SNACKING INTERVENTIONS DIFFERENTIALLY INFLUENCE SALIVA, SALIVARY ALPHA AMYLASE ACTIVITY, AND SENSATION

By

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For Mom, Dad, Joe, Tori, and Matt

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Thanks to all my lab members in the SPIT Lab for their assistance completing the study.

Thank you to Dr. Cordelia Running for her continued guidance and support.

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ABSTRACT

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Human saliva contains the enzyme alpha amylase, which greatly influences many facets of human health such as digestion, absorption of nutrients, and the sensory perception of certain foods. However, the complex relationships between chewing behavior, food texture preference, and salivary amylase require further investigation. In this study, we aim to observe salivary alpha amylase through a simple assay using pudding, and to examine whether salivary amylase activity relates to diet, the sensory properties of starchy foods, or mouth behavior. We hypothesized that the pudding/salivary amylase activity assay would show more activity (less pudding remaining) 1) at the end of the high dietary starch intervention week, with little or no change from baseline to the end of the low dietary starch intervention week and 2) for people with greater baseline starch consumption compared to less baseline starch consumption. A counter-balanced, crossover design was implemented for the study. 34 participants (11 Men, 23 Women, 0 Other) completed study tasks, consisting of a 3-day dietary recall, 2 separate weeks of dietary intervention consisting of high starch or low starch snacks, and 4 research visits. These research visits included participant taste and smell acuity assessments, sensory ratings of the study foods, a mouth behavior typing test, and our salivary amylase activity assay that determined flow rate of a mixture of participant saliva and starch-containing ready-to-eat pudding. After our higher and lower starch snack interventions, we saw minimal evidence of changes to salivary amylase activity in our assay; the only trend we observed was opposite our expectation (less amylase activity after the low starch intervention). However, we did observe mouth behavior grouping tended to associate with sensory ratings that validate the premise of the mouth behavior typing tool we utilized. Ultimately, more work on the consistency and usefulness of the salivary amylase activity assay will need to be conducted if it is to be utilized for research purposes, but our data do help validate the concept that different people prefer foods due to their preferred methods of orally manipulating foods.

CHAPTER 1. LITERATURE REVIEW

1.1. Introduction

Obesity and related diseases are a global health burden. Strategies to reduce energy intake are needed, and one area that many people focus on is reducing excess intake of simple carbohydrates: sugars and starch. Foods high in simple carbohydrates, though, are often palatable and convenient. To shift dietary patterns to improve health, the sensory properties of foods need to be considered. The role of starch in food sensory properties is complex and includes the phenomena of sweet taste as well as textural aspects that change as starchy food is processed in the mouth. Even with sugars, replacing the sugar's sweetness in lower sugar foods is only part of the formulation challenge when considering the textural and physical stability of the food. The roles of starches in food texture can be even more complex.

This narrative review will cover an overview of starch structure, a brief background on oral physiology and origins of salivary amylase, current knowledge of how salivary amylase influences sensory properties of foods, chewing behavior and its relation to food texture, and finally, will identify gaps in the research that inspired our study's concept.

1.2 An Overview of Starch Structure

Plant foods provide the adult diet with its main source of carbohydrates, one of the 3 macronutrients for human diets. Thus, understanding carbohydrates and their constituents is essential for creating advancements in this area of research and understanding the relationship between starch and human nutrition. Starch, a plant polysaccharide, is composed of the glucose polymers amylose and amylopectin. The amylose subunit is characterized by unbranched alpha (1→4) glycosidic bonds, several thousand D-glucose units long. By contrast, amylopectin is highly branched alpha (1→6) glycosidic linkages and contains hundreds of thousands of D-glucose units. Starch granules, cellular structures in which plants cells store their starch, vary in size and shape depending on plant source. The granules also display a unique birefringence. Birefringence refers to the optical property of splitting a ray of light into two distinct rays of light (Bragg & Pippard, 1953). Such phenomenon exhibited by these granules is indicative of highly branched molecular linkages (Baker & Whelan, 1950). These linkages allow starch granules to

retain their shape and composition, but in the presence of certain enzymes and at proper temperature and pH, the starch becomes subject to digestion into smaller oligomers and monomers. This breakdown of starch begins promptly once starch is exposed to saliva.

1.3 Oral Structure and the Origins of Salivary Amylase

Saliva is a complicated biofluid affecting many facets of foods and beverages, including physical breakdown of foods, enzymatic digestion, the formation of a bolus of food to aid in swallowing, control of oral pH, lubrication and antimicrobial capability, and numerous other functions. Saliva is secreted by a variety of major and minor glands in the mouth. It functions to keep the mouth moist, aids in mastication, and helps with taste, among other functions. Whole saliva is a mixture of secretions from the parotid, submandibular, sublingual, and minor salivary glands. Saliva is almost entirely composed of water; the remaining portion consists of various proteins, amino acids, and enzymes, such as salivary alpha amylase. Amylase makes up roughly 30% of all protein found in saliva (Bennick, 1982), but more recent studies have shown this concentration to vary dramatically across individuals (Crawford & Running, 2020; Davis & Running, 2021).

Amylase is a digestive enzyme that is secreted mainly from parotid salivary glands as well as the pancreas. It can be found in small levels in other tissues throughout the body and as serum amylase in the bloodstream (Pieper-Bigelow et al., 1990). Pancreatic amylase is secreted from the exocrine pancreas into the duodenum, and functions in the gastrointestinal tract to hydrolyze starch and glycogen – the stored forms of glucose in plants and animals, respectively (Goldberg & Spooner, 1975). Amylase can be classified as alpha, beta, or gamma amylase, dependent upon which of the glycosidic bonds of amylose or amylopectin that it affects (Jacobsen et al., 1972). While alpha amylase acts on locations throughout the starch chain to hydrolyze alpha (1→4) glycosidic linkages, beta and gamma amylase both function at the non-reducing end of the polysaccharide. Beta amylase is only able to hydrolyze alpha (1→4) glycosidic bonds, whereas gamma amylase can cleave both alpha (1→4) and alpha (1→6) glycosidic bonds (Jacobsen et al., 1972). In humans, sucrase-isomaltase is the only enzyme capable of cleaving alpha (1→6) glycosidic bonds (Conklin et al., 1975). Beta amylase is found in plants and microbes, gamma amylase is present in plants and animals, and alpha amylase is present in plants, microbes, animals, and is the only classification found in humans (Goldberg & Spooner, 1975). Human

saliva contains the enzyme alpha amylase, which breaks down amylose and amylopectin from starch into simplified carbohydrates such as maltose, maltotriose, and other oligosaccharides via hydrolysis of the alpha (1→4) glycosidic linkages. These smaller carbohydrate units are shorter chains of dextrins and maltose and can more easily move through the digestive system for further digestion and absorption.

Saliva composition and volume varies between individuals and is influenced by factors such as stimulation method, age, gender, circadian rhythm, overall nutrition, and environmental exposures (Dawes et al., 2015). The degree to which humans can be conditioned to salivate is still debated by the salivary research field, but recent work shows that this phenomenon can be established acutely within a single research session (Kershaw & Running, 2018). Prior research found significant positive differences in the volume of saliva secretion following conditioned stimuli of ringing bells or presenting participants with food (Brothers & Warden, 1950). They also saw increased amylase activity within the saliva during the conditioned response phase as compared to the unconditioned response phase for both the auditory (bells) and visual (food cues) stimuli (Brothers & Warden, 1950). Thus, conditioning impacts salivary secretion and amylase activity.

Previous research has also demonstrated the effects of stimulating saliva via chewing, taste, and starch content on salivary flow and composition. Froehlich, Pangborn, and Whitaker aimed to determine the effects of aqueous solutions of starch, sucrose, sodium chloride, and citric acid on parotid salivary flow (1987). The researchers were also interested in the effects of oral stimulation with these solutions on concentrations of protein, alpha amylase, and electrolytes in saliva (Froehlich et al., 1987). Interestingly, slight increases in salivary flow rate, protein secretion rate, and amylase secretion rate by concentration for salt and sucrose solutions were observed, as well as strong increases for the citric acid solution. Higher salivary protein content with starch stimulation was observed, but starch minimally effected flow rate. Subjects in this study with higher concentrations of protein and amylase in their saliva demonstrated lower flow rates (Froehlich et al., 1987). Similar research has been conducted using parotid saliva to measure salivary flow, with this experiment incorporating mastication of celery, bread, and parafilm wax (Mackie & Pangborn, 1990). The researchers found that salivary flow was independent of chewing rate, but chewing celery and bread instigated higher salivary flow rates than chewing parafilm wax alone. Notably, mastication increased the rate that saliva was

secreted, but the concentrations of protein and salivary alpha amylase were unaffected. Interestingly, bread mastication resulted in the highest rate of alpha amylase secretion, demonstrating the effect of a starch stimuli on salivary flow and alpha amylase secretion (Mackie & Pangborn, 1990). Newer research has investigated the relationship between salivary gland sizes and unstimulated whole saliva flow rates or chewing-stimulated whole saliva flow rates (Ono et al., 2007). Both unstimulated and chewing-stimulated whole saliva flow rates positively correlated with the size of the parotid and submandibular gland sizes, though notably, the effects were stronger with chewing-stimulated whole saliva flow rates. These findings suggest a correlation between salivary gland size and flow rate with chewing (Ono et al., 2007). Such variability of salivary flow rate, gland size, and amylase and protein concentration between individuals changes salivary composition, which can impact taste, flavor, texture perception, oral processing, and health.

However, much like saliva composition and function varies from person to person, individual salivary amylase activity varies in individuals depending on their *AMY1* expression. *AMY1* is the gene responsible for the production of salivary amylase, while *AMY2* is the gene responsible for the production of pancreatic amylase (Karn et al., 1974). Humans can have highly varied copy numbers of the *AMY1* gene, and literature suggests that dietary starch may correlate with how many copies an individual possess (Perry et al., 2007). The variation in the number of copies of the gene a person has is referred to as copy number variations. Usually, more copies (or a higher copy number variant) results in more AMY1 protein, and consequently, more amylase activity (Mandel et al., 2010). However, research has demonstrated that this pattern is not always the case (Mandel & Breslin, 2012). Individuals with high concentrations of amylase in saliva as well as high copy numbers of *AMY1* can rapidly hydrolyze a starch thickened solution *in vitro* (Mandel et al., 2010). Tracking the digestion of starch during oral manipulation demonstrates that individuals with higher copy numbers of the *AMY1* gene experienced faster decreases in the viscosity of starch thickened solutions (Mandel et al., 2010). This variation in copy numbers causes variation in the number of genes encoded into the AMY1 protein and the activity of this protein, leading to varied amylase concentration. Thus, copy number variations give insight into an individual's amylase levels. However, the enzymatic *activity* of salivary alpha amylase requires more research; the copy number variations of *AMY1* as well as the concentration of amylase present in a person may not perfectly predict enzymatic activity.

