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Effects of Preparation Method on Acid Diffusion into Red Beets during in Vitro Gastric Digestion in Relation to Buffering Capacity

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Preparation method may influence acid diffusion and buffering capacity of foods, which will consequently influence food breakdown and nutrient release during gastric digestion. The objective of this study was to determine the acid uptake into red beets during in vitro gastric digestion and characterize the buffering capacity of red beets as a result of different preparation methods. Red beets were cut into cylinders and they were examined as raw, steamed (15 min at 105°C) or pickled (steamed 15 min at 105°C, 7 days immersed in 2% acetic acid). Cylinders were covered to insulate their sides and back to have a one-dimensional diffusion system, before soaking in simulated gastric juice for up to 96 h (37°C). Before digestion, buffering capacity was measured by adding 0.2 M HCl to blended raw, steamed, or pickled beets until the pH value was below 1.5. After digestion, acidity measurements were performed. Acidity was significantly influenced by preparation method and digestion time ($p < 0.05$). Acidity of raw beets had the greatest increase from 0.46 mmol H^+/g dry matter to 3.71 mmol H^+/g dry matter during 96 hours of incubation. On the contrary, decrease of acidity was observed in pickled beets during 96 hours of incubation (15.28 mmol H^+/g dry matter to 7.50 mmol H^+/g dry matter). Preparation method significantly influenced buffering capacity ($p < 0.001$). Pickled beets had a higher buffering capacity of 0.051 mmol H^+/(pH g) compared to raw and steamed beets which had buffering capacity values of 0.035 and 0.034 mmol H^+/(pH g), respectively. Beet resistance to changes in pH, or buffering capacity, may cause differences in acid uptake of red beets in the gastric environment after different preparation methods. Higher acid uptake (3.25 mmol H^+/g dry matter) was observed in raw beets with respect to steamed beets (2.35 mmol H^+/g dry matter) during 96 hours of incubation as a result of higher buffering capacity and structural changes due to preparation method. The study of the relationship between food preparation and behavior during digestion can be important for development of innovative functional food products for specific consumer groups.

1. Introduction

Red beet (*Beta vulgaris*) is a plant rich in fiber that has widespread consumption in many countries (Latorre et al., 2013). It is grown throughout the Americas, Europe and Asia (Gupta et al., 2003). There are several methods to prepare red beets for consumption. They can be consumed as raw, steamed, boiled, roasted, pickled, or as a juice (Wruss et al., 2015; Tanumihardjo et al., 2016), depending on consumer preference.

The knowledge of how preparation methods affects gastric digestion and the underlying mechanisms (e.g. acid uptake) is critical to understand food breakdown in order to develop structured foods with targeted properties (Kong et al., 2013). According to previous studies with apples and sweet potatoes, processing modifies the rate of acid diffusion into food materials during simulated gastric digestion (Mennah-Govela and Bornhorst, 2016a; Dalmau et al., 2017).

During digestion, gastric acid interacts with foods, decreasing the pH and promoting acidic and enzymatic hydrolysis of food materials (Bornhorst and Singh, 2014). However, the food resistance to changes in pH, known as buffering capacity, influences the specific pH of the food material in the gastric environment. The pH is defined as free hydrogen ion concentration in a solution. In pure acid solutions, the normal concentration of the acid is proportional to the free hydrogen ion concentration. In the case of solutions of fruits or vegetables,

the free hydrogen ion concentration will be influenced by their colloid and buffer salts content, which will impact the pH measurement (Giger-Reverdin et al., 2002). For this reason, food buffering capacity is an important parameter needed to understand the gastric acid diffusion into food materials.
Acid diffusion into the red beets may have implications on the food breakdown and nutrient release (Mennah-Govela et al., 2015). The buffering capacity of red beets might be an important parameter to help describe the acid diffusion and food behavior during gastric digestion. Since the preparation method has a potential effect on food structure, it can influence the acid diffusion and buffering capacity of red beets. The objective of this study was to determine the acid uptake into red beets during in vitro gastric digestion and characterize the buffering capacity of red beets as a result of different preparation methods.

2. Materials and methods

2.1 Raw materials

Red beets (*Beta vulgaris*) were acquired from a local produce supplier (General Produce Company, Sacramento, CA, U.S.A.; Pedrick Produce, Dixon, CA, U.S.A.). Samples were stored in refrigerator at 4°C for use within 2 weeks. Vinegar (5% acetic acid of total volume) was purchased from a local supermarket (Target Inc., Minneapolis, MN, U.S.A.).

