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Impact of Green Vegetables and Green Smoothies on Enamel Mineral Composition In Vitro

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Abstract

Background: Recently, smoothies with a high vegetable content have become very popular, especially among young adults.
Objectives: This in vitro study was conducted to determine the erosive potential of selected green vegetables and green smoothies on human dental enamel.
Methods: A total of ten green smoothies and eight freshly mixed vegetables were chosen, and pH, calcium and oxalic acid contents were measured. The enamel specimens were obtained from wisdom teeth and incubated with 3 selected foods (spinach, parsley, or green smoothie) for 12 and 24 hours (N = 6). Control samples were incubated with a physiological salt solution. An electron probe microanalyzer (Jeol JXA 8900RL) was utilized to analyze the concentrations of calcium, phosphorus, magnesium and fluorine at various depths ranging from 5 to 50 μm within the enamel samples. A visual examination of the specimen was carried out with a digital microscope.
Results: The green smoothies showed pH values ranging from 3.6 to 4.2, and the values of the mixed vegetables ranged from 5.6 to 6.3. Only in the green smoothie group could a time-dependent release of different minerals be observed. After 12 and 24 hours of incubation, a pronounced loss of calcium and phosphorus was found to a depth of 15 μm. Digital microscopy of the enamel surfaces confirmed these findings.
Conclusions: In our in vitro study, incubation with the spinach and parsley showed no damage to the enamel specimens; in the case of the investigated smoothies, a mild loss of minerals to a depth of 25 μm was observed. It must be considered that numerous factors influence the enamel surface in vivo, translating these results to in vitro conditions must be done with great caution.

Keywords: Green Vegetables, Green Smoothie, Human Dental Enamel

1. Background

A well-balanced modern diet includes cereals, seeds, vegetables, fruits, dairy products, fish, meat, eggs, oils and beverages, in particular, water. Over the last 30 years, fast food cuisine has become established in the diet due to lifestyle changes. Frequent consumption of this type of nutrition with high amounts of fat and carbohydrate together with acidic soft drinks leads to the well-known effects of overweight and dental problems (1-3). Lifestyle habits arising from the permanent availability of fast food products and soft drinks in combination with intensified oral hygiene measures, chewing habits and bruxism, have led to an increased prevalence in dental erosion (4-9). In an in vitro study, Lussi et al. (10) showed that the erosive effect of modern popular beverages is characterized by a significant reduction of the surface hardness of enamel specimens and that in addition to the pH value, the titratable acidity and other components of the beverages, such as Ca or F, are also important. Ancient foodstuffs and nutritional habits vary to a large extent from today's dietary habits. In prehistoric times, humans were hunter-gatherers and had irregular food intake as well as limited food supply. An explanation for the dental damage was the diet of plant fibers, hard seeds and foods containing grinding stone grit. The major plant foods (e.g., agave, yucca, stool, prickly pear, wild onion, mesquite) are rich in calcium oxalate phytoliths. In addition to the erosive effects of oxalic acid, the presence of phytoliths, which are harder than enamel, may well have caused loss of dental hard tissue. Currently, there is an increased awareness of healthy nourishment

and a tendency toward a one-sided diet rich in vegetables. Various types of green vegetables, such as spinach, broccoli and parsley, contain high amounts of acidic components. Studies have reported that a vegetarian diet can provoke dental damage, such as abrasion or erosion (11-13). Consumption of acidic foods and soft drinks has been linked with the development of dental erosion (3, 5, 10). In general, dental erosion is primarily chemically induced by organic or inorganic acids from extrinsic and intrinsic sources. Tooth structures that are damaged and eroded by acidic foods are more sensitive to further breakdown by abrasion and attrition during tooth brushing (14). Recent health promotion actions recommend consumption of 5 servings of fruit or vegetables per day. Smoothies are promoted as an easy way to consume the necessary amounts of fruits and vegetables (6). There are only a few publications that discuss the effects of smoothies, in particular, fruit smoothies, on human enamel (6, 15).

2. Objectives

The aim of our in vitro study was to investigate the erosive potential of selected green vegetables and green smoothies on human enamel samples. To quantify the loss of essential minerals on the enamel surface, we selected the electron probe microanalyzer, since it is the most sensitive research method.

3. Methods

3.1. Green Vegetables and Green Smoothies

The experimental design was descriptive. For this study, only green vegetables and green smoothies were selected to determine the possible erosive effect on enamel surfaces. Green smoothies were selected since they represent a convenient way to increase children's fiber and vitamin intake. Green vegetables were further analyzed as controls to investigate the effect of the unprocessed foodstuff.