1.4 The Influence of Salivary Amylase on Sensory Properties of Foods

Salivary alpha amylase not only influences the digestion of starchy foods, but also has impact on the sensory properties of food. Hydrolysis of starch into maltose and dextrins is associated with the "melt-in-the-mouth" sensation of starch containing foods, influencing food sensory attributes like thickness and creaminess (Bridges et al., 2017). Researchers have observed high variance in perceived thickness of starch thickened puddings, but not for control yogurt produced with pectin (Bridges et al., 2017). Pectin is a polysaccharide with galacturonic acid as its main structural component. It is water soluble and able to form gel structures but given that it is a fiber rather than a starch, alpha amylase is unable to break any of its linkages. Ultimately, the researchers observed a high variability in the time taken to orally break down the pudding samples, while the breakdown of the pectin-thickened yogurt sample did not vary, demonstrating the effect of salivary amylase on starch thickened foods (Bridges et al., 2017).

Research from Lapis et al. that studied the effects of cooking on oral mastication and the perception of starch further explains this phenomenon and its effects on the sensory properties of food (2017). Researchers collected saliva from 5 participants of varying salivary amylase activities, and asked subjects to rate sweetness and other taste intensities of raw and cooked starch samples. *In vitro* starch hydrolysis was performed with samples of saliva donated from each participant, and it was determined that cooking heightened the ability to digest starch as seen through an increased hydrolysis (Lapis et al., 2017). Interestingly, cooking the starch did not result in different taste ratings, so the sensory implications of starch gelatinization still require further study. Similar research has been done to demonstrate this relationship between amylase and perception of starch containing foods. de Wijk et al. researched the role of alpha amylase with flavored custards, and how the amylase enzyme could modify attributes of oral texture and flavor (de Wijk et al., 2004). Starch based custards were mixed with either added bacterial alpha amylase or acarbose, a known inhibitor of amylase, to determine changes in the breakdown of the custard (de Wijk et al., 2004). The alpha amylase used in the study was extracted from *Bacillus licheniformis*, mixed with water at various concentrations, then added to the custards. Acarbose tablets were dissolved in water at various concentrations as well, then added to the custards. The control for this experiment was a non-starch based carboxymethylcellulose (CMC) vanilla custard (de Wijk et al., 2004). Participants rated the samples based on attributes of odor, flavor, and mouthfeel. While ratings of odor and flavor were

not significantly different between amylase and acarbose samples, the custard with amylase caused increased ratings of melting and decreased sensations of thickness and creaminess, while the custard containing acarbose caused decreased melting and increased perceived thickness and creaminess (de Wijk et al., 2004).

Similar research by Engelen et al. investigated the effect of adding either saliva or a solution of amylase and water to custard prior to ingestion, on sensory ratings of smell, taste, lip and mouthfeel (2003). Researchers collected samples of saliva from participants, and each participant received his/her own saliva to mix into the custard samples. The same pattern of amylase increasing melting while decreasing thickness and creaminess was observed, dependent on the volume of salivary fluid (Engelen et al., 2003). Interestingly, saliva produced stronger melting sensations than the amylase/water solution, implying that amylase from saliva may be stronger than added amylase alone (Engelen et al., 2003). Again, odor and flavor ratings were not affected significantly by either the saliva or the amylase/water solution. To further investigate how smell, taste, and chewing stimuli may be affected by salivary amylase, researchers collected participant saliva at rest and after exposure to an odor stimulus, a citric acid tastant, and following parafilm wax chewing (Engelen et al., 2007). Salivary flow rates, protein concentration, and alpha amylase activity were measured. Engelen et al. found that the greatest salivary flow rate was elicited by citric acid, while parafilm chewing and odor stimulation produced lower flow rates (2007). Total protein was highest in the unstimulated saliva and decreased with each stimulation (Engelen et al., 2007). This implies that protein in saliva dilutes once salivary flow is initiated, a phenomenon consistent with decades of research comparing stimulated and unstimulated salivary protein concentrations (Proctor, 2016).

The high variance in the time taken for starch thickened samples to break down can be attributed to the preparation and cooking method. Starch gelatinization is the breaking down of intermolecular starch bonds in the presence of heat or water, allowing hydrogen bonds to interact with water. The starch granule experiences swelling, melting, and eventual leaching of amylose, causing the granule to gelatinize (Badenhuizen, 1959). This process is irreversible, as the amorphous region of the starch granule is hydrated and swelled. Once gelatinization occurs, retrogradation, a recrystallization process, allows amylose and amylopectin molecules in the gelatinized starch to reassociate and form new, complex matrices (Badenhuizen, 1959). These matrices are harder for amylase to break down as quickly, leading to an increased variance in

product thinning and perceived thickness. Retrogradation affects the ultimate gel structure and crystallinity of the starch, and changes sensory attributes of texture, moisture, and viscosity (Badenhuizen, 1959). Gelatinization and retrogradation thus influence the accessibility of glycosidic bonds to alpha amylase enzymes and may cause more variability in the time it takes amylase to hydrolyze the bonds, which in turn causes variation in starch digestion via amylase. So, the size of the starch granules, the degree of gelatinization, and the formation of a new gel structure during retrogradation have an impact on starch mouthfeel and breakdown in addition to amylase concentration alone. Ultimately, relationships between salivary flow rate, concentration of salivary amylase, individual amylase activity, and sensory ratings of starch-containing foods remain complex and require continued research.

1.5 Chewing Behavior and Food Texture

Though texture perception and amylase activity are correlated, the relationship of individual texture preference and salivary amylase activity is still a relatively novel research question. Different chewing styles have been identified that suggest individuals experience stronger sensations of texture perception when using their preferred chewing pattern (Chen & Engelen, 2012). These chewing patterns differ from one another by how individuals use their tongue, teeth, and palate to orally process food (Chen & Engelen, 2012). "Mouth behavior" refers to the way individuals prefer to manipulate foods in their mouth, and this behavior relates to food texture preference (Jeltema et al., 2015). Researchers have developed a classification tool to better categorize and understand unique mouth behaviors. This mouth behavior tool categorizes individuals into four major groups: Crunchers, Chewers, Suckers, and Smooshers. These four categories can be paired as two subgroups divided by mouth action; crunchers and chewers are one group, while suckers and smooshers are the other (Jeltema et al., 2015). Crunchers and chewers prefer to use their teeth to orally process foods. Crunchers often use a strong force to break the food upon the initial bite, while chewers like foods that do not break down at first and can be chewed multiple times for a long while. Suckers and smooshers prefer to orally process foods between their tongue and the palate or roof of the mouth. Suckers like hard foods that can be sucked on for a long time, while smooshers like soft foods that can easily spread throughout the mouth and stay within the mouth for a long time (Jeltema et al., 2015). The Jeltema/Beckley Mouth Behavior Typing Tool consists of visual and narrative questions that ask individuals

about a broad range of differently textured foods (ice creams, granola bars, meats and cheeses, fruits and vegetables, baked goods) and which groups of foods individuals like or dislike. The assessment also gives individuals four different options for stories that would describe their chewing styles and asks individuals to select which story best resembles the way they eat (Jeltema et al., 2015). This classification tool has allowed researchers to categorize individuals based on their mouth behaviors, and subsequently gather information about how these mouth behaviors may influence texture and food preference. It has been demonstrated that individuals tend to like textures of foods that align with their mouth behavior grouping, and that these individuals may even perceive textures of the same food differently, dependent on their mouth behavior group (Jeltema et al., 2016). These individuals who tasted the same foods but reported different textures were able to manipulate the food in their mouths using chewing styles that align with their mouth behavior group, so that a desired texture could be achieved during the eating occasion (Jeltema et al., 2016, 2020). This suggests that texture is a dynamic food descriptor, as it is subject to change during oral processing in accordance with individual texture preference and chewing or mouth behavior.

To further examine this pattern, researchers have recorded subjects chewing foods in different ways so that a correlation between mouth behavior group and jaw/chewing movements could be observed (Wilson et al., 2018). Wilson et al. found that when participants were given foods that could be orally processed in a number of different ways, these participants opted to use chewing style and jaw movements that reflect their mouth behavior group (2018). Further testing of these individual jaw movements, chewing behaviors, and mouth behavior groups through recorded chewing of a food that can be processed in different ways, such as candies, snack crackers, baked goods, popsicles, and various nuts, is worth investigating in future work.

Mouth behavior groups may be translationally relevant in both the food industry and human health. Product development of new foods that consider mouth behavior groups during formulation may be able to tailor product textures to multiple mouth behavior groups, allowing for a more universally liked product (Jeltema et al., 2014). This would lead to more consumer acceptability, because foods could be made with the intention of satisfying the different mouth behavior subgroups – cruncher/chewer foods, and sucker/smoosher foods (Kim & Vickers, 2020). Mouth behavior may also have an impact on weight management. Individuals with knowledge of their own mouth behavior group and texture preferences may better adhere to

specific diet regimens. Future work should be done that investigates the food choice behaviors of people who wish to manage their weight. Further studies should focus on these individuals to determine if their selected foods are healthier, if they add balance to their meals, and if they satisfy their own mouth behavior groups.

1.6 Conclusions and Our Study Concept

Salivary alpha amylase has an impact on many aspects of human health such as digestion and absorption, and the sensory perception of starch-containing foods. Chewing behavior in relation to food texture likely relates to amylase or other salivary properties, however little research has been done on this area of interest. Thus, several gaps in the knowledge still exist. The intricacies between salivary flow, saliva volume, and amylase activity need further exploration, and how different stimuli may affect these factors. The relationships between salivary amylase activity and texture perception, chewing style, and mouth behavior should also be investigated. There has been extensive research thus far surrounding the different mouth behavior groups and texture preference, but the work has been done mostly within a few research groups. Additionally, the relationships between mouth behavior and salivary properties requires more study. Understanding the complexities surrounding mouth behavior and salivary amylase activity may further illustrate why individuals prefer certain textures, and this knowledge may be useful in food product development and improving nutrition. Exploring these gaps in the knowledge will lead to a better understanding of the many complicated processes associated with saliva, starch, amylase, and sensation, and will lead to improvements in human health. The work presented in the following chapters aims to address some of these gaps. Our study observes salivary alpha amylase activity through an easily accessible assay, and tests whether amylase activity correlates to diet, sensory properties of higher/lower starch foods, and/or mouth behavior.