2.2 Sample preparation

Red beets were cut into cylinders (length 30 mm, diameter 19 mm) using a size 14 cork borer. Beets were tested as raw, steamed or pickled. For steamed beets, 12 individual cylinders of raw beets were steamed for 15 min in a kitchen steamer (kept at 105 ºC) placed above 200 mL water. For pickled beets, 24 beet steamed cylinders (105 ºC for 15 min, as described above) were soaked in 300 mL of 2% acetic acid solution for 7 days (3 days at 25 ºC, 4 days at 4 ºC) following a home pickling procedure (Featherstone, 2016). All samples were tested immediately after preparation.

2.3 Simulated digestion

Simulated gastric juice was formulated following Bornhorst and Singh (2013). Briefly, simulated gastric juice was made by mixing 1.5 g/L of mucin from porcine stomach (Sigma-Aldrich, MO, U.S.A.), 8.78 g/L of NaCl (Fisher Science Education, IL, U.S.A.), and 1.0 g/L of pepsin (Fisher Science Education, IL, U.S.A.), with deionized water. The pH of the gastric juice was adjusted to 1.8 using 3 M HCl (Bornhorst & Singh, 2013).
Red beet cylinders were individually wrapped in parafilm and placed into a plastic holder (cylindrical shape: 21 mm external diameter, cut to 40 mm length) with one end closed with a 10 mm thick stopper, to provide a one-dimensional diffusion system, where gastric juice entered only through the face that was in contact with the gastric juice as shown in Figure 1. The sample was immersed in 250 mL of gastric juice (previously heated to 37°C), and was placed in an incubator (37°C) for up to 96 h (Figure 1) (Mennah-Govela et al., 2015). Samples were removed at ten time points: 0 (no digestion), 2, 4, 8, 12, 16, 24, 48, 72, and 96 h. Gastric digestion of red beet cylinders was evaluated up to 96 hours to be able to reach equilibrium conditions for acid uptake when the sample size was considered. After removal from the incubator, each cylinder was taken out of the holder and cut into 3 equal pieces (10 mm length/piece) for further studies. The mass average acidity values for each sample were calculated using the values obtained from all three pieces for the analysis in the current study. Digestions were completed in triplicate for each preparation method-time combination. In each digestion replicate, duplicate samples were analyzed for acidity.

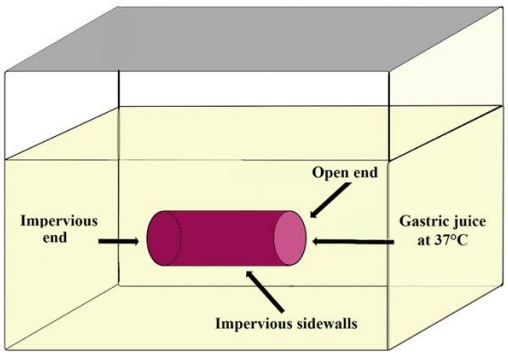

Figure 1: Simulated gastric digestion conditions for red beet cylinders

2.4 Acidity and pH measurements

Each piece of red beet (one region from one preparation-digestion time, ~ 2.5 g) was weighed and homogenized with 20 mL of deionized water for 1 min at 5,000 rpm (IKA T18 Ultra Turrax, Wilmington, NC, U.S.A.). The pH of samples was measured using an IQ150 portable IFSET pH meter (Hach Co., Loveland, CO, U.S.A.). Sample acidity was determined via potentiometric titration. For raw and steamed red beets, 0.01 M NaOH was used for titration, and for pickled red beets 0.1 M NaOH was used for titration until the sample had a pH of 8.2 ± 0.05 (Mennah-Govela et al., 2015).

2.5 Buffering capacity measurement

Raw, steamed, and pickled red beet cylinders were blended in a coffee grinder (Mr. Coffee Blade Grinder, IDS77-RB, Jarden Corporation, Hoboken, NJ, U.S.A.) for 10-15 second intervals for 9 minutes before buffering capacity measurement. Six pieces of cylinder were used for each blending batch (~ 60 g). Ten grams of blended red beet were measured into a 50 mL beaker. 0.2 M HCl was added to the sample in 0.5 mL increments. The sample was stirred using a spatula after each addition of HCl for 15 seconds and the pH was recorded. HCl was added to the samples and the pH was monitored until the pH was below 1.5. Measurements were completed for 3 replicates and for each replicate, duplicate samples were analyzed.
Buffering capacity was calculated and expressed in mmol H^+/(pH g sample) according to Eq(1) (Tan et. al, 2014).

$$BC = {}^{-\Delta C_a}/_{\Delta pH} \quad (1)$$

where BC indicates buffering capacity, in mmol H^+/(pH g sample); ΔC_a is the amount of H^+ added into 10 g sample, as mmol H+/ g sample; ΔpH represents the corresponding change in pH.

