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ORIGINAL CONTRIBUTION

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Intra-oral lactic acid production during clearance of different foods containing various carbohydrates

Produktion von Milchsäure in der Mundhöhle während des Verzehrens von verschiedenen kohlenhydrathaltigen Nahrungsmitteln

Summary Oral carbohydrate clearance and acid production were monitored over a two hour time period following the ingestion of six foods (chocolate bar, potato chip, oreo cookie, sugar cube, raisin and jelly bean). Each food was evaluated intra-orally in eight volunteers. Oral fluid samples were obtained from each volunteer at 30 min intervals at five different tooth sites using absorbent paper points. The oral fluid samples were analyzed qualitatively and quantitatively for carbohydrates and organic acids using high performance liquid chromatography. Data obtained for each food were averaged and subjected to statistical analysis. The quantity of lactic acid produced 30 min after ingestion was found to be in the following order: (highest) raisin > chocolate bar > sugar cube > jelly bean > oreo cookie > potato chip (least). Two hours after food intake the order had changed significantly: potato chip > jelly bean > sugar cube > chocolate bar > oreo cookie > raisin. A direct linear relationship existed between lactic acid production and the presence of glucose. In foods containing cooked starch prolonged clearance occurs via the intermediate metabolites maltotriose, maltose and glucose. Results indicated that the term 'stickiness', when used to label certain foods such as jelly bean and chocolate bar, should be used cautiously. Foods containing only cooked starch or cooked starch and sugars can be considered as 'sticky', since glucose arising from their intra-oral degradation contributed to acid production over prolonged periods of time.

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Zusammenfassung Nach dem Genuß von sechs zucker- und/oder stärkehaltigen Nahrungsmitteln (Schokoladenriegel, Kartoffelchips, gefüllter Keks, Würfelzucker, Rosinen und Geleebohnen) wurde der Kohlenhydratabbau und die Säureproduktion im Mund über zwei Stunden verfolgt und analysiert. Jedes Nahrungsmittel wurde an acht Probanden in Zeitintervallen von 30 min untersucht. Die Freisetzung von Glukose und die Bildung von Milchsäure im Mund wurden mittels HPLC-Analyse qualitativ und quantitativ über zwei Stunden verfolgt und statistisch ausgewertet. Innerhalb der ersten 30 Minuten wurde Milchsäure in folgender Rangordnung produziert: (höchster Wert) Rosinen > Schokoladenriegel > Würfelzucker > Geleebohnen > gefüllter Keks > Kartoffelchips (niedrigster Wert); nach 120 Minuten änderte sich diese Rangordnung wie folgt: Kartoffelchips > Geleebohnen > Würfelzucker > Schokoladenriegel > gefüllter Keks > Rosinen. Die Menge der produzierten Milchsäure war linear abhängig von der Menge der von den Speisen freigesetzten Glukose. Gekochte Stärke wurde im Mund über Maltotriose und Maltose zu Glukose abgebaut. Klebrige (zuckerhaltige) Nahrungsmittel (Schokoladenriegel) verlassen die Mundhöhle schneller als Nahrungsmittel, die gekochte Stärke enthalten (Kartoffelchips); letztere produzieren daher über einen längeren Zeitraum (2 h) im Munde mehr Milchsäure.

Key words Cooked starch – diet – oral breakdown – glucose – HPLC analysis – lactic acid – oral fluid – organic acids – sugars – saliva

Schlüsselwörter gekochte Stärke – Nahrung – oraler Abbau – Glukose – HPLC-Analyse – Milchsäure – Mundflüssigkeit – organische Säuren – Zucker – Speichel

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Introduction

Several in-vivo and in-vitro methods have been previously introduced for the estimation of intra-oral acid production from carbohydrates. These methods include harvesting of pooled plaque, touch electrodes, intraoral enamel demineralization/remineralization test and the telemetric plaque pH monitoring method, and have been described (1, 2, 3, 4) and discussed (5, 6, 7). Most of these methods are not comparable and provide only qualitative data (8). Some studies reported on the 'short-term' (up to 30 min) plaque acid production following ingestion of reference foods by human subjects (9, 10). The present study used a method (11) based on high performance liquid chromatography (HPLC) to qualitatively and quantitatively follow oral food clearance and intra-oral acid production of some foods, containing various carbohydrates, perceived as 'sticky' and 'non-sticky' (12) for up to two hours after ingestion. Some of the preliminary results using this method were abstracted earlier (13, 14, 15).