CHAPTER 2. MATERIALS AND METHODS

All methods were approved by the Purdue University Institutional Review Board, and all participants provided written informed consent.

2.1 Participants

Participants were recruited through online and print materials on the Purdue University West Lafayette campus and through the Saliva, Perception, Ingestion, and Tongues (SPIT) lab participant database. Exclusion criteria included individuals who: had food allergies or dietary restrictions to any of the ingredients included in the study, or severe food allergies of any kind; had known salivary problems, i.e., dry mouth; had a history of choking, trouble swallowing, or dysphagia; currently smoked, vaped, or used other tobacco products; or had self-reported Type I or Type II diabetes. Participants were recruited between the ages of 18-45. Women older than 45 were not considered for inclusion to avoid the potentially confounding effect of menopause on taste, smell, salivary flow, and oral health; therefore, all individuals over the age of 45 were excluded to maintain balance among genders. Participants qualified for the study if they: were willing and able to consume the study's products; agreed not to alter diet, physical activity, or medication routine during the intervention weeks; agreed to comply with study protocols including video calls or in person visits; agreed to complete study surveys and questionnaires; and, if participating remotely, had access to video quality internet and a computer or smart device for taking the surveys. In total, 34 participants consented and completed all study tasks (11 Men, 23 Women, 0 Other). The overall average participant age was 27 years (range: 19-42), and average BMI was 24.8 kg/m^2 (range: 19.5-44.5 kg/m^2). 8 participants (5 Men, 3 Women, 0 Other) dropped out of the study at some point after the first visit. Details are in Table 1.

Table 1. Participant information, including the number of visits completed, the intervention week order assigned, gender*, average age (years), and average BMI (kg/m^2).								
	High then low starch				Low then High starch			
	Counts	Age	BMI	Dropouts	Counts	Age	BMI	Dropouts
Visit 1	7 men 13 women	26 ± 6	24.5 ± 6.1	--	9 men 13 women	27 ± 6	25.8 ± 6.3	--
Visit 2	6 men 12 women	26 ± 6	23.4 ± 3.2	-1 man -1 woman	9 men 13 women	27 ± 6	25.8 ± 6.3	None
Visit 3	4 men 12 women	26 ± 5	23.5 ± 3.4	-2 men	9 men 13 women	27 ± 6	25.8 ± 6.3	None
Visit 4	4 men 11 women	26 ± 5	23.8 ± 3.3	-1 woman	7 men 12 women	28 ± 6	25.7 ± 6.6	-2 men -1 woman
*An option for "Other" was provided, but no participants selected this gender identity.								

2.2 Intervention Overview

We used a counter-balanced, crossover design for our study. Figure 1 illustrates the study design.

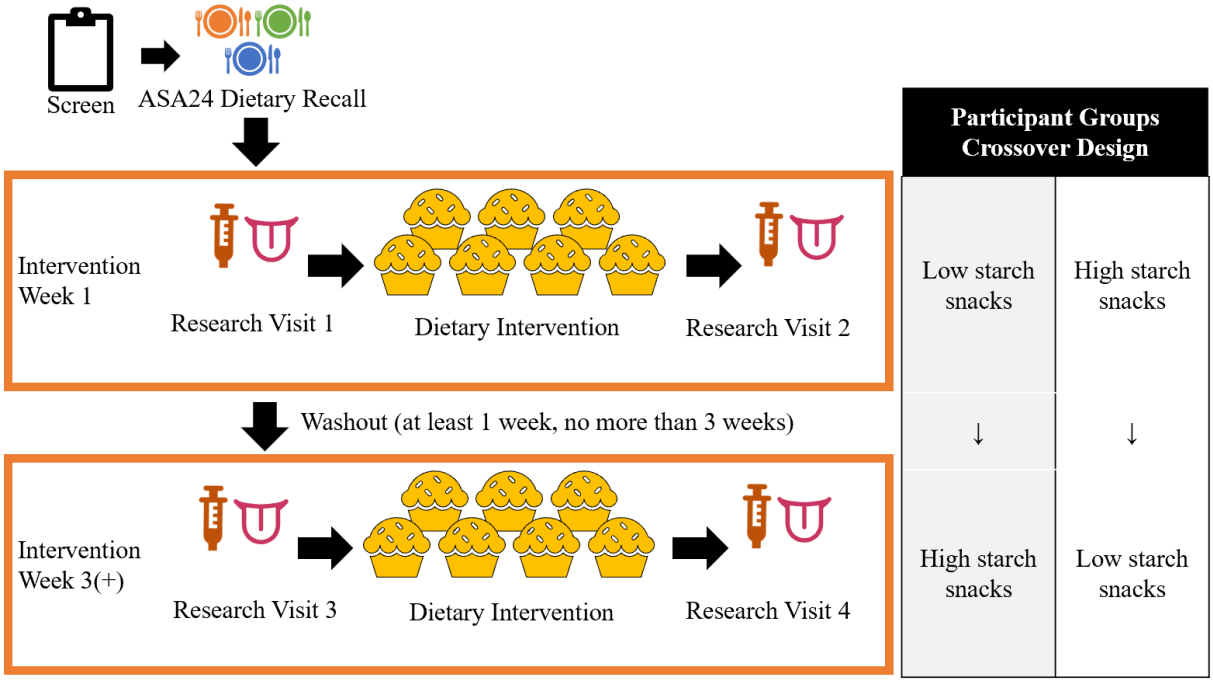

Figure 1. 4-week study design schematic.

After interested individuals completed the online screening survey via Qualtrics (Qualtrics International Inc., Seattle, WA), qualified individuals were invited to ask any questions about the four-week study and were emailed the consent form via DocuSign (DocuSign Inc., San Francisco, CA) if still interested. After consenting, subjects were sent a brief online demographics questionnaire to collect additional data including year of birth, ethnic background, height, weight, medication use, and availability. Prior to the first visit, dietary intake data were gathered using the Automated Self-Administered 24-hour (ASA24) Dietary Assessment Tool, version 2021, developed by the National Cancer Institute, Bethesda, MD (https://epi.grants.cancer.gov/asa24). Each participant was asked to complete 3 records of what foods/beverages they consumed over the past 24 hours. Participants were randomly assigned 2 weekdays and 1 weekend day to complete these recalls. Days were randomized by the researchers. All these dietary data were gathered prior to the start of the research visits.

2.3 Study Foods

The snack foods for this study were intentionally selected to be similar in total energy but different in total starch content. We also designed the study to use foods that were commercially available (not made in our laboratory) locally. The options were thus somewhat constricted, especially as this study occurred in fall 2021 when many supply disruptions were occurring due to the COVID19 pandemic. All these factors combined meant that by necessity other food constituents also differed among our "high starch" and "low starch" snack foods. In other words, the amount of starch in a food cannot be reduced while maintaining energy content without increasing some other energy-yielding ingredients (mostly sugar, fat, or protein), which can then also lead to nutritional or texture differences. We fully acknowledge this is a limitation of the work. But as one of the first studies to investigate the effects of an intervention on salivary amylase activity, we made choices aimed to allow us to gather initial data on the question as best we could at the time.

During the intervention weeks, snacks with either more or less starch were provided to participants. Three total snacks were provided per day, and the options for each day's snacks were grouped to balance the total calories and starch content across all days. Details for fat, protein, and sugar content are shown in Table 2 to demonstrate how our interventions had other nutrients confounded with the starch content.

The foods for the high/low starch groupings in this study were selected to be conceptually similar. This is the primary reason for the high proportion of nut/granola bars in the study, as these items are very similar in concept and dietary usage and yet yield different total starch. By staying mostly within one brand, we also kept the foods conceptually similar. Notably, the KIND brand did not fund or support this study in any way (to our knowledge they are not aware of the study's existence). The brand simply was consistently available and had different starch profiles that fit the purpose of the study. All items were purchased locally.

Within the starch snack kits, participants received 7 days' worth of snacks with 3 snacks for each day. The 3 snacks were from 3 groups of snacks, swapped out amongst the days to allow for variety while balancing total energy content. Participants could eat the "day packs" in any order across the 7-day intervention but were asked not to eat from multiple packs within a single day or mix packs across days. Total daily calories within a day's package of snacks were approximately 390-440. Total daily starch within a day's package for the high starch week was approximately 44-47g and for the low starch week was approximately 14-18g. We balanced the packs offered to each participant so that the total energy for the entire week was balanced for both the high and low starch intervention weeks.

Table 2. Study foods categorized into low and high starch groups, balanced for calories and starch content. Total carbohydrates, total protein, and total fat are also reported below.							
Starch Type	Group Number	Foods	Calories (kcal)	Starch (g)	Total Carbohydrate (g)	Total Protein (g)	Total Fat (g)
Low Starch	Group 1	KIND Caramel Almond & Sea Salt Bars	170	4	16	6	15
		KIND Dark Chocolate Nuts & Sea Salt Bars	180	4	16	6	15
	Group 2	Hershey's Kisses (7 pieces)	160	<1	19	2	9
		Fun Size Skittles (2 packs)	120	6	28	0	1
	Group 3	KIND Minis Peanut Butter Bars	100	2	8	3	7
		KIND Minis Peanut Butter Dark Chocolate Bars	100	2	8	3	7
High Starch	Group 1	KIND Blueberry Almond Breakfast Bars	220	17	32	3	9
		KIND Honey Oat Breakfast Bars	220	18	33	3	7
	Group 2	OREO Minis	130	9	21	1	5
		Snyder's of Hanover Mini Pretzels	100	19	22	2	0
	Group 3	KIND Mini Chewy Peanut Butter Bars	100	8	15	2	4
		KIND Mini Chewy Dark Chocolate Bars	100	9	16	1	3.5

2.4 Research Kits

In additional to the foods, participants received a kit of supplies for the research visits. These research kits were packaged separately from the snacks as well as for the start and end visit each week, to minimize accidental consumption of research test items as snacks. Table 3 below lists out all materials included in the supply kits.

Table 3. Materials included in the research kits. Company and location included.

