10, 15 and 25 min later. The flow rate and buffering capacity of stimulated saliva, and the buffering capacity, calcium and phosphorus contents of the beverages were measured. The statistical evaluation of the data was performed employing the Wilcoxon test for paired samples.

Results: The mean base value of the pH of whole saliva was 7.0 ± 0.2 (children), and 6.8 ± 0.3 (adults). The differences in the flow rates of stimulated saliva in children and adults were statistically significant ($p = 0.0003$). The intake of mineral water led to a statistically significant rise in the salivary pH-value ($p < 0.05$). Undiluted and diluted apple juice caused a reduction in the salivary pH-values. Within the first 10 min the changes of pH in saliva only differed significantly between mineral water and the other beverages ($p < 0.01$). In the pH range of 5.8 – 7.0, mineral water had a weak buffering capacity, while undiluted apple juice had a high buffering capacity.

Conclusions: With regard to dental and general health, only mineral water can be recommended for children.

Key words: apple juice, salivary pH, children, adults

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The economic development of a country can be associated with changes in the dietary habits of its population. Epidemiological studies confirm a high level of sugar consumption in countries with an established market economy as well as in developing nations with high or medium income (Diehnelt and Kiyak, 2001). An increased consumption of soft drinks, including fruit-flavored drinks, fruit juices and carbonated beverages has also been observed over the past decades (Nielsen et al, 2002). Even the preference among fruit juices has changed in children: nowadays apple juice is the preferred juice in children under 5 years of age (Dennison et al, 1997).

The changes in drinking patterns also have implications for dental health. The number of sugar-containing snacks and beverages consumed between meals, and a late onset of oral hygiene measures correlate positively with plaque accumulation and

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caries incidence in the primary dentition (Mattos-Graner et al, 1998; Habibian et al, 2001; Vanobbergen et al, 2001). Soft drinks not only contain sugars but also different organic acids, and they are implicated as an extrinsic cause in the development of dental erosions (Rugg-Gunn et al, 1998; Al-Dlaigan et al, 2001). In the presence of good oral hygiene, a moderate consumption of acidic and sugar-containing beverages has no adverse effects on the teeth of healthy individuals (Tenovuo and Rekola, 1977). However, various host factors like salivary flow rate, buffering capacity and pH, as well as the concentration of calcium and phosphate in saliva, and the frequency of fluid intake can influence the extent of dental erosion (Millward et al, 1994; O'Sullivan and Curzon, 2000).

Fruit juices contain several types of sugars and organic acids as natural ingredients. The variety, the geographical origin, the time of harvesting, the treatment and the processing methods of the fruits all influence the composition of the fruit juices and thus the amounts of sugars and organic acids (Hernandez et al, 1997; Drake and Eisele, 1999; Gökmen et al, 2001). Apple juices for example contain several sugars (glucose, fructose and sorbitol), phenolic compounds (such as hydroxymethylfurfural) and non-volatile organic acids such as quinic, malic, citric and fumaric acids (Mattick and Moyer, 1983; Lee and Wrolstad, 1988). Fructose and glucose are considered to be less cariogenic than sucrose, but the dental plaque, formed in the presence of a mixture of these two sugars also leads to a decrease in the micro-hardness of the enamel (Cury et al, 2000). Furthermore, it was shown that the various naturally occurring acids in fruit juices can cause enamel erosions (Lussi et al, 1995; Lussi et al, 2000; Al-Dlaigan et al, 2001). After the consumption of acidic drinks, the pH on the tooth surface decreases within a short time, whereas the form of intake e.g. with a glass, straw or feeder cup influences the resting time of low pH on the enamel surface and the extent of the enamel damage (Millward et al, 1997, Edwards et al, 1998). The erosive potential of each dietary acid is different, but also the concentration and temperature of the acids affect the extent of dental hard tissue loss (Hughes et al, 2000, West et al, 2000). Furthermore, the erosive effect of acidic beverages on the tooth surface also depends on other components in the beverages, especially, the amount of calcium and phosphate, which can modify the buffering capacity (Edwards et al, 1999; Larsen and Nyvad, 1999; Hughes et al, 2000).