Materials and methods

Test foods

The following foods, containing different types or mixtures of carbohydrates, were each tested in eight volunteers: Milky way bar (Mars Inc., Hackettstown, NJ); potato chip (Wise Foods, Borden Inc., Columbus, OH); oreo chocolate sandwich cookie (Nabisco Brands Inc., East Hanover, NJ); sugar cube (Domino, Amstar Sugar Corp., New York, NY); raisin (Sun-Maid Growers of California, Pleasanton, CA); jelly bean (E.J. Brach & Sons Inc., Chicago, IL).

Collecting of clinical specimens

Eight healthy dental student volunteers were offered the test foods in single portions of approximately 25 g. The volunteers were not preconditioned, i.e., they received no instructions on oral hygiene and eating habits preceding the experiment. However, they were advised to refrain from food intake for two hours before commencement of the initial dental examination. Oral fluid samples were taken from 5 sites, using one sterile paper point (No. 2717, Johnson & Johnson) for each of the following oral sites: interproximal surface of the last two teeth in the lower right quadrant, interproximal surface of the last two teeth of the upper left quadrant, occlusal surface of the last tooth in the lower left quadrant, occlusal surface of the last tooth in the upper right quadrant, and on the lingual surface of the last tooth in the lower right quadrant. Oral fluid samples were collected at times 0, immediately before eating the test food ('base line'), and at 30, 60, 90, and 120 min after ingestion of food. The vials with the five pooled paper points were kept frozen at -80 °C (Revco Freezer) until extraction.

Extraction of carbohydrates and organic acids

To five pooled paper points (containing approximately 60 mg combined oral fluid) 1.0 ml of dist. water was added; the vial was shaken for 10 sec., and again for 10 sec. after a 20 min time period. The supernatant was filtered through a 0.2 µ membrane filter (Acro LC13, Gelman Sciences), and then 80 µl portions were analyzed using high performance liquid chromatography (HPLC).

HPLC

A liquid chromatographic system was constructed from commercially available components and interconnected with stainless steel capillary tubing (1/16" OD x 0.007" ID; Alltech Associates, Deerfield, IL) to keep the dead volume to a minimum. This new HPLC technique was discussed earlier (16).

Pump: A Liquid Chromatograph Series 3B dual pump (Perkin-Elmer Corp., Norwalk, CT) was used. The mobile phase used was 0.01 N degassed and nitrogen purged sulfuric acid (Fisher Scientific Co., Fair Lawn, NJ). The system was run at a flow rate of 0.6 ml/min, which generated a pressure of 700 to 800 psi (maximum pressure set to 1,000 psi).

Column: A 300 x 7.8 mm ion exclusion column Aminex HPX-87H (Bio-Rad Laboratories, Richmond, CA) was employed. The main column was preceded by a 40 x 4.6 mm guard column of the same type. The columns were maintained at 30 °C in a Column Oven LC-100 (Perkin-Elmer Corp.). On-stream injections of 80 µl per sample were made through a loop injector (Rheodyne, 175 µl sample loop).

Detector: A Differential Refractometer R401 (Waters Associates, Morristown, NJ), set to positive polarity and run at an attenuator setting of 32x was used. The refractive index of the eluate was determined at room temperature. The reference cell was calibrated with 0.01 N sulfuric acid.

Standardization, data collection and integration: The concentration of standard compounds was 1 µg/1 µl. For the standardization curve 5, 10 and 15 µl of the following compounds were injected: maltotriose, maltose, sucrose, lactose, glucose, fructose, lactic acid, formic acid, acetic acid, propionic acid and ethanol (Sigma Chemical Co., St. Louis, MO). After injection of the standard compound sample the integrator interface was activated. Obtained retention times (and corresponding HPLC peak

area) for 10 µg glucose were 9.667 min (462,248) and for 10 µg lactic acid were 13.947 min (46,956). Each HPLC analysis of standard compounds (11) or extracted oral fluid sample (injection volume 80 µl) was run for 26 min. The chromatographic data were collected and simultaneously integrated using an Omega-2 (Perkin-Elmer, Omega-2 software V 2.5) computerized (Epson Equity I+ computer with interface board and high resolution Amdek monitor) data station connected to an Epson LQ-500 printer. The sensitivity of the computerized integration method allowed for a detection sensitivity of 0.1 µg substance per HPLC assay (80 µl).