Product	Company	Company Location
Snack Pack Chocolate Pudding	ConAgra Foods, Inc.	Chicago, IL
10 mL Non-Leur Lock Syringes	BH Supplies	Jackson, NJ
Parafilm Wax Strips (5 cm x 5 cm squares)	Fisher Scientific L.L.C.	Waltham, MA
Purple Nitrile Gloves	Fisher Scientific L.L.C.	Waltham, MA
Clear Plastic Spoons	Comfy Package	Brooklyn, NY
Pocket Smell Tests (Versions: Universal, Power Plant Company, Gas Company, Smoke)	Sensonics International (https://sensonics.com/product/pocket-smell-test/)	Haddon Heights, NJ
Crystal Geyser Bottled Water OR Dasani Bottled Water	Crystal Geyser Water Company; The Coca-Cola Company	Moultonborough, NH; Atlanta, GA

2.5 Snacking Intervention Weeks

Participants had two, 1 week intervention phases. The research visits occurred at the beginning and end of each of these weeks (day 1 and day 7). At least 1 week was required in between each intervention week, resulting in a minimum of 3 weeks from the beginning of the study to the end. During the intervention weeks, participants received either the low-starch snacks or the high-starch snacks, as described above, as well as two research visit kits. We instructed the participants to consume the snacks as part of their normal eating patterns. Participants consumed 3 snacks a day for each assigned week. Participants were randomized to the order in which they ate the intervention snacks, with half assigned to low starch snacks then high starch snacks and the other vice versa. We asked participants after the intervention if they substituted the snacks into their day or if they added the snacks on top of their regular food consumption. To ensure compliance with consumption of the snacks, we asked participants to

photograph themselves eating their snacks (“take a selfie”) and upload these photos at the end of the week.

2.6 Research Visits

Each subject participated in four total research visits, one at the beginning and end of each intervention week. Participants completed these research visits at the same time of day and were given the option to choose which time slot worked best for their individual schedules. Whichever time of day the participant chose for their first visit, they completed the subsequent visits at the same time. This was done to minimize effects of circadian rhythm on salivary flow rate and composition (Dawes, 1972). Two participants were able to complete visits in-person, and the remaining participants completed the research visits virtually due to COVID19 precautions. Research visits lasted approximately one hour, and participants were instructed to refrain from eating, drinking, or using oral care products for at least one hour prior to their scheduled visit time. Participants completed surveys for the research visits using RedJade Sensory Software (Redwood City, CA). Procedures for each research visit were the same and are as follows.

2.6.1 Taste Acuity Test

The taste acuity test was conducted used salty, bitter, sour, and sweet flavored solutions. The solutions each contained water and concentrations of 1.16% w/w sodium chloride (salty), 0.002% w/w sucrose octa acetate (bitter), 0.27% w/w citric acid (sour), and 5% w/w sucrose (sweet). We were unable to keep the water used for the tasting solutions and rinses consistent due to lab access, supply issues, and shipping delays. Thus, tasting solutions were mixed using DI water for participants 1001-1013. Tasting solutions for participants 1014-1029 were mixed using Crystal Geyser Alpine Spring Water (natural spring water). Tasting solutions for participants 1030-1045 were mixed using Dasani Purified Water (purified water, magnesium sulfate, potassium chloride, salt). Samples were prepared at concentrations designed to be around moderate intensity, so the water differences should have had minimal effects on identification.

Prior to each in-person research visit, tasting solutions were prepared and stored in 30 mL sample cups with lids, labeled with unique 3-digit identifiers. The solutions were stored in a refrigerator at 4°C overnight to preserve freshness and to allow the tastant to fully dissolve. The tasting solutions were removed from the refrigerator 1 hour before each research visit so that the

solutions warmed to room temperature (20°C). For remote/virtual participants, tasting solutions were prepared, then approximately 10 mL of each solution was pipetted into 15 mL glass vials with screw cap lids. Vials were labeled with each solution's 3-digit identifier. The tasting solutions were frozen at -20°C in vials until the scheduled kit pickup time. Upon kit pickup, participants were instructed to place the vials into their home's freezer as soon as possible. The night before each research visit, participants were instructed to defrost the vials to room temperature.

During the research visit, participants first rinsed their mouths with water. One vial at a time, the survey prompted participants to take a sip of the tasting solution and identify whether that solution was salty, bitter, sour, or sweet. The tasting order assigned to participants was counterbalanced via the survey software, and participants were unable to skip a question and come back to it, eliminating the option to change answers after tasting the solutions. If participants incorrectly identified 2 or more of the tasting solutions, they were coded as a "fail" for the taste acuity test.

2.6.2 Smell Assessment Test

Olfactory function was assessed using the Sensonics International Pocket Smell Test (*Pocket Smell Test*^{TM}, n.d.). This test is a 3-odor, forced choice screening test that briefly determines if a subject may have olfactory dysfunction. The pocket test works like a scratch-and-sniff card. Each item's odor patch is scratched with a pen or pencil to release an odor. The card (and matching RedJade survey we used to capture the remote participants' responses) prompted participants to smell each odor one at a time, then choose one response from 4 options (one correct response and 3 distractors). If a subject incorrectly identified one or more odors, this implies a degree of olfactory dysfunction ((*Pocket Smell Test*^{TM}, n.d.). There are four different versions of the smell test each with unique odors. Participants were assigned a different version of the smell test for each of the four research visits.

2.6.3 Pudding/Salivary Amylase Assay

To assess the activity of participants' salivary amylase, we observed how quickly their saliva liquified a starch-thickened pudding. To do this, we adapted methods from the International Dysphagia Diet Standardization Initiative (*IDDSI - IDDSI Testing Methods*, n.d.).

This initiative invented a method to observe the viscosity of fluids using a 10mL syringe. The liquid is placed in the syringe and the user observes how much liquid flows through the syringe in 10 seconds. We piloted this test to observe how different individuals' saliva caused a commercial pudding to thin after pre-set time periods (Pacheco & Running, 2022). The current research study will be the first to publish the method for observing salivary amylase with a larger sample of subjects.

Participants first rinsed their mouth with water. For their first visit, participants 1001 and 1002 used deionized water at room temperature for rinses between samplings. For all other visits and participants, bottled water was used for rinses. Participants were then given disposable nitrile gloves to wear during the experiment if they preferred. Next, they were given a 5 cm X 5 cm square of wax (Parafilm) to chew like a piece of gum. They chewed the wax for 30 seconds, spitting out any accumulated saliva into a provided 60 mL plastic cup. Participants then used a 10 mL syringe to gather the saliva from the plastic cup. Participants photographed this syringe at eye level (Figure 2A), then submitted this picture in a later survey so researchers could measure the volume of saliva generated. Next, participants opened the provided pudding and stirred the contents thoroughly. Participants then emptied the syringe of saliva onto the top of the pudding (Figure 2B), then mixed the saliva into the pudding for 10 seconds (Figure 2C). The pudding/saliva mixture then rested for 30 seconds. After 30 seconds, participants used another syringe to gather 10mL of the pudding/saliva mixture (Figure 2D). Next, they transferred the pudding/saliva mixture into a new syringe without the plunger, holding their finger at the bottom of the syringe tip to prevent the mixture from flowing out (Figure 2E). Subjects then removed their finger, allowing the pudding/saliva mixture to flow out of the bottom of the syringe (into a cup, Figure 2F). After 10 seconds, the participant recorded how much pudding/saliva mixture remained in the syringe (Figure 2G). Participants took a photograph of this syringe at eye level to submit in a later survey. This assay was repeated after 4 minutes of the same pudding/saliva mixture resting. Figure 2 below shows photographs of the assay step-by-step, which were also provided to participants to help them through the process.

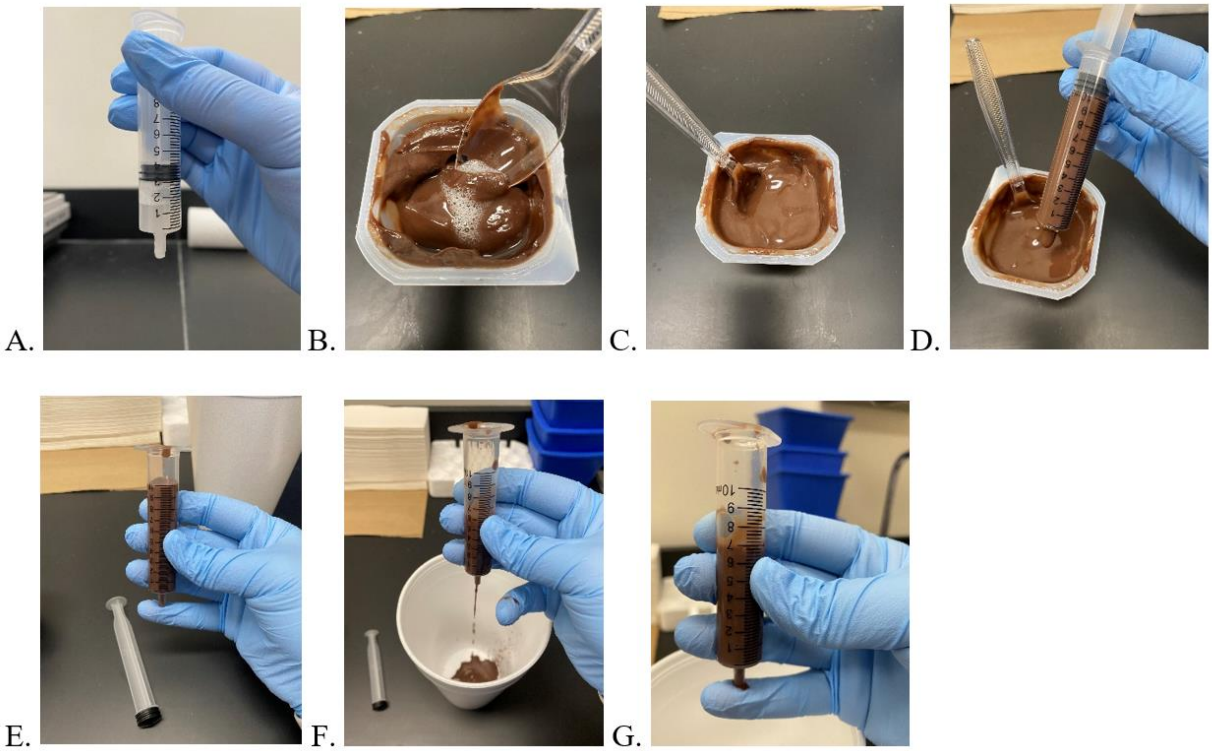

Figure 2. Photographs of the Pudding/Salivary Amylase Assay. A) After chewing wax for 30 seconds and spitting accumulated saliva into a cup, the volume of saliva is collected from the cup using a 10 mL syringe. B) The saliva is added to the pudding cup. C) The saliva is mixed with the pudding for 10 seconds. The pudding rests for 30 seconds. D) 10 mL of the pudding/saliva mixture is collected using a new 10 mL syringe. E) The pudding/saliva mixture is transferred to a new, plunger-less syringe while sealing the tip with a finger. F) For 10 seconds, the pudding/saliva mixture free flows from the syringe into a cup. G) The volume of pudding remaining in the syringe is measured.