The salivary properties of the host, which affect the tooth damage, are age-dependent. While the flow rate of stimulated saliva remains stable in adults (> 20 years of age), the flow rate of unstimulated saliva decreases with age (Percival et al, 1994). While the phosphate concentrations in resting and stimulated whole saliva were found to be similar in children and adults, the calcium concentration was considerably lower in children; in addition, the critical pH-value was significantly higher for children than adults (Anderson et al, 2001).

The aim of this study was to determine the possible differences in the decrease of pH-values of whole saliva in children and young adults following the intake of apple juice. In addition, the potentially beneficial effects of diluted apple juice, a popular beverage among German children, were to be evaluated.

MATERIALS AND METHODS

Thirty healthy subjects, 15 children (5 male, 10 female) with a mean age of 4.4 ± 0.9 years, attending kindergarten, and 15 adults (6 male, 9 female; 30 ± 2.4 years of age) participated in this study. Written consent was obtained from the adults and from the parents of the children. None of the subjects had unrestored cavities in any of their teeth.

The following beverages were tested: carbonated mineral water (pH = 5.8, Ca^{2+} 0.25 mmol/l), undiluted apple juice (pH = 3.3, Ca^{2+} 0.08 mmol/l) and various dilutions of apple juice with carbonated mineral water (2:3, pH = 3.7; 1:2, pH = 4.2; 1:3, pH = 4.8). In order to account for the circadian rhythm, all the beverages were always given at the same time of day on subsequent days. At 9 a.m., at least 60 min after the last meal (breakfast) and 15 min after tooth cleaning (which was supervised in the children), the pH-value of unstimulated whole saliva (base value) was measured. Whole saliva was collected in graduated Falcon tubes® (1 – 15 ml) with the help of plastic funnels, and the pH-value of saliva as well as the buffering properties of the tested beverages were determined with a portable pH-Meter (Novodirect GmbH, Germany) equipped with a microelectrode (Standard, diameter x length: 6 x 115, Novodirect GmbH, Germany). The portable pH-meter with an integrated printer and microprocessor had a range of pH 0.00 – 14.00 with an accuracy of pH ± 0.01. The pH-meter was calibrated each day prior to the first measurement with buffering solutions from IUPAC (Radiometer, Copenhagen). 50 ml of each beverage were given to the subjects in special cups (Nuk®, Germany), to ensure a uniform intake of the fluids (Fig 1). Immediately after the intake of a beverage, and 5, 10, 15 and 25 min later, whole saliva was collected and the pH-value was measured again.

Since a gustatory stimulation after intake of the beverages was expected, the flow rate and the buffering capacity of stimulated saliva was determined in both groups. The subjects chewed on a piece of paraffin wax for one minute, and the saliva, which had collected during this period of time in the oral cavity, was swallowed. Afterwards, saliva was collected for 5 min in a 30 ml beaker. The total amount of saliva was measured and the flow rate expressed as ml per minute. Afterwards, the buffering capacity of saliva was determined employing the CRT buffer test (Ivoclar/Vivadent®, Schaan/Liechtenstein). The test strips were moistened with saliva by means of a plastic pipette and the color of the test area was compared with the color pattern supplied by the manufacturer. The color blue indicated a high (pH $\geq$ 6.00), green a medium (pH = 4.5 – 5.5), and yellow a low (pH = $\leq$ 4) buffering capacity.

In addition, the buffering capacity, the calcium and the phosphorus contents of the beverages were measured. The buffering capacity was determined by titration with 0.2 N NaOH and subsequent measurements of the resulting pH values. The phosphorus and calcium contents of the beverages were determined spectrophotometrically (Shimadzu UV-1202/UV-VIS, Shimadzu Europe GmbH) by means of a colorimetric assay, using commercially available test kits (Sigma®, Deisenhofen).

The statistical evaluation of the data was performed using the SPSS program at the Institute for Medical Biometrics, Epidemiology and Computer Sciences, Johannes-Gutenberg University, Mainz, employing the Wilcoxon test for paired samples.

RESULTS

The mean base value of the pH of whole saliva was 7.0 $\pm$ 0.2 in children, and 6.8 $\pm$ 0.3 in the adults. The differences in the flow rates of stimulated saliva in children (median value = 1ml/h) and adults (median value = 2ml/h) were statistically significant ($p = 0.0003$). All adult subjects showed a high buffering capacity of whole saliva (pH > 6.00), but 26% of the children had only a medium buffering capacity (pH = 4.5 – 5.5), and these differences were statistically significant ($p = 0.038$).

