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Short communication

Quantitative comparison of adsorption and desorption of commonly used sweeteners in the oral cavity

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ABSTRACT

Adsorption-desorption properties of different sweeteners in the oral cavity were evaluated using high performance liquid chromatography-based methodology. Three low calorie artificial sweeteners (aspartame, acesulfame potassium and sucralose), one steviol glycoside (rebaudioside A), and high fructose corn syrup (HFCS) were examined and compared with sucrose at pH 3 and 7 in a model beverage matrix. Results indicated that HFCS had the highest adsorption in the oral cavity, followed by rebaudioside A and the artificial sweeteners. The physicochemical interaction between sweeteners and salivary proteins did not affect the adsorption properties significantly as validated from a series of characterization techniques.

1. Introduction

Non-nutritive sweeteners account for more than 60% of the commercial sweetener market (Musto, Lim, & Suslick, 2009). The interest continues to grow due to an increase in consumer preference for healthier alternatives. Currently, there are six artificial sweeteners approved by the Food and Drug Administration on the US market, namely acesulfame potassium (ASK), aspartame (ASP), saccharin, sucralose, neotame and advantame. These sweeteners have been used in a broad range of food products, including diet beverages, frozen desserts and chewing gum. However, they present some disadvantages in comparison to other caloric sweeteners which can hinder consumer acceptance, such as delayed sweetness perception, bitter aftertaste and lingering sweetness (Musto et al., 2009).

Although lingering has been studied in terms of receptor binding mechanisms (Miele et al., 2017; Musto, Lim, & Suslick, 2009; Wiet & Beyts, 1992), the role of sweetener adsorption-desorption in the oral cavity is nearly unexplored. This study investigated the extent of sweetener adsorption and desorption in the oral cavity by a high-performance liquid chromatography (HPLC)-based methodology. Here, we examined and compared adsorption of five commercially used sweeteners in the oral cavity, namely ASK, ASP, rebaudioside A (Reb-A), sucralose and high fructose corn syrup (HFCS) in comparison to sucrose (their chemical structures are shown in Fig. S1). Additionally, the interaction of sweeteners with salivary proteins as well as the possibility of sweetener adsorption saturation in oral cavity was also studied. We developed and described a strategy to analyze the adsorption of five commercially used sweeteners in the oral cavity in comparison to aqueous sucrose solutions. This could provide some insight on quantitative comparison of interaction of the sweeteners with saliva components and lingering effects, given that the sensitivity and aftertaste perceptions are subjective measurements.

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.foodchem.2018.07.221.

2. Material & methods

2.1. Materials

Food-grade citric acid and sodium citrate dihydrate were obtained from Sigma Aldrich (St. Louis, MO, USA); potassium phosphate monobasic and potassium phosphate tribasic were acquired from Spectrum Chemical (New Brunswick, NJ, USA). Food-grade sucrose, ASK (99%, w/w), ASP (97%, w/w), HFCS (77% sugar solids, 55% fructose, 45% glucose), Reb-A (95%, w/w), and sucralose (25%, w/w) were provided by PepsiCo Inc. (Hawthorne, NY, USA). HPLC-grade acetonitrile was purchased from Sigma Aldrich, methanol was obtained from J. T. Baker (Phillipsburg, NJ, USA), acetic acid glacial was from EMD Millipore (Burlington, MA, USA), and sodium acetate anhydrous was from VWR (Randnor, PA, USA).

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Table 1
HPLC operating conditions for each sweetener analyzed in this study.

	Sucrose	HFCS	Sucralose	Reb-A	ASK	ASP
Mobile phase	100% Milli-Q water	100% Milli-Q water	Acetate buffer: DI water: methanol, 20:60:20	Water: acetonitrile, 78:22	Acetate buffer: DI water: methanol, 20:58.5:21.5	Acetate buffer: DI water: methanol, 20:58.5:21.5
Flow rate (ml/min)	0.6	0.6	1.0	1.0	0.9	0.9
Column temperature (°C)	80	80	30	30	30	30
Injection volume (μl)	10	10	50	10	5	5
Run time (min)	15	17	5	16	5	12
Detection	RID	RID	RID	UV (204 nm)	UV (254 nm)	UV (214 nm)

DI – deionized; RID – refractive index (RI) detector; UV – ultraviolet detector.

2.2. Apparatus

An Agilent 1200 LC System (Agilent Technologies, Waldbronn, Germany) equipped with a binary pump SL, a Shodex RI-501 refractive index (RI) detector (single channel), and an Agilent 1100 thermostatted column compartment G1316 were used in this study. Zetasizer Nano ZS from Malvern Instruments Ltd. (Worcestershire, UK) was used to measure the zeta-potential of saliva samples with and without sweeteners. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-Page) in a gel electrophoresis system from Bio-Rad (Hercules, CA, USA) was used to determine the molecular weight distribution of salivary proteins.