We conducted a practice session with the participants on this method during the first research visit. For the practice, we did not have the participants spit or use actual pudding. Instead, they went through the motions of how to chew, spit, collect the spit, and mix the spit with pudding. The instructions in Figure 2 were also given to our participants to guide them through the assay at every visit. Remote participants had a researcher live on a video chat in case they needed assistance.

2.6.4 Sensory Testing

During each research visit, participants rated all study foods for sensory qualities. The snacks were provided in their original packaging for all remote participants. This was because we were not masking the visual appearance of the snacks anyway, and most of them are easily identified—especially as the participants had been eating the snacks for a week. Additionally, as participants were not informed of our targeted difference (higher or lower starch) among the snacks, their ability to identify the items was not a key concern for our work. Thus, the foods remained in their original packaging, labeled with 3-digit identifiers, and stored in gallon size zip-closure plastic bags.

During the research visit, participants were instructed to rinse their mouth with water prior to tasting the foods. Next, the survey instructed participants to take a bite of the sample (sample order was counter-balanced across participants and visits), then rate the sample on generalized, labeled visual analog scales for sweetness, hardness, chewiness, texture liking, overall liking, and desire to eat the sample (scales adapted from (Kershaw & Running, 2019)). For sweetness, hardness, and chewiness the scale was a 110-point generalized visual analog scale with labels at “None” (0), “Barely detectable” (5), “Weak” (25), “Moderate” (45), “Strong” (65), “Very strong” (85), and “Strongest ever” (105). Texture liking and overall liking were measured on a 220-point scale with labels at “Worst ever” (-100), “Dislike” (-50), “Neutral” (0), “Like” (50), and “Best ever” (100). Desire to eat used a generalized labeled visual analog 220-point scale with points ranging from “Not at all strong” (-100), “Very weak” (-50), “Neutral” (0), “Very strong” (50), to “As strong as I’ve ever felt” (100). Figure 2 shows the line scales used. Participants were allowed to re-taste the sample as needed to accurately rate the sensations experienced, but they were given a cup and the option to spit out samples to avoid becoming overly full.

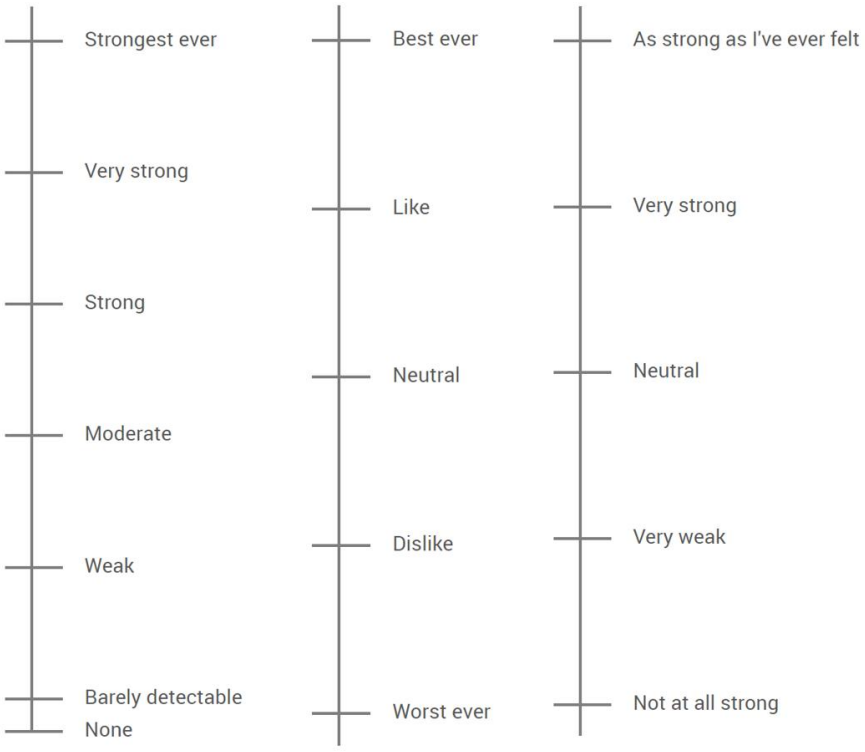

Figure 3. Scales used for sensory ratings of intervention snack foods. Sweetness, hardness, and chewiness used the left-hand scale, texture liking and overall liking used the middle scale, and desire to eat used the right-hand scale.

2.6.5 Jeltema/Beckley Mouth Behavior Typing Test

Participants completed an assessment of their preferred "mouth behavior" for orally manipulating foods at each research visit. We used the Jeltema/Beckley Mouth Behavior Typing Tool to conduct this test (Jeltema et al., 2015). The test asked participants a series of hypothetical/memory questions about typical foods they would eat, and how they would go about oral processing each of these everyday foods. The survey also showed participants four pictures of different groups of foods that fit with different mouth behaviors. The different groups were labeled: "I like foods that I can crunch", "I like foods that I can chew", "I like foods that I can smoosh. I even smoosh foods that I could chew" and "I like foods that I can suck on a long time, and I often suck on them until they dissolve." The survey asked participants which group they liked most, which group(s) they disliked, how hard of a decision it was to pick their favorite group. Finally, the survey gave four different narratives about textures of foods and eating experiences in general, and participants were asked to select which story best represented them. Once complete, the test grouped participants into four categories based on the answers they gave

in the survey – Crunchers, Chewers, Smooshers, or Suckers. According to models developed by Jeltema/Beckley, Crunchers like to use their teeth to break down foods and prefer a strong bite that breaks foods upon biting. Chewers like to use their teeth to break down foods, and prefer foods that do not break upon biting, and can be chewed for longer periods of time. Smooshers prefer to manipulate foods between the tongue and the roof of the mouth and prefer soft and creamy foods that spread throughout the mouth and can be held in the oral cavity for long periods of time. Suckers also prefer to manipulate foods between the tongue and roof of the mouth, but they prefer harder foods that can be sucked on for long periods of time (Jeltema et al., 2015). These categories later helped to determine associations between mouth behavior, salivary amylase activity, and outcomes from the sensory testing.

2.7 Statistical Analysis

Linear mixed models with subjects as a repeated measure (when appropriate) were performed using SAS OnDemand (Cary, NC) in Jupyter Lab to evaluate the fixed effects. Proc MIXED statements were used. We used the Kenward-Roger approximation for degrees of freedom with the option set for restricted maximum likelihood estimation. We also used a compound symmetry covariance structure, and verified this structure was a better fit that the default of variance components. Categorical variables were analyzed using LSMEANS statements, and we did not adjust any post-hoc comparisons due to the large number of non-logical comparisons possible in our data (example: it does not make sense to compare data from the start of the high starch week to the end of the low starch week; rather we need to compare start to end within week type, and start to start across week types, and end to end across week types). Because of this, the post hoc comparisons when many comparisons are possible should be considered preliminary. The full code is available in the appendix section. OriginPro 2022 (Northampton, Massachusetts, USA) was used to create figures with boxplots. Boxplots show the median line, boxes are the 25-75th percentile, whiskers are the 5-95th percentile, and data points are shown next to the boxes.

We assessed our participants' reports of dietary intake and screened for inaccuracies to determine the number of plausible dietary reporters. To calculate plausible dietary reporters, we first used the formula and method from (McCrory et al., 2002) to calculate the predicted total energy expenditure (pTEE) in Megajoules (MJ):

*pTEE (MJ) = 7.377 - (0.073*age) + (0.0806*weight) + (0.0135*height) - (1.363*gender)

We used units of kilograms for weight, centimeters for height, 0 for males and 1 for females.

Next, we converted the reported energy intake (rEI) from our dietary recall data from kilocalories (kcal) to Megajoules using the below formula:

rEI (Reported (from dietary recalls) energy intake in kcal) ÷ 238.8 = rEI in MJ

Next, we calculated the ratio of reported energy intake to predicted total energy expenditure using the below formula, resulting in a percentage:

% pTEE= rEI / pTEE * 100

Next, we calculated the ± 1 standard deviation (SD) for the population by first calculating the coefficient of variation (CV) for each participant using individual days of reported energy intake, mean, and standard deviation for each participant.

CV=standard deviation ÷ mean*100

Finally, we then took the average coefficient of variation for the population using the following formula, where d = the number of days of dietary intake data, and 17.7 and 8.2 are constants (McCrory et al., 2002):

$$\pm 1 \text{ SD} = \sqrt{[CV^2_{\text{rEI}} / \text{d} + (8.2)^2 + (17.7)^2]}$$

The resulting ± 2 SD were used to identify under and over reporters (McCrory et al., 2002). Our calculations resulted in 19 plausible reporters, 12 under reporters, and 3 over reporters.

While we started with models including all the factors of potential interest, we serially reduced these models to limit over-parametrization of the models. This was done by sequentially removing factors with the highest p-values over 0.1, starting with interaction effects and then moving to main effects. If an interaction was significant, the main effects within that interaction were kept in the model.

For the pudding/salivary amylase assay, factors tested included: pudding time (either 40 seconds or 240 seconds), order assigned, gender, intervention week, visit type, saliva volume,

and medication usage. While we tested baseline dietary intake of carbohydrate, total starch, added sugar, total sugar, and various micronutrients, no dietary factors were significant when analyzing data with only the plausible dietary reporters. Thus, all dietary factors were removed from the models. The final model after we removed non-significant effects was:

*PuddingRemaining = InterventionWeek SalivaVolume VisitType Medication InterventionWeek*VisitType*

This model was run individually for both the 40s and 240s timepoints. *PuddingRemaining* refers to the amount of pudding in mL that remained in the syringe after the pudding assay. *InterventionWeek* refers to whether participants were currently assigned to the high starch snacks or the low starch snacks. *SalivaVolume* is the amount of saliva participants generated after 30 seconds of chewing on the parafilm wax. *VisitType* is whether it was the start or the end of the intervention week. *Medication* refers to whether participants were taking any medications at all (Yes), or no medications at all (No).