Statistics

Data were analyzed using the statistical DOS package SPSS/PC+, V 4.0 (SPSS Inc., Chicago, IL). Subjective ratings were compared by analysis of variance. Lactic acid data of the different foods were analyzed by oneway analysis (multiple range test) using Tukey-HSD and Scheffe procedures (ranges for the 0.05 level).

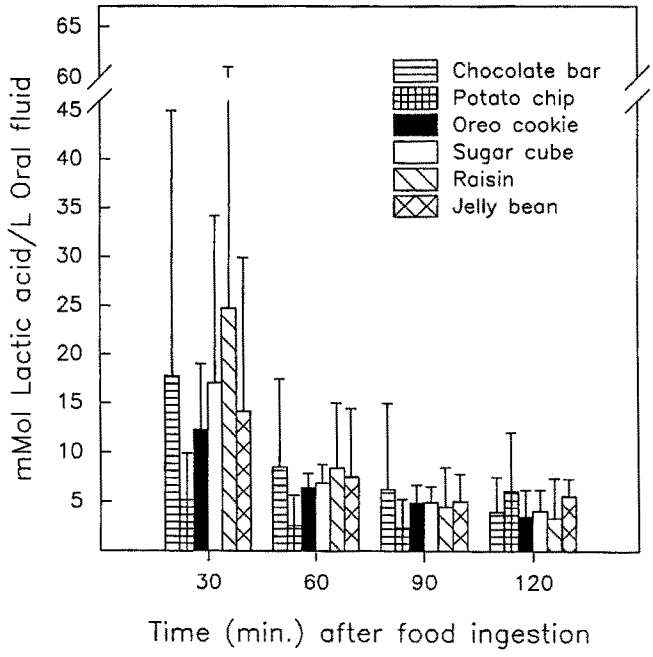

Fig. 1 Lactic acid production from various foods during 120 minutes of oral clearance

Results

Chocolate bar

The chocolate bar is composed of different carbohydrate sweeteners, but does not contain cooked starch. The glucose clearance proceeded in an anticipated pattern. As expected, the highest glucose concentration was found 30 min after eating the food, and then slowly cleared the oral cavity within 120 min after ingestion. Lactic acid levels were highest 30 min after food intake, and subsequently decreased within 120 min (Table 1, Fig. 1 and Fig. 2).

Potato chip

The potato chip is composed mainly of cooked starch, but does not contain any carbohydrate sweeteners. The cooked starch was slowly hydrolyzed into glucose, reaching a concentration peak 90 min after the outset of the study. Glucose levels were still higher after 120 min than at the outset of the experiment. This is reflected in a relative constant lactic acid level at all times tested after the initial ingestion of potato chips. The concentration of lactic acid reached its highest level 120 min after in gestion of the food (Table 1, Fig. 1 and Fig. 2).

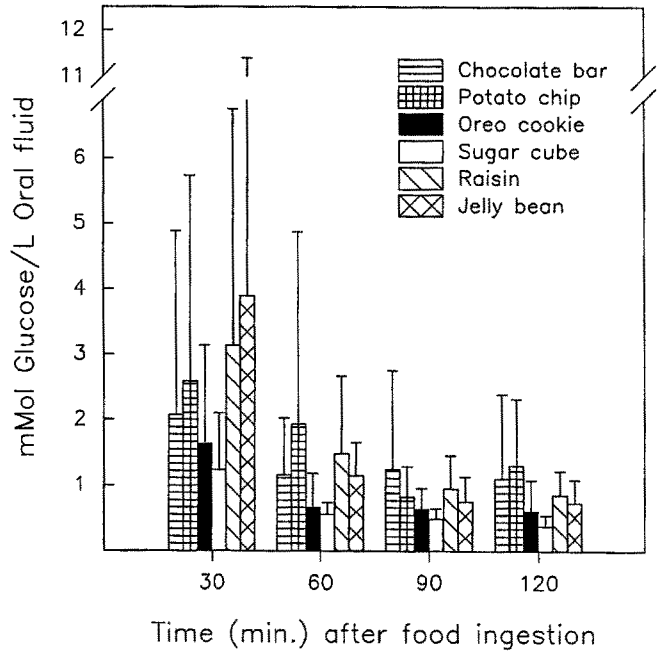

Fig. 2 Formation of glucose from various foods during 120 minutes of oral clearance

Oreo cookie

The oreo cookie contains both carbohydrate sweeteners and cooked starch. Glucose levels were highest 30 min after ingestion and then remained almost constant for two hours of the experiment. Lactic acid concentrations reached a peak 30 min after ingestion and slowly decreased until termination of the experiment (Table 1, Fig. 1 and Fig. 2).