2.3. Preparation of standard solutions

Stock solutions consisting of 10% (w/v) of sucrose and HFCS were prepared by dissolving 1 g of the pure compound in 10 ml of 1 mM citrate (pH 3, to simulate an acidified beverage) or phosphate (pH 7, to simulate the human saliva) buffer at room temperature. Stock solutions of the other sweeteners were prepared by dissolving 10 mg of the pure sweetener in 10 ml of buffer (1000 ppm). A series of working standard solutions were prepared with a concentration ranging between 1.25–10 g/100 ml of HFCS, 0.625–5 g/100 ml of sucrose, and 62.5–500 ppm of the other sweeteners by diluting the stock solutions with an appropriate amount of buffer. All stock solutions were prepared freshly every day prior to use.

2.4. Preparation of samples and expectorate collection

Two subjects were asked to maintain a uniform regimen of diet and oral hygiene to reduce variability throughout the study. Briefly, 10 ml of corresponding sweetener solution in buffer was introduced in the oral cavity and gently spread throughout it for 10 s. Expectorate was then collected into 15-ml centrifuge filter units (Amicon Ultra-15, Millipore) with a 10 kDa membrane. This dialysis tube was used to filter the macromolecules from the saliva-sweetener mixture by centrifugation at 7000*g* for 35 min immediately after the expectorate collection. The filtrate was collected into a freeze-drying vial, and the sample volume was recorded. The vials were then freeze-dried overnight in a Labconco FreeZone 2.5 L Bench-top Freeze Dry System (Kansas City, MO, USA). Freeze-dried samples were reconstituted into minimal volume of buffer (8 ml for sucrose and 1 ml for all the others) to have a concentrated solution for analysis. Concentration of the sweetener present in the filtrate was determined by HPLC using the corresponding calibration curve. All measurements were carried out in triplicate.

The same protocol was used to determine the desorption of sweeteners from the oral cavity; i.e., 10 ml of the corresponding sweetener solution in citrate buffer (pH 3) was introduced into the oral cavity and gently spread throughout it for 10 s. After the sweetener expectoration, 10 ml of deionized water was introduced in the oral cavity, and washed thoroughly for 10 s. The washing step was repeated three times. The time interval between the washing steps was 10 s. All the samples were measured in triplicate. A control study was carried out to determine the losses of sweeteners due to freeze-drying process, and the results are shown in Fig. S2. Overall, this process caused less than 10% loss for all the sweeteners studies. The loss of ASP at pH 7 will be discussed later in the text. These results were taken into account when determining the effective amount of sweetener that adsorbed in the oral cavity.

2.5. Quantitative determination by HPLC

Prior to HPLC, a preliminary gel electrophoresis experiment was carried out to assess the filtration of saliva proteins (without sweeteners) by the dialysis tube subjected to centrifugation. This was done by introducing 10 ml of the corresponding buffer into the oral cavity and rinsing thoroughly for 10 s. An SDS-Page gel was run and compared with the un-centrifuged saliva, as shown in Fig. S3. The un-centrifuged saliva sample exhibited several protein bands whereas the filtered saliva in phosphate (A) and citrate (B) buffers did not show any protein bands. This observation concluded that no interference from proteins would occur in the HPLC spectra obtained from expectorates.

Reverse phase HPLC was carried out on a Phenomenex Luna analytical column (100 × 4.6 mm, 3 μm) preceded by Security Guard cartridges (with 3.2–8.0 mm internal diameters) to separate and analyze ASK and ASP. A cation exchange Bio-Rad Aminex HPX-87C column (300 × 7.8 mm, 9 μm) preceded by Micro-Guard Carbo-C Refill cartridges was used to quantify sucrose and HFCS. Reverse phase HPLC was performed on a Cortecs C18 column (100 × 4.6 mm, 2.7 μm) to separate and analyze sucralose and Reb-A.

A summary of the HPLC operating conditions used in this study is provided in Table 1. All runs were carried out under an isocratic regime. All solvents were filtered using a stericup vacuum filtration system prior to use, and samples were filtered in a Celltreat Syringe Filter with a 0.22-μm pore size. The five-minute run time for ASK, 12 min for ASP, 16 min for Reb-A, 17 min for HFCS, and 15 min for sucrose were optimized for determination and quantification of these compounds without any interferents. A simple protocol from Agilent was followed to detect Reb-A (DuBois & Prakash, 2012). ASK, ASP, and Reb-A were detected by ultraviolet (UV) absorption and quantified against known standards. A RI detector was used to detect and quantify sucrose, sucralose and HFCS. Calibration curves were plotted at detection wavelengths of 204 nm for Reb-A, 254 nm for ASK, and 210 nm for ASP. All calibration curves were linear over the concentration ranges tested ($R^2 > 0.99$). Fig. S4 shows the HPLC chromatograms and the corresponding calibration curves of the selected sweeteners and sucrose in citrate buffer (pH 3).

2.6. Characterization of protein binding

SDS-Page was used to determine the degree of interaction between food sweeteners and salivary proteins. TGX Fast Cast acrylamide solutions from Bio-Rad were used to hand-cast 1-mm thick Tris-glycine acrylamide gels. A 10X Tris-glycine running buffer (pH ~8.2) was prepared by mixing water, Tris base, glycine, and sodium dodecyl sulfate in a ratio of 100:3:14.4:1, which was further diluted 1 in 10 for running gel electrophoresis. β-mercaptoethanol was used as the