For sensory data, factors tested included: intervention week, order assigned, gender, visit type, taste acuity, smell acuity, food type, mouth behavior, pudding time (40 seconds or 240 seconds). The final models after we removed non-significant effects were:

Sweetness = TasteAcuity FoodType

*Hardness = VisitType FoodType MouthBehavior MouthBehavior*FoodType*

Chewiness = Pudding40s MouthBehavior

*TextureLiking = FoodType MouthBehavior MouthBehavior*FoodType*

*OverallLiking = FoodType MouthBehavior MouthBehavior*FoodType*

*DesireToEat = Pudding40s VisitType FoodType MouthBehavior Pudding40s*VisitType MouthBehavior*FoodType*

These models were run separately for each intervention week (high starch week and low starch week) to avoid the need to analyze a high number of two- and three-way interaction effects. *Sweetness, Hardness, Chewiness, TextureLiking, OverallLiking,* and *DesireToEat* refer to the intensity of the attributes participants rated on generalized, labeled visual analog scales in

the sensory testing of the intervention snack foods. *TasteAcuity* refers to whether participants passed or failed the taste acuity assessment during the research visit. *FoodType* refers to whether the item being rated was in the high starch or low starch snack group (per Table 2). *MouthBehavior* refers to the categories that participants were assigned using the mouth behavior typing tool – crunchers, chewers, suckers, or smooshers – using the outcome from the "story" based question. *Pudding40s* refers to the amount of pudding in mL remaining in the syringe at the 40 second timepoint.

Our primary hypotheses to be evaluated by these models were:

- The pudding/salivary amylase activity assay would show more activity (less pudding remaining):
    - At the end of the high dietary starch intervention week, with less or no change from start to end of the low dietary starch intervention week
    - For people with greater baseline starch consumption compared to less baseline starch consumption

Secondary hypotheses we analyzed with these models were:

- If amylase activity changed from the intervention, then sensory ratings would shift for the intervention foods. For example, higher starch foods would become less chewy and/or less hard. Lower starch foods would not change in sensory properties in response to amylase changes.
- Sensory ratings would differ among the mouth behavior groups, especially for overall liking and texture liking. High starch snacks (generally chewier) would be better liked by the chewers.
- Participants who preferred chewing over crunching, smooshing, or sucking on food would exhibit higher salivary amylase activity.

The final models allow for additional comparisons that will be discussed, but the above were our original hypotheses.

CHAPTER 3. RESULTS

3.1 Pudding/Salivary Amylase Assay Analysis

Most significant effects were limited to the 40s timepoint (Tables 4 and 5). The exception was saliva volume, which was significant in both time points' models. For both models, more saliva volume corresponded to less pudding remaining in the syringe, which would indicate more amylase activity.

Additionally, for the 40s timepoint, we saw an overall trend for more pudding remaining (less amylase activity) at the end of the week compared to the beginning ($p=0.062$). Observing post-hoc comparisons for the interaction, this trend was driven by the low starch intervention week, though the patterns were in the same direction for both interventions ($p=0.055$ low starch intervention, $p=0.48$ high starch intervention; Figure 4).

A trend for medication use associating with more pudding remaining (less amylase activity; $p=0.079$) at the 40s timepoint. We checked our data briefly to see if this was confounded by saliva volume, as medication use is known to cause dry mouth (Bardow et al., 2001). However, means for saliva volume were, if anything, higher with medication use (opposite the expected direction if the effect were truly mediated through saliva volume, which as already shown associates with less pudding remaining/more amylase activity).

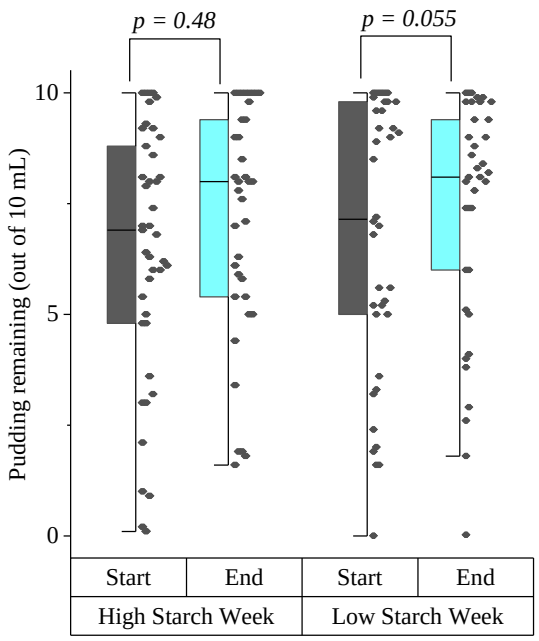

Figure 4. The effects of visit type and intervention week on volume of pudding remaining in the syringe after 40 seconds. Boxes indicate the 25th to 75th percentile, the median is the horizontal line, and whiskers go from the 5th to the 95th percentile.

Table 4: Pudding/Salivary Amylase Activity Assay at 40 Seconds					
Model: (Pudding Remaining) = (Intervention week) (Saliva volume) (Visit type) (Medications) (Visit type) x (Intervention week) *Analysis run separately for 40s and 240s timepoints*					
Pudding remaining at 40s *(more pudding remaining means less salivary amylase activity)*					
Effect	***Estimate (SE)***	***F (DF)***	***p-value***	***Comparisons p-value****	***Comparison (t, DF)***
Intervention week	High starch week: 7.5 (0.4) Low starch week: 7.3 (0.4)	0.20 (1/93.1)	0.66		
*Saliva volume***	-0.92 (0.4)	6.29 (1/109)	0.014		
Visit type	Start: 7.1 (0.4) End: 7.7 (0.4)	3.58 (1/92)	0.062		
Medications (Yes or No)	Yes: 8.0 (0.5) No: 6.8 (0.4)	3.29 (1/31.5)	0.079		
Visit type x Intervention week	Start of High starch week: 7.3 (0.4)	0.77 (1/92.8)	0.38	Within High starch week, start to end: 0.48	0.72 (92)
	End of High starch Week: 7.6 (0.4)			Within Low starch week, start to end: 0.055	1.95 (92.7)
	Start of Low starch week: 6.9 (0.4)			High to Low starch weeks, start: 0.36	0.92 (93.6)
	End of Low starch week: 7.8 (0.4)			High to Low starch weeks, end: 0.76	-0.31 (92.3)
*p-values from unadjusted pos-hoc comparisons. These are left unadjusted as many comparisons run by the analyses are not logical (example: End of high starch week to beginning of low starch week), and as these comparisons were planned a priori. **Saliva volume, unlike our other effects, is a continuous variable. The estimate here is a slope, with units of mL saliva/mL pudding. SE: standard error					

Table 5: Pudding/Salivary Amylase Activity Assay at 240 Seconds

Model: (Pudding Remaining) = (Intervention week) (Saliva volume) (Visit type) (Medications) (Visit type) x (Intervention week)
Analysis run separately for 40s and 240s timepoints

Pudding remaining at 240s *(more pudding remaining means less salivary amylase activity)*

Effect	***Estimate (SE)***	***F (DF)***	***p-value***	***Comparisons p-value****	***Comparison (t, DF)***
Intervention week	High starch week: 5.5 (0.5) Low starch week: 5.3 (0.5)	0.25 (1/94.5)	0.61		
*Saliva volume***	-1.3 (0.5)	8.13 (1/101)	0.0053		
Visit type	Start: 5.2 (0.5) End: 5.7 (0.5)	2.04 (1/93.3)	0.16		
Medications (Yes or No)	Yes: 6.0 (0.7) No: 4.9 (0.5)	1.97 (1/32.7)	0.17		
Visit type x Intervention week	Start of High starch week: 5.3 (0.5)	0.04 (1/94.1)	0.84	Within High starch week, start to end: 0.39	0.87 (93.4)
	End of High starch Week: 5.8 (0.5)			Within Low starch week, start to end: 0.26	1.14 (94)
	Start of Low starch week: 5.0 (0.6)			High to Low starch weeks, start: 0.63	0.49 (95)
	End of Low starch week: 5.7 (0.5)			High to Low starch weeks, end: 0.83	0.22 (93.6)

*p-values from unadjusted pos-hoc comparisons. These are left unadjusted as many comparisons run by the analyses are not logical (example: End of high starch week to beginning of low starch week), and as these comparisons were planned a priori.
**Saliva volume, unlike our other effects, is a continuous variable. The estimate here is a slope, with units of mL saliva/mL pudding
SE: standard error

3.2 Sensory and Mouth Behavior Analysis

3.2.1 Sweetness

For sweetness ratings, we saw a trend that failing the taste acuity test tended to associate with reduced sweetness intensity during the high starch intervention week (p=0.063) but did not find any significance for the low starch intervention week (p=0.76), though the pattern is in the same direction (Figure 5, Table 6).

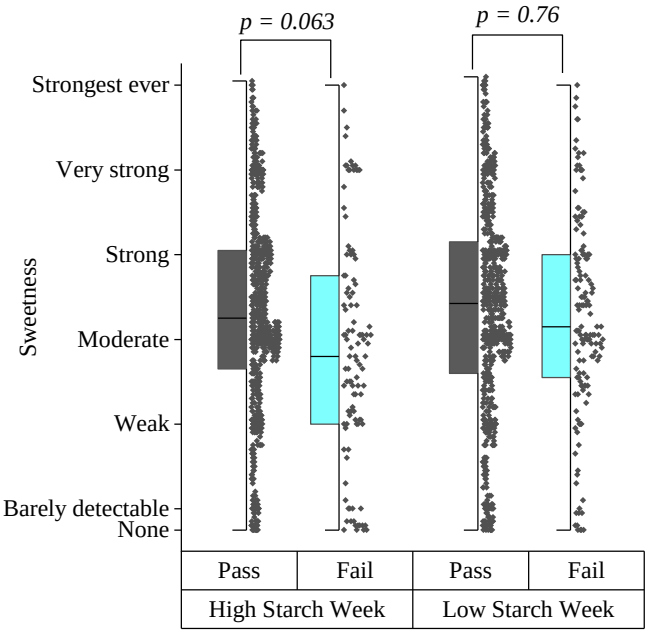

Figure 5. The effects of taste acuity status (pass or fail) and intervention week on sweetness ratings. Boxes indicate the 25th to 75th percentile, the median is the horizontal line, and whiskers go from the 5th to the 95th percentile.

Higher starch foods were perceived as less sweet than lower starch foods, regardless of intervention week ($p<0.0001$ for either week). A quick assessment of the individual foods indicates this is driven by pretzels (high starch) with low sweetness ratings. Foods rated highest for sweetness were the Skittles (low starch), Hershey Kisses (low starch), and Oreo Minis (high starch). Combined, having two of the highest sweetness items in the low starch food group, and having only one high sweetness as well as the lowest sweetness food in the high starch food group likely drove this pattern for less sweetness overall in the low compared to high starch food types (see Figures 6 and Figure 7).