Therefore, eight different fresh green vegetables (spinach, parsley, mangold, broccoli, arugula, kale, leek, and romaine lettuce hearts) were each homogenized in a blender for 5 minutes. Next, the vegetable supernatant was separated from the residue with a centrifuge for 10 minutes (3000 U/min). For all examinations, only the supernatants of the vegetables were used (Table 1). A total of ten green smoothies were chosen, which were mixtures of different vegetables and fruits (Table 2). For further analyses, the smoothies were also centrifuged for 10 minutes at 3000 U/min. The pH values and concentrations of calcium and oxalic acid were determined from the vegetable supernatants and the green smoothies. Among all green vegetables, we selected spinach due to its high concentration of oxalic acid and parsley because of its high vitamin C content (Table 1). Within the green smoothie group, we selected a smoothie with a relatively high percentage of green vegetables and apple juice as well as a low pH value (Table 2).

Table 1. Concentrations of Calcium and Oxalic Acid and pH Values in the Supernatants of Eight Green Vegetables

Green Vegetables	Oxalic Acid, mg/mL	pH	Ca, mg/mL
Spinach	391.3	6.3	0.014
Parsley	83.4	5.6	0.036
Mangold	225.4	6.2	0.025
Broccoli	Not detectable	6.2	0.016
Arugula	Not detectable	5.8	0.100
Kale	Not detectable	6.2	0.018
Leek	Not detectable	5.6	0.039
Romaine lettuce	Not detectable	6.0	0.171

3.2. Human Enamel Specimen Preparation

For this investigation, twelve fully retained wisdom teeth from young adult patients were selected. The patients were informed about the purpose of this investigation and gave consent for the use of their teeth. Furthermore, the protocol for the experimental analysis of human samples was approved by the Ethics Commission of the University Medical Center, Mainz, Germany. The collected teeth were visually examined with a digital microscope, and all teeth with mineralization irregularities or damage were excluded. The roots were separated from the crowns, and the enamel surfaces were polished with silicon carbide and aluminum oxide discs and sliced horizontally into pieces of approximately 3 mm thickness (16). All crowns were then divided into up to six slices, resulting in a total of 52 samples. All samples were washed with and stored in physiological sodium chloride solution until further analysis.

3.3. Physiochemical Measurements

To select characteristic green vegetables, the pH of the supernatant of the vegetables and of the green smoothies were determined with a microelectrode (Novodirect, Kehl, Germany). The calcium concentration of the samples

Table 2. List of Ingredients of Ten Different Green Smoothies, Which Are Available in German Supermarkets

Name of Green Smoothies	Ingredients	pH	Ca, mg/mL
Saftig smoothie; Fresco y Zumos, S. A. Spain	Apple juice 48%, apple puree 25%, cucumber puree 12%, banana puree 10%, lemon juice 4%, spinach and nettle extract	3.69	0.05
Bio-Alnavit Grüner Held, Alnavit GmbH; Bickenbach, Germany	Pear puree 47%, kiwi puree 25%, acerola puree, broccoli puree 9.5%, bell pepper puree, dandelion juice 2%, stinging juice 2%, spirulina 0.1%, matcha powder 0.1%	3.77	0.28
Voelkel fair to go; 100% direct juice; Demeter e.V.; Germany	Tomato juice 66%, carrot juice 13%, sauerkraut juice 6%, beetroot juice 5%, celery juice 4%, cucumber juice 2%, bell pepper paste 2%, onion juice, bean juice, dill juice, herbal sea salt	4.18	0.11
True fruits green smoothie; spinach; Bonn, Germany	Apple juice 54.9%, banana puree 21%, organic spinach puree 11%, pear puree 8%, organic kale puree 4%, ginger puree 1%, organic matcha tea 0.1%; percentage of organic ingredients: 15.1%	3.86	0.03
True fruits green smoothie; arugula; Bonn, Germany	Apple juice 48.9%, mango puree 19%, arrugula puree 15%, pear puree 11%, carrot puree 5%, mint 1%, chlorella 0.1%	3.85	0.21
Grüner Smoothie Voelkel; orange; Demeter Germany	Apple juice 20%, orange juice 20%, pear juice 19%, banana puree 4%, kale 8%, peach pulp 7%, spinach 5%, acerola pulp 5%, wheatgrass, matcha	4.10	0.12
Grüner Smoothie Voelkel[a]; Demeter Germany	Apple juice 52.5%, mango pulp 17.5%, beetroot juice 16%, spinach 8%, kale 6%	3.89	0.05
Grüner Smoothie Voelkel, mango; Höhbeck, Germany	Apple juice 48%, mango pulp 15%, banana puree 13%, kale 6%, ginger juice 6%, ginger, mineral water, lemon juice concentrate, acerola pulp 5%, spinach 8%,	3.96	0.18
Voelkel vegan to go; Kraftprotz gluten frei Demeter e.V.; Germany	Cucumber juice 35%, apple juice 20.5%, mango pulp 10%, orange juice 10%, spinach 6.5%, kale 6.5%, banana puree 6%, quinoa 2.5%, wheatgrass juice, lemon juice, acerola pulp, spirulina powder, matcha 0.1%	4.19	0.14
Innocent smoothie-; Innocent Alps; Salzburg, Austria	Apple juice 48%, pineapple juice, kiwi puree 16%, grape juice, lime juice 1.2%, spinach and stinging nettle extract	3.58	0.10