Fig 1 Children using feeder cups (Nuk®, Germany) to ensure a uniform intake of the beverages.

The intake of mineral water in adults within the first five minutes and in children over the whole period of measurements led to a statistically significant rise in the salivary pH-value (positive ΔpH) ($p < 0.05$) (Table 1). Undiluted and diluted apple juice, however, caused a reduction in the salivary pH-values in all subjects (negative ΔpH) (Table 2). The pH reduction in whole saliva, caused by drinking various dilutions of apple juice, was statistically significant for the first 15 minutes in the adults' group, whereas significant reductions in the pH of whole saliva could only be determined in children during the first 10 minutes. Therefore, the period of 0–10 min was chosen for the statistical evaluation of the differences between ΔpH of whole saliva after the intake of the various beverages. During this period, the course of the changes of ΔpH in whole saliva differed significantly between mineral water and undiluted apple juice or its various dilutions in both groups ($p < 0.01$). No significant differences could be found between pure apple juice and its various dilutions during this period, except in the group of children between pure apple juice and its dilution 1:3 ($p = 0.03$) (Table 3). No significant differences in the changes of ΔpH, caused by the intake of any of the beverages, were observed between children and adults (Tables 1 and 2).

While in mineral water the calcium concentration was three times higher (0.25 mmol/l) than in pure apple juice (0.08 mmol/l), apple juice contained considerably more phosphorus (0.262 mmol/l) than

Table 1 The ΔpH of whole saliva at the various measurements and the p values (Wilcoxon-test for paired samples) comparing the various ΔpH to the baseline pH of whole saliva for children and adults after drinking of mineral water, and comparison of the differences between children and adults (the lowest and highest p values at different measurements)

Beverage	mineral water			
	children		adults	
Time of measurement	ΔpH	p-value	ΔpH	p-value
immediately after intake	0.32	0.0026*	0.18	0.0238*
after 5 minutes	0.29	0.0043*	0.23	0.013*
after 10 minutes	0.08	0.0267*	0.11	0.1924
after 15 minutes	0.11	0.0468*	0.03	0.6484
after 25 minutes	0.13	0.0477*	0.27	0.1826
p-values (children/adults)	0.1583 – 0.9009			

*statistically significant $p < 0.05$

mineral water (0.001 mmol/l) (Table 4). In the pH range of 5.8 – 7.0, mineral water had a weak buffering capacity, while undiluted apple juice had a high buffering capacity; the buffering capacity of apple juice decreased with the degree of dilution (Fig 2).

DISCUSSION

The stimulated flow rate and the buffering capacity of saliva are important individual factors which influence the development of dental erosions by means of eliminating or neutralizing acids from saliva and plaque. These two properties of saliva, as well as its pH, calcium and phosphate concentrations vary during the day and undergo strong fluctuations, even within the same individual at the same time of day on different days (Larsen et al, 1999). Therefore, in the present investigation, all experiments were performed at the same time of day and under identical conditions.

A significantly lower stimulated salivary flow rate and a lower buffering capacity of saliva were found in the group of children in comparison to the adults. Although values for the salivary flow rates can vary considerably between different studies, an increase in the stimulated salivary flow rate with increasing age was demonstrated in previous studies (Andersson et al, 1974; Crossner, 1984; Soderling et al, 1993). In healthy individuals the stimulated salivary flow rate became stable, beginning at an age of 15 to 20 years (Heft and Baum, 1984; Soderling et al, 1993; Bergdahl, 2000). This low salivary flow rate in children might be correlated with physiological factors like the growth of the parenchymal tissue of the salivary glands or the lower number of teeth, which might also partly influence the stimulated salivary flow rate (Crossner, 1984; Bergdahl, 2000). However, other authors have been unable to establish a connection between the number of teeth and the flow rate of stimulated saliva (Heft and Baum, 1984). The lower values for the stimulated salivary flow rate, found in children in the present study, might also be attributed to the observation that in this particular age group (3–5 years) some of the children were not capable of completely following the instructions. Andersson et al (1974) also considered the possibility that at times the children might swallow the saliva instead of actually collecting it over the whole period of time.