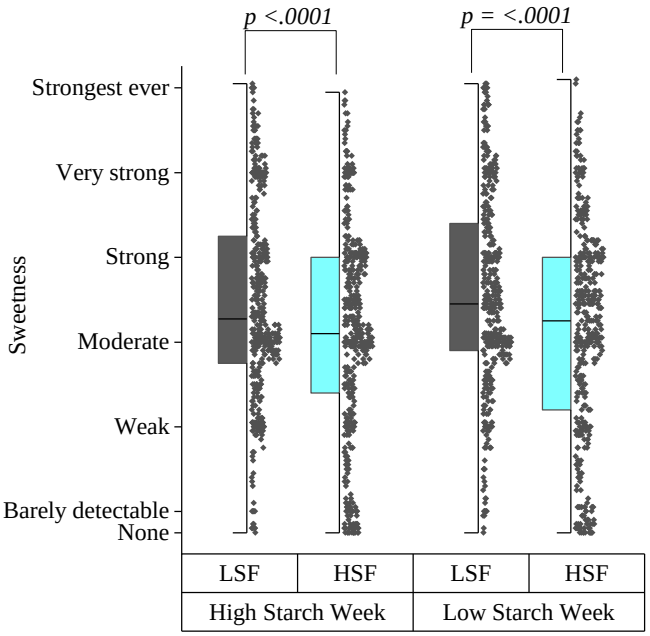

Figure 6. The effects of food type (high starch food or low starch food, abbreviated HSF and LSF) and intervention week on sweetness ratings. Boxes indicate the 25th to 75th percentile, the median is the horizontal line, and whiskers go from the 5th to the 95th percentile.

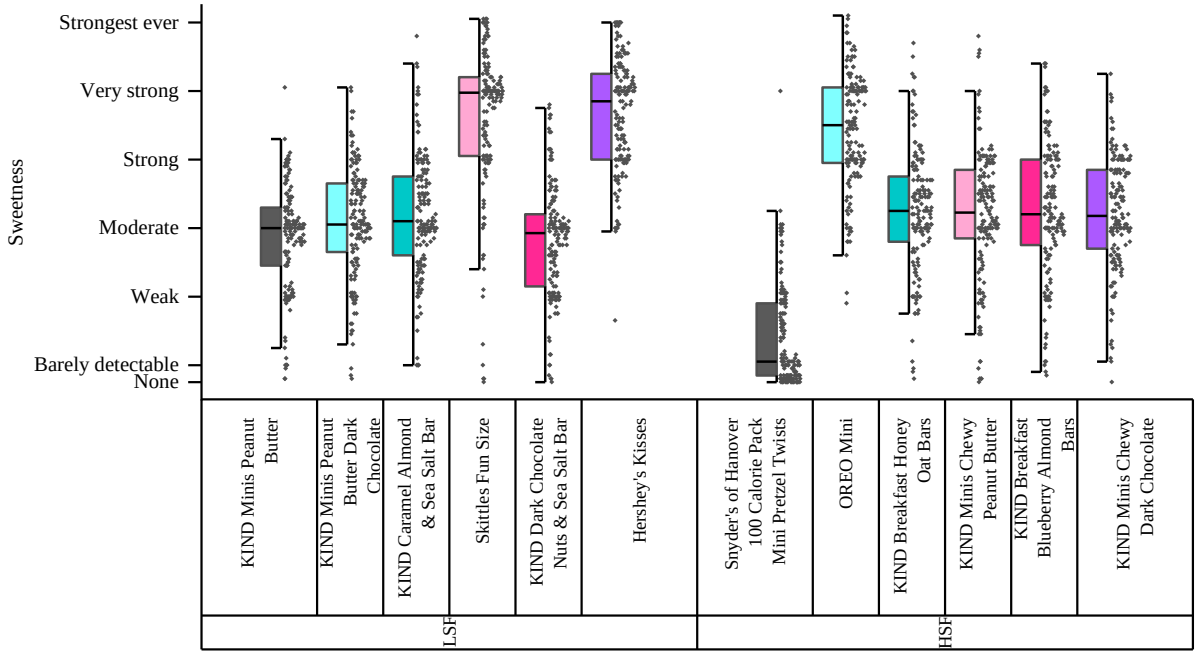

Figure 7. Sweetness ratings of low starch and high starch intervention foods. Low Starch Food is abbreviated "LSF", and High Starch Food is abbreviated "HSF". Boxes indicate the 25th to 75th percentile, the median is the horizontal line, and whiskers go from the 5th to the 95th percentile.

Table 6: Sweetness Intensity			
Model: Sweetness = (Taste acuity) (Food type)			
High starch week			
Effect	***Estimate (SE)***	***F (DF)***	***p-value***
Taste acuity	Pass: 52 (1.5) Fail: 44 (4.0)	3.57 (1/64.5)	0.063
Food type	Low starch food: 51 (2.3) High starch food: 44 (2.3)	21.93 (1/894)	<.0001
Low starch week			
Effect	***Estimate (SE)***	***F (DF)***	***p-value***
Taste acuity	Pass: 52 (1.8) Fail: 51 (3.0)	0.10 (1/470)	0.76
Food type	Low starch food: 56 (2.1) High starch food: 47 (2.1)	35.17 (1/861)	<.0001
SE: standard error Analyses were run separately for the two intervention weeks in order to avoid having to test and interpret a large number of two- and three-way interaction effects.			

3.2.2 Hardness

For hardness, there was a significant effect for visit type during the high starch intervention (p=0.025), where participants rated foods as harder at the end of the week. During the low starch week, there was no difference in hardness ratings (Figure 8, Table 7).

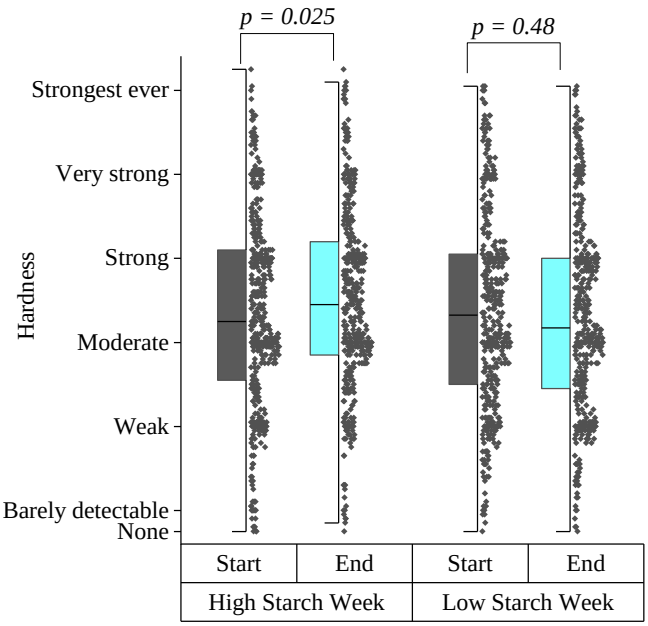

Figure 8. Effect of visit type on hardness ratings during the high and low starch interventions. Boxes indicate the 25th to 75th percentile, the median is the horizontal line, and whiskers go from the 5th to the 95th percentile.

Low starch foods were rated as harder than high starch foods during both the high and low starch interventions ($p<0.0001$ for either week; Figure 9, Table 7).

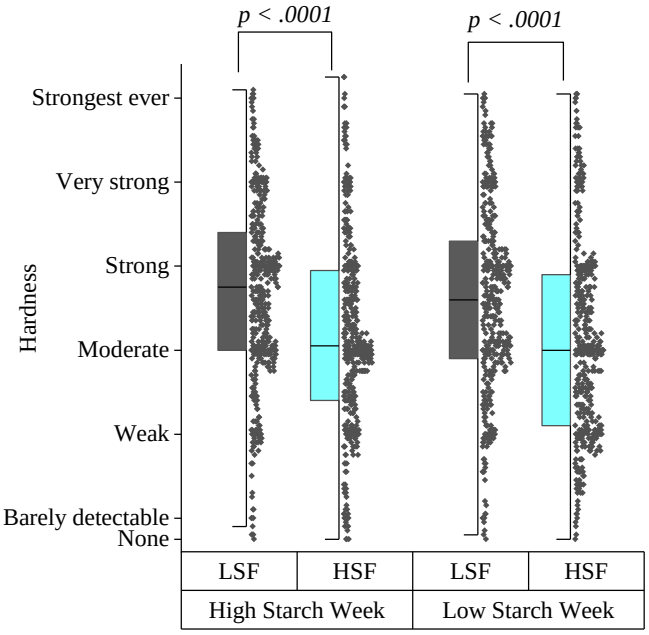

Figure 9. Effect of food type on hardness ratings during the high and low starch intervention weeks. Low Starch Food is abbreviated "LSF", and High Starch Food is abbreviated "HSF". Boxes indicate the 25th to 75th percentile, the median is the horizontal line, and whiskers go from the 5th to the 95th percentile.