Table 1 Total production of glucose and lactic acid derived from various foods during two hours of oral clearance*

Food	Clearance time (min)**		Glucose Mean	Glucose Std Dev	Lactic acid Mean	Lactic acid Std Dev
Chocolate bar	**average**		**1.290**	**1.576**	**7.771**	**13.984**
	after	30	2.067	2.816	17.799	27.208
		60	1.165	0.853	8.519	8.939
		90	1.248	1.509	6.233	8.733
		120	1.112	1.284	3.974	3.550
Potato chip	**average**		**1.567**	**2.026**	**3.518**	**4.428**
	after	30	2.589	3.143	5.149	4.751
		60	1.928	2.937	2.565	3.092
		90	0.834	0.459	2.385	2.892
		120	1.305	1.014	6.085	5.897
Oreo cookie	**average**		**0.815**	**0.838**	**5.412**	**5.197**
	after	30	1.637	1.499	12.267	6.753
		60	0.672	0.514	6.360	1.485
		90	0.649	0.316	4.903	1.776
		120	0.620	0.464	3.532	2.641
Sugar cube	**average**		**0.622**	**0.504**	**6.676**	**9.304**
	after	30	1.238	0.856	17.046	17.075
		60	0.569	0.179	6.865	1.903
		90	0.499	0.158	4.950	1.606
		120	0.387	0.163	4.113	2.113
Raisin	**average**		**1.510**	**1.901**	**8.799**	**17.936**
	after	30	3.140	3.618	24.744	36.312
		60	1.495	1.175	8.449	6.592
		90	0.959	0.499	4.507	3.961
		120	0.862	0.360	3.380	4.002
Jelly bean	**average**		**1.475**	**3.426**	**7.775**	**8.216**
	after	30	3.893	7.504	14.126	15.735
		60	1.157	0.507	7.522	6.952
		90	0.767	0.373	5.063	2.752
		120	0.746	0.348	5.586	1.757

* Glucose and lactic acid data for each food averaged from 8 volunteers; concentrations expressed as mMol/L oral fluid.
** **Average** glucose and lactic acid data for the duration of 120 min after food intake (sampling at time 0 excluded).

Sugar cube

The sugar cube was chosen because it is composed of a single carbohydrate sweetener, sucrose. During the experiment, the highest concentrations of glucose was observed 30 min after intake. After 30 min the glucose concentration slowly decreased. Lactic acid levels slowly decreased over the duration of the experiment, but were on the average higher than with the oreo cookie (Table 1, Fig. 1 and Fig. 2). Individual clearance data for glucose and lactic acid are shown in Table 3.

Raisin

The raisin contains different carbohydrate sweeteners. Clearance of glucose proceeded slow but at a steady pace; its concentration reached a peak at 30 min, then slowly decreased, but at 120 min there still remained approximately 30 % of the initial concentration level of that at 30 min. The concentration of lactic acid was highest at 30 min and then slowly decreased for the duration of the experiment (Table 1, Fig. 1 and Fig. 2).

Jelly bean

The jelly bean contains different carbohydrate sweeteners. During oral clearance it produced the highest concentration of glucose 30 min after intake. This was higher than with any other food tested. The concentration of lactic acid was highest 30 min after ingestion, but then its production slowly diminished. However, the lactic acid concentration at the 120 min sampling period was the second highest. At the duration of the experiment glucose and lactic acid levels were lower than with the potato chip (Table 1, Fig. 1 and Fig. 2).