2.6 Particle size measurement

Particle size distribution of the raw, steamed, and pickled red beets were determined using a Mastersize 2000 (Malvern Instruments, Malvern, UK). Blended red beets (prepared for buffering capacity measurements) were used as samples for particle size distribution measurements. Particles were characterized under high dilution conditions by dispersing the samples in a distilled water-filled tank (10% obscuration). Air bubbles can lead to uncertainties in the interpretation of the results since they appear as large particles, so care was taken to eliminate bubbles prior to the addition of samples in the tank. Two samples were analyzed for each preparation method and 10 readings were performed for each sample.

2.7 Statistical analysis

SPSS 18 (SPSS Inc, Chiicago, ILL, U.S.A.) was used for statistical analysis. A statistical data analysis was conducted on the acidity values by analysis of variance (ANOVA) with 2 factors. The factors were type of process (raw, steamed, pickled) and digestion time (0, 2, 4, 8, 12, 16, 24, 48, 72, 96 h). Also differences in buffering capacity, initial acidity, and particle size distribution values as the result of different preparation methods were determined by one-way ANOVA. A post-hoc Tukey's test was conducted to establish the differences among mean values when main effects were significant. Statistical significance was assessed at level of $p < 0.05$.

3. Results and discussion

The titratable acidity of red beets was significantly influenced by preparation method and digestion time ($p < 0.05$). In Table 1, initial acidity values of red beet cylinders for all preparation methods are given. The initial acidity of raw red beets (0.46 ± 0.15 mmol H^+/g dry matter) was the lowest among all initial acidity values. Steamed red beets did not have significantly different acidity prior to digestion (0.65 ± 0.09 mmol H^+/g dry matter) compared to raw red beets. However, the initial acidity of pickled red beets (15.28 ± 7.1 mmol H^+/g dry matter) was significantly higher compared to raw and steamed samples. The reason of this increase was likely due to the acetic acid content of pickled samples.

Table 1: Initial acidity and calculated buffering capacity values of red beets. Different letters within each column represent means that are significantly different ($p < 0.05$).

Preparation Method	Acidity (mmol H^+/g dry matter)	Buffering Capacity (mmol H^+/ pH g)
Raw	0.46 ± 0.15^b	0.035 ± 0.0002^b
Steamed	0.65 ± 0.09^b	0.034 ± 0.0005^b
Pickled	15.3 ± 7.10^a	0.051 ± 0.0300^a

In Figure 2, normalized acidity values of red beet cylinders during 96 hours of in vitro gastric digestion for all preparation methods are shown. For each trial, the acidity after each digestion time was normalized with the initial sample acidity of that digestion trial. When the acid uptake of samples was compared, raw red beets had a higher acid uptake than steamed red beets. In the raw red beets, the acidity increased of 8-fold, from 0.46 ± 0.15 to 3.71± 0.3 mmol H^+/g dry matter over 96 hours of in vitro gastric digestion. In steamed red beets, a 4.6-fold increase of acidity (from 0.65 ± 0.09 to 3.00 ± 0.3 mmol H^+/g dry matter) was observed after 96 hours of in vitro gastric digestion. Previous studies with rice and sweet potatoes have shown that an increase in acidity values were observed during in vitro gastric digestion (Mennah-Govela et al., 2015; Mennah-Govela & Bornhorst, 2016a). Preparation methods (including thermal treatments like steaming or boiling) might change the microstructure of red beets, which may influence the acid diffusion into those samples during gastric digestion. According to previous studies performed on red beets (Mennah-Govela et al., 2016b), the acid uptake during in vitro gastric digestion of raw red beets was greater than boiled red beets, with canned red beets having the greatest acid uptake. These findings may support the results in the current study since both steamed and boiled red beets are exposed to high temperature and have a certain degree of moisture uptake during the steaming and boiling processes. As such, the similar behavior compared to raw red beets may have been due to the thermal treatment. In addition, a previous study (Mennah-Govela and Bornhorst, 2016a) showed that boiling and steaming resulted in different rates of acid diffusion into sweet potatoes, so it may be expected that boiled and steamed red beets may show different rates of acid uptake as well.