As in the present study, a lower buffering capacity of saliva in the younger children was also shown by Andersson et al (1974), who investigated subjects between the ages of 5 to 13 years. Soderling et al (1993) showed a decrease in the buffer effect of the stimulated saliva in a five-year follow-up study.

In the present study, no significant differences in the initial salivary pH-values could be found be-

Table 2 The ΔpH of whole saliva at the various measurements and the p values comparing the various ΔpH to the baseline pH of whole saliva for children and adults after drinking apple juice and its various dilutions, and comparison of the differences between the children and adults (the lowest and highest p values at different measurements)

Beverage	apple juice				apple juice:mineral water 2:1				apple juice:mineral water 1:1				apple juice:mineral water 1:2			
	children		adults		children		adults		children		adults		children		adults	
Time of measurement	ΔpH	p-value	ΔpH	p-value	ΔpH	p-value	ΔpH	p-value	ΔpH	p-value	ΔpH	p-value	ΔpH	p-value	ΔpH	p-value
immediately after intake	-0.20	0.0266*	-0.57	0.0225*	-0.28	0.1688	-0.79	0.0015*	-0.29	0.0151*	-0.51	0.0009*	-0.17	0.0730	-0.25	0.0118*
after 5 minutes	-0.50	0.0001*	-0.44	0.0003*	-0.32	0.0131*	-0.23	0.0034*	-0.28	0.0004*	-0.49	0.0001*	-0.29	0.0108*	-0.53	0.0001*
after 10 minutes	-0.24	0.0198*	-0.44	0.0028*	-0.01	0.4293	-0.25	0.0052*	-0.25	0.0001*	-0.25	0.0026*	-0.13	0.0287*	-0.30	0.0012*
after 15 minutes	-0.16	0.0833	-0.20	0.0040*	-0.04	0.2576	-0.27	0.0067*	-0.05	0.4628	-0.21	0.0174*	0.01	0.3028	-0.19	0.0145*
after 25 minutes	-0.01	0.9012	0.12	0.4625	0.00	0.6698	-0.04	0.8256	-0.05	0.4212	-0.03	0.1924	0.10	0.3817	-0.01	0.9891
p-values (children / adults)	0.2807–0.9357				0.1057–0.5337				0.2625–0.8682				0.1249–0.9339			

*statistically significant p<0.05

tween children and adults. However, the pH-values of the whole saliva of the children changed to a lesser, but not significant, extent compared to adults over the whole period of 25 minutes, except after intake of mineral water. Although we tried to standardize the intake of the fluids by using special cups, the children in this age group were more used to this way of drinking than the adults. This could have contributed to the fact that it took the adults longer to swallow than the children, which might explain the small differences in the ΔpH values of saliva between the two age groups.

Another difference in the ΔpH of saliva was observed between children and adults after the intake of undiluted or diluted apple juice. The salivary pH-values of the children dropped to the lowest level within 5 min after drinking, while in adults this was the case immediately after fluid intake. In our study this could be connected with the distribution of the beverages within the oral cavities of adults and children, and with the differences in swallowing in children. When drinking from a feeding cup, the region of the maxillary front is covered first with fluid, followed next by the maxillary molars. The fluid's molecules reach the area of the mandibular molars after some delay, and lastly cover the area of the mandibular front and thus the sublingual area (Weatherell et al, 1989; Smith and Shaw, 1993). While the adults deliberately collected the saliva before expectorating it into the container, thereby allowing a regular distribution of the molecules retained in the mouth in whole saliva, the children tended to expectorate smaller amounts, and thus only the saliva, which was present at this time in the sublingual area. Another reason for slightly more alkaline salivary pH-values in the children could be that the children needed a longer time than the adults for the collection of a sufficient amount of saliva in the containers, during which carbon dioxide could evaporate from the saliva samples (Andersson et al, 1974; Bardow et al, 2000).