[a] The marked smoothie was selected for the in vitro study.

was determined spectrophotometrically using a colorimetric assay (Randox Laboratories, Krefeld, Germany). The concentration of oxalic acid was measured spectrophotometrically using a colorimetric assay test kit (Enzytec, R-Biopharm AG, Darmstadt, Germany).

3.4. Exposure to Selected Green Vegetables and Green Smoothies

Exposure to selected green vegetables and green smoothies for the specific and sensitive analysis of the elements Ca, Mg, F, and P with the electron probe microanalyzer was repeated in six independent experiments.

For each test solution (spinach, parsley, green smoothie), one sample per tooth served as the enamel control and was incubated with 3 mL of the physiological salt solution.

This in vitro study aimed to investigate the maximal erosive potential of selected vegetables and smoothies on dental enamel without further possibility of remineralization. Therefore, we consciously incubated our samples for up to 24 hours and abstained from employing a further incubation period with artificial saliva solution.

The selected enamel slices (N = 48) were placed into multiwell plates (Greiner, Frickenhausen, Germany) and then incubated with 3 mL of the respective test supernatants at 37°C in a humidified atmosphere in a gas incubator (Heraeus, type Function Line, Hanau, Germany) for 12 hours and 24 hours. After exposure, the supernatants were removed, and the enamel samples were thoroughly washed in sterile distilled water, air-dried and prepared for electron probe microanalysis.

3.5. Electron Probe Microanalyses

The quantitative measurement of the elements Ca, F, P and Mg in the enamel surfaces was conducted with an electron probe microanalyzer (JEOL JXA 8900 RL, JEOL, Eching, Germany). For this analysis, the dental slices were embedded in epoxy resin and processed according to a previously published protocol (16).

3.6. Data Analysis

The statistical analysis was carried out with the SPSS software (version 23.0; SPSS Inc., Chicago, IL, USA). For the measurement of the concentrations of the various elements in the enamel slices, only descriptive analyses were

performed, which are expressed as the means ± SD (standard deviations).

4. Results

The ten selected green smoothies showed pH values ranging from 3.6 to 4.2 and calcium concentrations ranging from 0.03 to 0.28 mg/mL (Table 2). For all smoothies, the oxalic acid concentration was lower than the detection limit. The pH values of the eight green vegetables ranged from 5.6 (parsley) to 6.3 (spinach), and the concentration of free calcium in the supernatants was low for all vegetables (Table 1). Oxalic acid was only detectable in spinach (391.3 mg/L), mangold (255.4 mg/L) and parsley (83.4 mg/L). Since qualitative and quantitative erosive damages can be detected by the Electron Probe Microanalyzer, which allows the detection of characteristic elements, we purposely abstained from the unspecific investigation of the surface roughness.

With the electron beam microprobe analyzer, the elemental concentrations of Ca, P, Mg and F in the enamel samples were measured at depths of up to 50 μm (Figure 1). The incubation of the enamel samples with spinach and parsley showed no erosive potential over the incubation time of 24 hours. In contrast, the incubation of the dental samples with the green smoothie showed, in comparison to the controls, a mild erosive potential. The incubation time of 12 hours showed a loss of Ca and P at a depth of 20 μm, while the longer exposure time demonstrated a loss of minerals up to 25 μm. The fluorine content showed considerable variation at all depths and in all specimens. The visual examination of the enamel samples with the digital microscope showed only a slight alteration of the surface topography for the specimens incubated for 24 hours with the green smoothie (Figure 2).