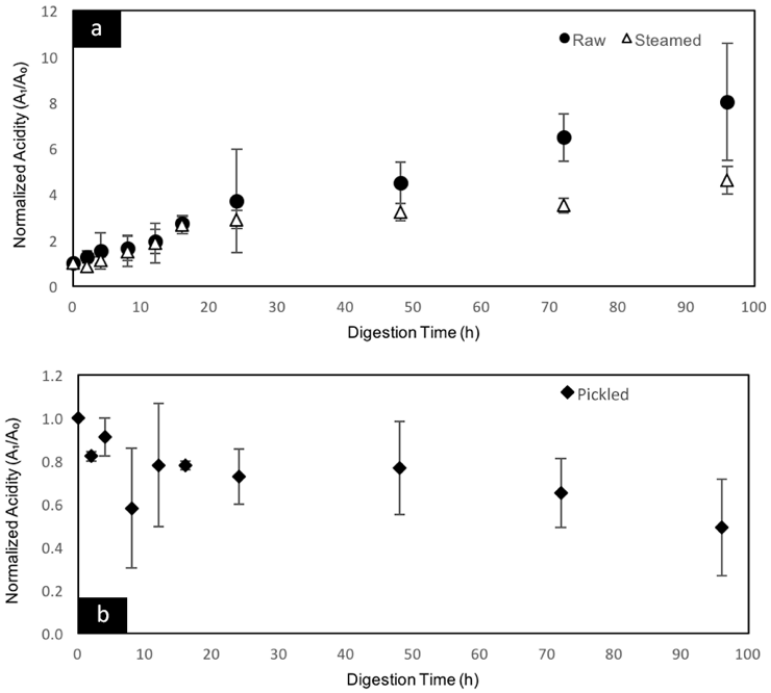

Figure 2: Acidity values of red beets during in vitro gastric digestion: a) raw & steamed red beets, b) pickled red beets. Markers represent average experimental value (n=6) ± standard error of the mean. It should be noted that the graphs have different y-axis scales.

On the contrary, a slow decrease of titratable acidity was observed in pickled beets over 96 hours of in vitro gastric digestion (reducing nearly 50%, from 15.28± 7.1 to 7.50 ± 0.7 mmol H^+/g dry matter). This trend may be due to the initial value of pickled beets' titratable acidity, which is much higher than the acidity of the simulated gastric juice (15.28 ± 7.1 vs 2.42 ± 0.3 mmol H^+/g dry matter). This concentration gradient may promote titratable acidity transfer from the pickled beets toward the gastric juice. Also there might be interference of the acetic acid content in the pickles during the potentiometric titrations. The weak acid content in certain foods may have an adverse effect on acidity measurements after gastric digestion. Similarly, acidity values decreased during 180 min of gastric digestion in apples (var. *Granny Smith*), which contain malic acid (Dalmau et al., 2017). However, the pH values of the pickled beets decreased during 96 hours of gastric digestion (Figure 3), indicating that a higher number of protons, coming from strong acid available in the simulated gastric juice reached the pickled samples.

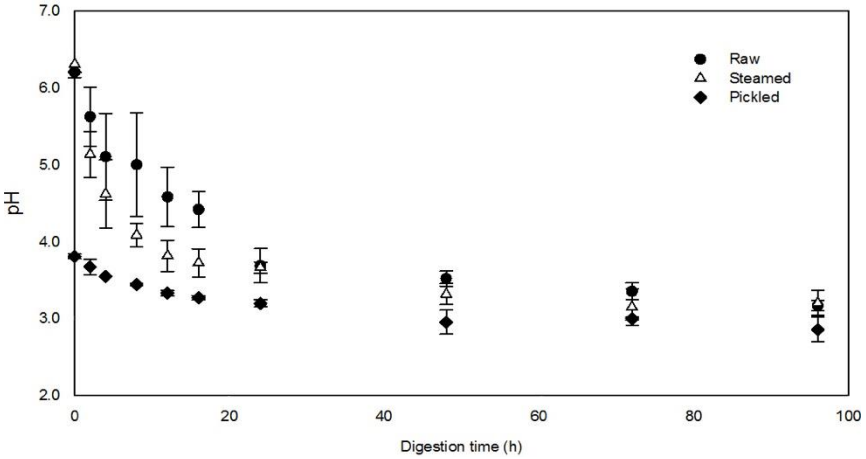

Figure 3: Evaluation of pH changes during in vitro gastric digestion. pH value of cylinders was calculated from average pH values of 3 regions. Markers represent average experimental values (n=6) and error bars represent the standard error of the mean.