Changes in pH-values of saliva are not only influenced by the individual properties of saliva but also by acids and sugars and their concentrations in the beverages, their base pH-values, and the temperature of the oral environment. The inherent buffering capacity of the beverages competes with the salivary buffering systems. The changes in the salivary pH, induced by the buffering system of saliva, become compensated by the buffering capacity intrinsic to the beverages, so that lower pH-values in saliva and in plaque are maintained longer and cause

Table 3 The median values of the differences in the ΔpH of whole saliva within the first 10 minutes after drinking of various beverages and the p value comparing these median values for children and adults

Beverages		children		adults	
		Median of differences	p-value	Median of differences	p-value
apple juice	mineral water	-0.62	0.0003*	-0.63	0.0001*
apple juice	apple juice:mineral water 2:1	0.03	0.0637	-0.05	0.5711
apple juice	apple juice:mineral water 1:1	0.14	0.3303	0.09	1.0000
apple juice	apple juice:mineral water 1:2	-0.18	0.0302*	-0.03	0.2832
apple juice:mineral water 2:1	apple juice:mineral water 1:1	0.14	0.1876	-0.05	0.8040
apple juice:mineral water 2:1	apple juice:mineral water 1:2	0.01	0.7321	-0.04	0.3375
apple juice:mineral water 1:1	apple juice:mineral water 1:2	-0.12	0.1050	-0.07	0.3028
mineral water	apple juice:mineral water 2:1	0.45	0.0103*	0.59	0.0001*
mineral water	apple juice:mineral water 1:1	0.53	0.0003*	0.58	0.0001*
mineral water	apple juice:mineral water 1:2	0.47	0.0015*	0.41	0.0001*

*statistically significant p<0.05

Table 4 Calcium and phosphorus contents of the tested beverages

Beverages	Calcium		Phosphorus	
	Absorption	mmol/l	Absorption	mmol/l
mineral water	0.368	0.25	0.003	0.001
apple juice	0.122	0.08	0.397	0.262
apple juice:mineral water 2:1:	0.207	0.14	0.230	0.151
apple juice:mineral water 1:1	0.156	0.11	0.293	0.193
apple juice:mineral water 1:2	0.270	0.15	0.175	0.115

deeper erosive damage on the tooth surfaces (Edwards et al, 1999; Larsen and Nyvad, 1999; Lussi et al, 2000).

The apple juice, which was utilized in the present study, had a high buffering capacity, which was also found in previous investigations of other fruit juices (Touyz, 1994; Larsen and Nyvad, 1999; Lussi et al, 2000). Using pH telemetry of mixed saliva, Imfeld and Mühlemann (1978) could even show that the buffering capacity of apple juice proved to be six times higher than that of coca cola, whereby the buffering in the oral cavity was slowed down considerably by the salivary buffering system. The mineral water had, as in other studies, a lower buffering capacity (Parry et al, 2001). Therefore, the buffering capacity of the beverages decreased accordingly when apple juice was diluted with increasing amounts of mineral water. In spite of this, the ΔpH values of saliva scarcely differed within the first 10 min after the intake of various dilutions of apple juice. Diluting the apple juice with mineral water also reduced the sweetness of the beverage. Both 'sour' and 'sweet', but also 'bitter' and 'salty' are stimulants for the salivary flow (Dawes and Watanabe, 1987; Edgar, 1992). By diluting the apple juice, the concentrations of sugars and acids became lower and this also led to a weaker buffering capacity of the beverages. Therefore, a decrease in the salivary flow, and thus a slower buffering of the low salivary pH-value occurred.

The gustatory stimulation can also explain the rise of the pH-value in whole saliva after the intake of mineral water, although mineral water by itself had a pH-value of 5.8, which was considerably low-