5. Discussion

Nutritional habits can influence dental health. In particular, frequent consumption of diets rich in sugar or acids can lead to dental decay or dental erosion (4, 8, 17). Recently, increased health awareness has evoked increasing popularity of fruit smoothies (6, 15) and so-called superfoods such as chia seeds or goji berries. Green smoothies, a blended mixture of fruits and vegetables, are viscous fruit juices with high fiber content [10% - 19%], and these beverages are very popular for adolescents and young adults. Our in vitro study aimed to elucidate the possible erosive effects of green smoothies and, in particular, green vegetables as a major component of these modern beverages.

The etiology of erosive tooth wear is a complex process with different causative and individual factors (18); it is important to recognize initial changes and to assess possible erosive processes with sensitive techniques. We investigated parsley and spinach as representative green vegetables, which are also known for being especially healthy because they contain numerous minerals and are rich in vitamin C. Spinach also has high oxalic acid content, which binds especially effectively to calcium as a chelator. However, Hannig et al. (19) showed in an in vitro study that incubation of bovine enamel specimens with oxalic acid did not have a pronounced erosive effect. The present study showed that a green smoothie with a high content of green vegetables and apple juice led to a notable loss of the essential minerals calcium, phosphate and magnesium. Oxalic acid was not detectable in this smoothie and therefore cannot be responsible for the mineral loss. However, the low pH and considerable titratable acidity reflect the content of other acidic fruit components. In contrast, the supernatants of spinach and parsley induced only a slight loss of calcium and phosphate on the enamel surface.

When assessing the concentration of different elements in human teeth, it must be taken into consideration that even within the same tooth, greater variability in elements exists. The concentrations of magnesium and fluoride, e.g., as opposed to calcium and phosphate, are more likely to be influenced by environmental factors such as nutrition or fluoride supplementation (20, 21). Both the pH value and other factors, such as acid type, buffer capacity and mineral components, must be taken into consideration. For example, soft drinks contain considerable amounts of fruit acids, whereas green vegetables very often contain high amounts of vitamin C and considerable quantities of oxalic acid (22). Vegetarian or vegan diets that are naturally rich in raw fruits and vegetables can particularly damage teeth by erosion or abrasion (11). Linkosalo and Markkanen (12) found in Finnish vegetarians a 77% prevalence of erosion of teeth when compared to individuals consuming a balanced diet. On the other hand, Herman et al. (11) observed erosive changes in teeth in 39% of vegetarians compared to 24% in the control group. Similar results were reported by Staufenbiel et al. (23), who examined the oral health of 100 vegetarians and used a questionnaire concerning nutritional and eating habits. The vegetarians showed a significantly higher number of teeth with dental erosion. To study the erosive effects of green vegetables, we selected wisdom teeth of young adults, whereas