Table 2 'Base line' data of carbohydrates and acids in oral fluid before food ingestion*

Compound (mMol/L oral fluid)	Mean	Std Dev
Maltotriose	0.033	0.160
Maltose	0.013	0.045
Sucrose	0.007	0.030
Lactose	**	
Glucose	0.810	0.692
Fructose	0.073	0.160
Lactic acid	2.273	3.516
Formic acid	5.418	3.208
Acetic acid	5.443	3.686
Propionic acid	0.378	0.642
Ethanol	0.033	0.230

* mean from 48 volunteers.
** compound not analyzed in this series.

The 'base line' data obtained and averaged from 48 volunteers are summarized in Table 2. The concentration of glucose was found to be 0.810 mMol/L oral fluid. There was always a small amount of lactic acid (2.273 mMol/L oral fluid) present. Formic and acetic acid were consistently found in these samples (5.418 and 5.443 mMol/L oral fluid, respectively).

Discussion

To obtain a representative oral fluid sample, it was decided to obtain samples from five different sites rather than from one. There is little evidence that unstimulated saliva is well mixed in the mouth, and buffering capacity of saliva from the different glands significantly varies (17). Other investigators reported that glucose clearance varied at the different sites in the oral cavity (18). The analytical HPLC method is sensitive enough to pick up carbohydrates during the first examination of the volunteers, possibly from previous food intake. This is evident by examining the 'base line' data (Table 2). The high standard deviation also reflects the fact that some volunteers had a higher salivary flow than others. Ingestion of one and the same test food varied from 3 to 20 min among the volunteers; some individual ingestion times are shown in Table 3, using the test food sugar cube. Other factors contributing to the high standard deviation between individuals may include variations in amount of dental plaque at the five sampling sites.

Small amounts of glucose were always found in oral fluid before ingestion of the test foods. Other investigators found glucose (400 mg/L) in whole saliva (19) and detected glucose (5 to 25 μMol/L) in parotid saliva before carbohydrate intake (20). Before carbohydrate ingestion the observed concentration of organic acids (Table 2) were unexpectedly high. Using a different analytical method, other investigators found similar concentrations of organic acids in starved plaque fluid (21, 22). These acids could have originated either from metabolism of carbohydrates in food remnants or from soluble plaque storage polysaccharides, such as levan. After ingestion of the test foods formic and acetic concentration levels dropped whereas lactic acid levels increased significantly (Table 1). The concentrations of formic, acetic and propionic acids are not reported here because they contribute only 5 % or less of the total amount of acids produced during 'long-term' (up to one to two hours) carbohydrate plaque metabolism (23, 24, 25). Two hours after food ingestion lactic acid levels approached 'baseline' levels again (Table 1); a similar observation was made by other investigators (26, 27).

Significant differences were observed among the various foodstuffs tested, especially in regard to their oral retention time. Retention times, or 'stickiness', of the

Table 3 Individual clearance data of glucose and lactic acid after ingestion of the food sugar cube*

		Glucose				Lactic Acid			
	Ingestion	Clearance Time (min)				Clearance Time (min)			
Volunteer	Time**	30	60	90	120	30	60	90	120
1	2.67	0.741	0.546	0.492	0.483	9.608	6.226	3.354	3.576
2	7.83	1.318	0.676	0.586	0.554	10.570	8.069	5.365	5.219
3	8.58	1.455	0.454	0.426	0.182	51.297	8.005	6.923	4.664
4	7.67	0.478	0.230	0.252	0.287	9.188	3.243	5.043	3.966
5	9.83	3.196	0.805	0.644	0.257	35.982	7.869	7.057	6.720
6	7.17	1.119	0.510	0.333	0.464	8.105	9.391	5.252	6.049
7	19.25	0.719	0.607	0.540	0.246	8.785	6.494	2.442	0.000
8	12.00	0.877	0.723	0.723	0.620	2.834	5.624	4.166	2.713
average	9.38	1.238	0.569	0.499	0.387	17.046	6.865	4.950	4.113

* Concentrations expressed as mMol/L oral fluid.
** Time required in min to chew 25 g food and swallow bolus.

different foodstuffs profoundly effect bacterial acid production in the oral cavity (12). The effect of the different foods on retention and acid production may be explained as follows: in general, foods containing starch are insoluble in saliva, as such they take longer to clear than those foods containing only sugar. During clearance starch foods provide substrate for bacteria leading to increased and prolonged organic acid production. Foods containing mono- and disaccharides but no starch clear the oral cavity more rapidly; maximum acid production occurs 30 min after ingestion. Beyond 30 min the concentration of acids diminishes rapidly, similar to the classical Stephan curve (28). More complex foods that are composed of various carbohydrates behave differently in regard to clearance and acid production. Such foods are more difficult to evaluate, and they follow a pattern of clearance and acid production that places them in between the two other food categories.