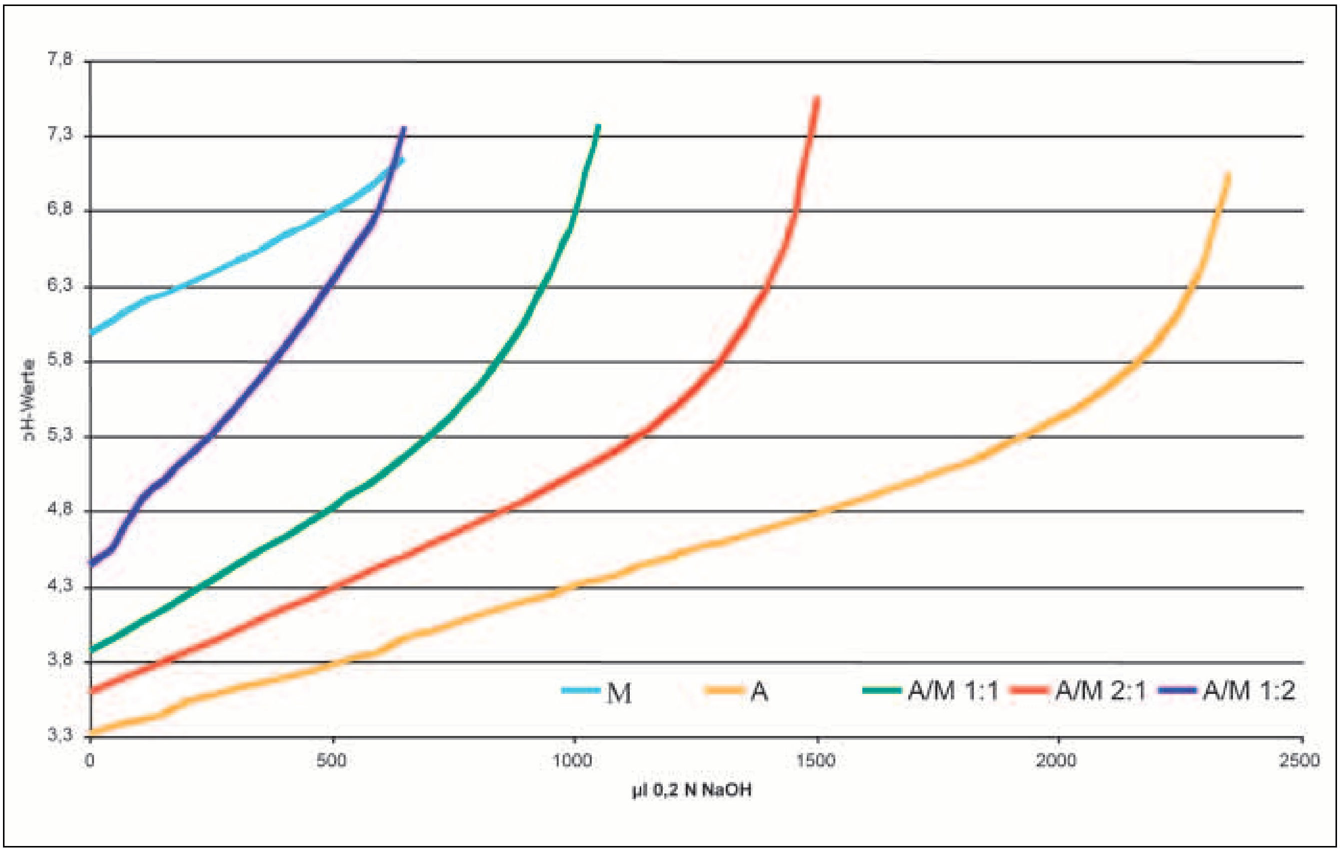

Fig 2 Buffering properties of the beverages. M = mineral water, A = apple juice.

er than the base pH-values of whole saliva in both groups of subjects. Within the first 5 min, this rise was significantly different in both groups from the base values. In contrast to the adults, this significance lasted in children up to the measurement at 25 min. In the present study, the mineral water had a strikingly salty taste and therefore could have led to a more pronounced stimulation of saliva, especially in children. The difference in time before the pH in both groups returned to the base value, probably depends on the fact that adults respond less strongly to this stimulus, because the detection of a particular kind of taste depends also on the concentration of the respective stimulus in saliva.

Fruit juices and other soft drinks can not only lead to dental erosions, but can also be associated with several problems regarding the general health of children. The excessive consumption especially of apple juice, can also cause non-specific gastrointestinal symptoms like diarrhea, which is a result of the malabsorption of fructose and sorbitol that are present in these juices (Ribeiro et al, 2001). In conclusion, with regard to dental but also to general health, only uncarbonated or carbonated mineral water can be recommended for children without reservation (Parry et al, 2001). The regular consumption of apple juice, also in a diluted form, should be discouraged.

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