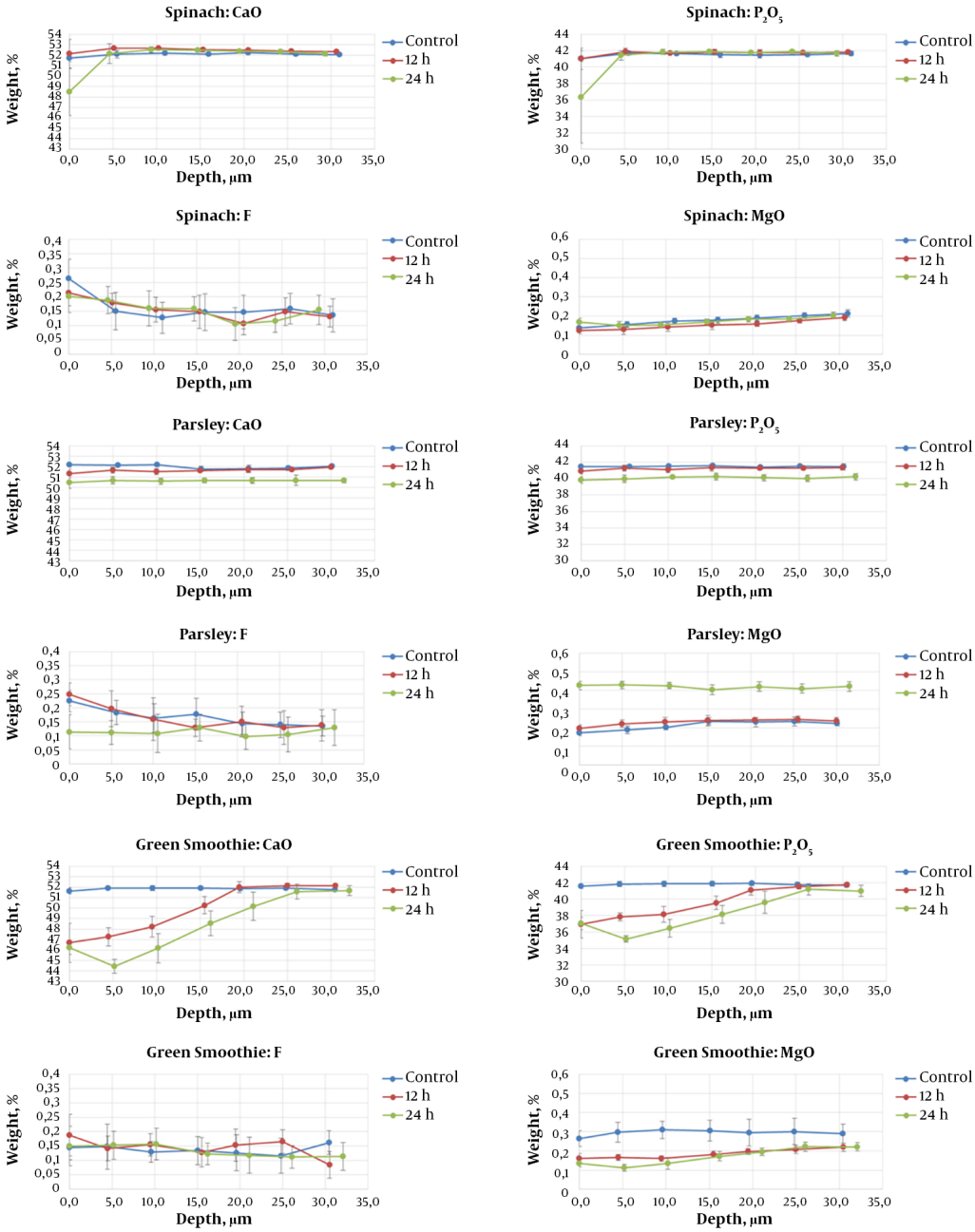

Figure 1. Concentrations of the minerals calcium, phosphorus, magnesium and fluorine (determined as CaO, P2O5, MgO, and F) in human enamel specimens (N = 6; mean, SD) after an incubation time of 12 and 24 hours with different green vegetable or green smoothie supernatants (spinach: pH = 6.3; parsley: pH = 5.6 and green smoothie: pH = 3.9)

previous studies were performed on bovine teeth, as they are larger and easier to obtain but do not necessarily reflect the human situation (3, 19, 24).

5.1. Conclusions

Our in vitro study demonstrated that green smoothies containing a high percentage of acidic fruit induced a loss of calcium and phosphate from enamel, whereas the selected green vegetables alone had no substantial effect on

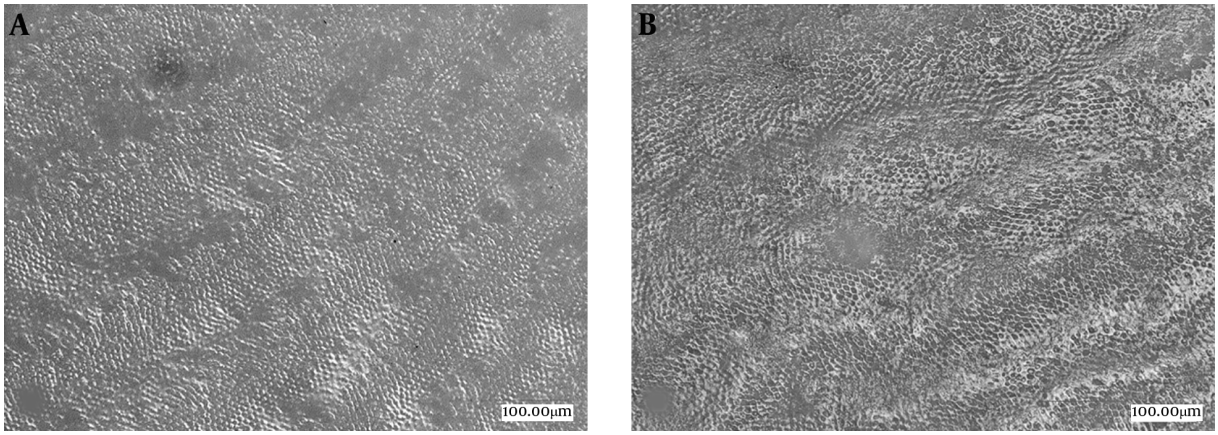

Figure 2. Digital microscopy of human enamel surfaces. A, control sample, B, enamel sample after incubation with a green smoothie for 24 hours (Magnification 500 ×)

the enamel surface.

Since multiple factors, such as remineralization, reduce the erosive effect within the oral cavity, green smoothies can be considered a tooth-friendly beverage when consumed in moderation.

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Footnotes

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