The particle size distribution results of raw, steamed and pickled red beets prepared for buffering capacity measurements are given in Table 2. Particle size was the greatest in the raw samples compared to steamed and pickled samples. Pickled red beets had greater particle size values than steamed red beets ($p < 0.05$). The knowledge of particle size distribution of samples is an important parameter to evaluate significance of the buffering capacity measurements. However, it is not related to acid diffusion into red beets during digestion, since the samples used for particle size distribution measurements were not exposed to gastric digestion conditions.

Table 2: Particle size distribution of raw steamed and pickled red beets. d10, d50, d90 are cumulative volume percentiles representing the 10th, 50th and 90th percentile diameters, respectively. Superscripts of different letters within each column represent significant differences ($p < 0.05$).

	Particle Size Distribution (µm)		
Preparation Method	d10	d50	d90
Raw	235.0 ± 29.4^{a}	452.6 ± 5.2^{a}	568.8 ± 0.9^{a}
Steamed	107.4 ± 2.9^{b}	306.4 ± 6.5^{c}	528.7 ± 2.6^{c}
Pickled	109.4 ± 1.9^{b}	339.2 ± 4.9^{b}	542.1 ± 3.8^{b}

The buffering capacity was calculated by Eq(1) and is shown in Table 1. The buffering capacity was significantly influenced by the red beet preparation method ($p < 0.001$), with pickled red beets having the highest buffering capacity. Pickled beets had a buffering capacity of 0.051 ±0.03 mmol H^+/(pH g) compared to raw and steamed beets which had similar buffering capacity values of 0.035 ± 0.0002 and 0.034 ± 0.0005 mmol H^+/(pH g), respectively. After the same blending procedure, raw beets had larger particles compared to steamed beets. This might be the reason for the similar buffering capacity values for raw and steamed red beets. Buffering capacity of raw beets might be significantly higher than steamed ones with similar particle size distribution values. The smaller particle size of samples will create a larger contact surface between H^+ molecules and the sample. In this case, more ions can be released from the red beets and interact with H^+ molecules during the buffering capacity measurements. For this reason, there might be an important influence of particle size on buffering capacity. The sample preparation and particle size distribution should be carefully considered in future studies of food buffering capacity.

Buffering capacity may have an effect on the acid uptake of red beets in the gastric environment after different preparation methods. Higher acid uptake (3.25 mmol H+/g dry matter) was observed in raw beets with respect to steamed beets (2.35 mmol H+/g dry matter) during 96 hours of incubation as a result of a slightly higher buffering capacity (0.035 ± 0.0002 mmol H^+/(pH g) compared to 0.034 ± 0.0005 mmol H^+/(pH g)) due to preparation method. If the particle size distribution of blended raw red beets were the same as steamed and pickled red beets (resulting in modifications to the measured buffering capacity value), the influence of buffering capacity on acid uptake between raw and steamed red beets may have been clearer. Nevertheless, pickled red beets, which have the highest buffering capacity (0.051 ± 0.03 mmol H^+/(pH g)), showed the highest resistance to change its pH (with 0.95 ± 0.19 pH difference value) during 96 hours incubation. The

trend of pH change in raw red beets was similar to steamed red beets, but during in vitro gastric digestion the raw red beets showed slightly higher resistance to pH changes (3.03 ± 0.07 pH difference) with respect to the steamed red beets (3.11 ± 0.14 pH difference).

4. Conclusions

This study has demonstrated that acid uptake in red beets was influenced by preparation method, buffering capacity, and in vitro gastric digestion time. The acid uptake in raw and steamed red beets during in vitro gastric digestion increased over 96 hours. However, pickled red beets did not follow the same trend and had decreasing acidity during in vitro gastric digestion because of acetic acid interference in potentiometric titrations and a greater initial acidity value compared to the gastric juice. However, pH changes in pickled red beets followed a decreasing trend (from pH 3.81 to pH 2.86) similar to raw and steamed samples. Raw red beets had the greatest acid uptake with a similar buffering capacity value compared to steamed red beets, which had the lowest buffering capacity. A possible explanation for this might be differences in particle size between raw and steamed red beets that were tested for buffering capacity. Overall, these results indicate that red beet digestion is influenced by preparation method, which may influence the rate of gastric emptying and nutrient availability. Acid diffusion rate into the food matrix may be important for estimation of food breakdown rate and nutrient release in the later stages of digestion. Development of relationships between these parameters and digestion behavior of foods can be used to develop innovative functional products with targeted properties. Future studies are recommended to correlate differences in acid uptake during digestion with nutrient bioaccessibility and bioavailability.

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