Results from testing the oral clearance and acid production of the six foods (Table 1) indicated that the amount of acid produced 30 minutes after food intake was: (lowest) potato chip < oreo cookie < jelly bean < sugar cube < chocolate bar < raisin (highest). 120 min after food ingestion the order had changed significantly: raisin < oreo cookie < jelly bean < sugar cube < chocolate bar < potato chip. Comparison of the total amount of lactic acid produced by the different foods over the 2-hour time period yielded the following order (Table 1): (highest) raisin > jelly bean > chocolate bar > sugar cube > oreo cookie > potato chip (lowest). A direct linear relationship existed between the presence of glucose and lactic acid production. The predominantly starch-containing food (potato chip) generated its peak in acid production between 90 and 120 minutes. The foods containing carbohydrate sweeteners only (chocolate bar, sugar cube) displayed a marked decrease in acid production after the first 30 minutes. The complex foods composed of both sugars and starches (cookie and jelly bean) were more difficult to evaluate; they followed a pattern of clearance and acid production that differentiated them from the other tested foods. This difference was also significant when compared to the starch-food (potato chip). Statistical analysis of total lactic acid production for the duration of the experiment (120 min) indicated that the raisin produced the largest amount of acid whereas the potato chip produced the least amount. Oneway analysis using the multiple range test (Tukey-HSD procedure) on the variables 'lactic acid' by 'time' produced significant differences (ranges for the 0.05 level) for the foods 'oreo cookie' (30′ vs. 60′, 30′ vs. 90′, 30′ vs. 120′) and sugar cube (30′ vs. 90′, 30′ vs. 120′).

No foodstuff can be considered a 'pure' food; most sweet foodstuffs are composed of a multitude of sugars and are very often combined with starch and other compounds. There is also an intricate relationship between the individual sugars present in a food and oral clearance time. Some sugars stimulate saliva flow at a different rate and therefore, this may determine the rate of foods clearance in the oral cavity. It was observed that among the volunteers there was a significant difference in salivary flow. While the 'sugar cube' portion in one volunteer ('wet mouth') cleared the oral cavity within 2 to 3 min, in another volunteer ('dry mouth') the same portion needed 19 to 20 min to clear (Table 3). In the latter case this resulted in a delay in peak lactic acid production. This is also reflected in the higher standard deviation of these data (Table 1). From previous studies it is known that monosaccharides interfere with the *Streptococcus mutans*-sucrose-system that less lactic acid is produced (25). This would imply that a food containing solely sucrose would contribute to a larger amount of acid produced by plaque bacteria than a food that contains besides sucrose also monosaccharides, e.g., fructose or glucose. All these factors must be considered before a meaningful clinical interpretation of the results obtained in this study can be made.

Moreover, results indicate that the starch containing foods gave rise to significant amounts of glucose about 30 min after ingestion. Production of glucose apparently occurs by the action of α-amylase on cooked starch via the intermediate degradation products maltotriose and maltose (29, 30, 31, 32).

A more detailed clearance study on foods containing cooked starch is indicated. One would expect to be able to obtain two important additional pieces of information. First, a detailed knowledge of the mechanism of action and a time-line for intra-oral starch breakdown (maltotriose → maltose → glucose). Second, with such data one would be able to hypothesize the relative cariogenicity of different types of starches (corn, potato, wheat) that are ingested commonly.

The most significant finding of this study is the demonstration of a marked difference between oral clearance time of foods containing primarily carbohydrate sweeteners and those containing primarily cooked starches. The acid production from predominantly sugar-containing foods diminishes over a two-hour time period, whereas the acid production from predominantly starch-containing foods continues unreduced. This would indicate, as reported also by other investigators (12), that starch-containing foods, which consumers commonly perceive as 'non-sticky', appear more retentive than foods containing primarily sugars, i.e., the foods commonly perceived by consumers as 'sticky'. This consumer perception about sticky foods has to be reevaluated, including the perception that the more sugar is present in a particular food the more acid will be produced from it by the oral bacteria. The latter concept needs to be explored further.

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