Table 7: Hardness intensity					
Model: Hardness = VisitType FoodType MouthBehavior MouthBehavior*Food Type					
High starch week					
Effect	***Estimate (SE)***	***F (DF)***	***p-value***	***Comparisons***	***p-value* (t, DF)***
Visit Type	Start: 51 (2)	5.05 (1/918)	0.025		
	End: 54 (2)				
Food Type	Lower starch food: 59 (2)	67.63 (1/889)	<.0001		
	Higher starch food: 47 (2)				
Mouth Behavior	Cruncher: 55 (2)	1.17 (3/155)	0.32		
	Chewer: 55 (3)				
	Sucker: 53 (4)				
	Smoosher: 49 (3)				
*MouthBehavior*FoodType*	Low Starch Cruncher: 59 (2)	4.26 (3/889)	0.0053	High to Low Starch Cruncher	<.0001 (-4.49, 889)
	High Starch Cruncher: 51 (2)			High to Low Starch Chewer:	0.027 (-2.21, 889)
	Low Starch Chewer: 58 (3)			High to Low Starch Sucker:	<.0001 (-4.58, 889)
	High Starch Chewer: 52 (3)			High to Low Starch Smoosher:	<.0001 (-5.75, 889)
	Low Starch Sucker: 62 (5)			Within high starch food type, Smoosher to Cruncher:	0.0096 (2.62, 163)
	High Starch Sucker: 43 (5)			Within high starch food type, Smoosher to Chewer:	0.018 (2.39, 144)
	Low Starch Smoosher: 57 (4)			Within high starch food type, Smoosher to Sucker:	0.67 (-0.42, 178)
	High Starch Smoosher: 41 (4)				

Table 7 (continued): Hardness intensity

Model: Hardness = VisitType FoodType MouthBehavior MouthBehavior*Food Type

Low starch week

Effect	***Estimate (SE)***	***F (DF)***	***p-value***	***Comparisons***	***p-value* (t, DF)***
Visit Type	Start: 52 (2)	0.50 (1/880)	0.48		
	End: 51 (2)				
Food Type	Lower starch food: 56 (2)	31.23 (1/856)	<.0001		
	Higher starch food: 47 (2)				
Mouth Behavior	Cruncher: 54 (2)	0.94 (3/105)	0.42		
	Chewer: 50 (4)				
	Sucker: 53 (4)				
	Smoosher: 49 (3)				
*MouthBehavior*FoodType*	Low Starch Cruncher: 59 (2)	0.28 (3/856)	0.84	High to Low Starch Cruncher:	<.0001 (-4.66, 856)
	High Starch Cruncher: 50 (2)			High to Low Starch Chewer:	0.014 (-2.48, 856)
	Low Starch Chewer: 54 (4)			High to Low Starch Sucker:	0.083 (-1.74, 856)
	High Starch Chewer: 46 (4)			High to Low Starch Smoosher:	<.0001 (-4.05, 856)
	Low Starch Sucker: 57 (5)			Within high starch food type, Smoosher to Cruncher:	0.11 (1.63, 112)
	High Starch Sucker: 49 (5)			Within high starch food type, Smoosher to Chewer:	0.51 (0.67, 94.2)
	Low Starch Smoosher: 55 (3)			Within high starch food type, Smoosher to Sucker:	0.25 (-1.15, 275)
	High Starch Smoosher: 43 (3)				

*p-values from unadjusted pos-hoc comparisons. These are left unadjusted as many comparisons run by the analyses are not logical (example: End of high starch week to beginning of low starch week), and as these comparisons were planned a priori.
SE: standard error
Analyses were run separately for the two intervention weeks in order to avoid having to test and interpret a large number of two- and three-way interaction effects.

We saw a significant interaction effect for food type (high vs low starch) and mouth behavior (p=0.0053). This was driven by the smooshers giving lower ratings to the high starch foods compared to the crunchers or chewers (Table 6, Figure 10) during the high starch intervention week.

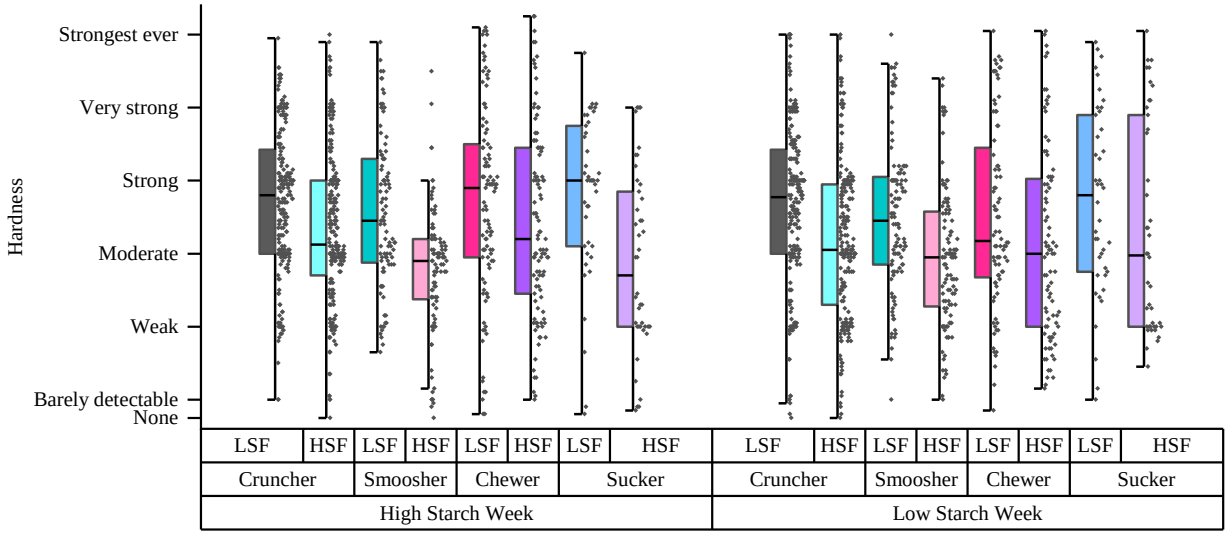

Figure 10. Effects of food type and mouth behavior on hardness ratings during the high and low starch intervention weeks. Low Starch Food is abbreviated "LSF", and High Starch Food is abbreviated "HSF". Boxes indicate the 25th to 75th percentile, the median is the horizontal line, and whiskers go from the 5th to the 95th percentile.

3.2.3 Chewiness

Overall, no factors had significant effects on chewiness (all p>0.1). Effects with the values closest to p=0.1 are shown in Table 8.

Table 8: Chewiness intensity

Model: Chewiness = Pudding40s MouthBehavior

High starch week			
Effect	***Estimate (SE)***	***F (DF)***	***p-value***
Pudding40s		1.95 (1/145)	0.16
Mouth Behavior	Cruncher: 54 (2) Chewer: 51 (3) Sucker: 62 (4) Smoosher: 50 (3)	2.12 (3/120)	0.10
Low starch week			
Effect	***Estimate (SE)***	***F (DF)***	***p-value***
Pudding40s		2.78 (1/197)	0.097
Mouth Behavior	Cruncher: 53 (2) Chewer: 48 (3) Sucker: 59 (4) Smoosher: 52 (3)	1.77 (3/96.5)	0.16

SE: standard error
Analyses were run separately for the two intervention weeks to avoid having to test and interpret a large number of two- and three-way interaction effects.

3.2.4 Texture Liking

For texture liking, ratings differed by mouth behavior groups for both intervention weeks (Tables 9 and 10). Crunchers liked the textures of low starch foods more than high starch foods during both intervention weeks (p=0.0082 high starch week and p=0.0009 low starch week). No differences in liking were observed between the food types for chewers. Smooshers rated textures of the high starch foods as more liked than the low starch foods for both intervention weeks (p<0.0001 high starch week and p=0.0007 low starch week). Smooshers also gave lower texture liking ratings for the high starch food compared to crunchers (high starch intervention week, p=0.043) and perhaps chewers (low starch intervention week, p=0.086). Suckers liked the low starch foods' texture more during the low starch intervention week (p=0.047) but showed no pattern during the high starch week (p=0.83). Notably, there were very few suckers and smooshers in our dataset, so these patterns should be interpreted cautiously. Data can be seen in Figure 11 below.

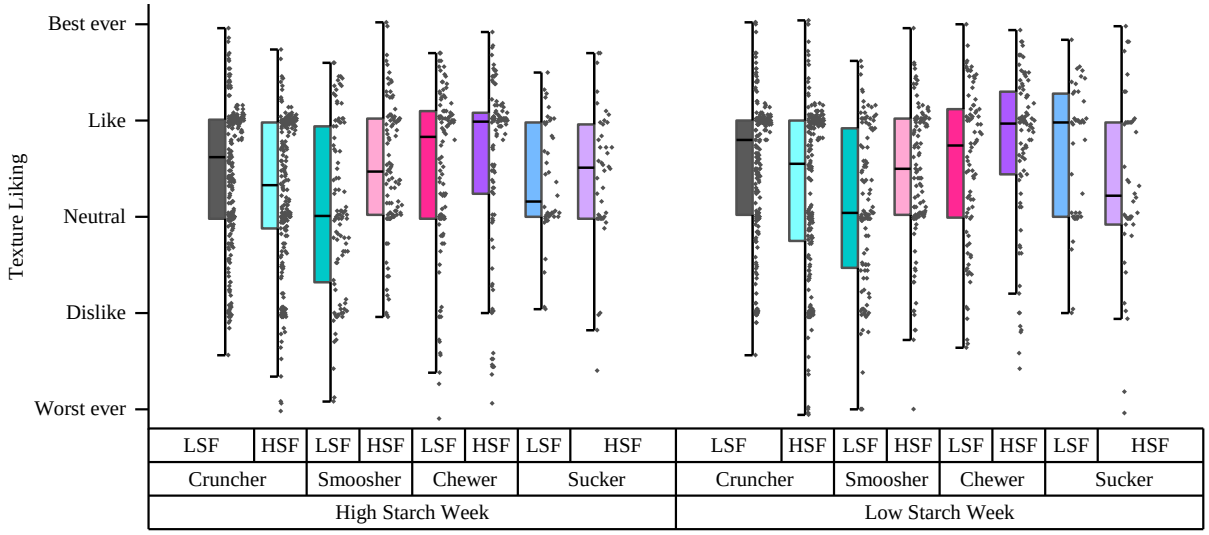

Figure 11. The effects of food type, intervention week, and mouth behavior categories on texture liking ratings. Low Starch Food is abbreviated "LSF" and High Starch Food is abbreviated "HSF". Boxes indicate the 25th to 75th percentile, the median is the horizontal line, and whiskers go from the 5th to the 95th percentile.

Table 9: Texture Liking Ratings					
Model: TextureLiking = FoodType MouthBehavior MouthBehavior*FoodType					
High starch week					
Effect	***Estimate (SE)***	***F (DF)***	***p-value***	***Comparisons***	***p-value* (t, DF)***
Food Type	Low starch food: 17 (3) High starch food: 22 (3)	2.44 (1/891)	0.12		
Mouth Behavior	Cruncher: 17 (3) Chewer: 25 (5) Sucker: 21 (7) Smoosher: 15 (5)	0.96 (3/98.7)	0.42		
*MouthBehavior*FoodType*	Low Starch Cruncher: 22 (4) High Starch Cruncher: 13 (4) Low Starch Chewer: 24 (5) High Starch Chewer: 27 (5) Low Starch Sucker: 20 (8) High Starch Sucker: 22 (8) Low Starch Smoosher: 4.0 (6) High Starch Smoosher: 26 (6)	8.09 (3/891)	<.0001	High to Low Starch Cruncher: High to Low Starch Chewer: High to Low Starch Sucker: High to Low Starch Smoosher: Within high starch food type, Smoosher to Cruncher: Within high starch food type, Smoosher to Chewer: Within high starch food type, Smoosher to Sucker:	0.0082 (-2.65, 891) 0.50 (0.68, 891) 0.83 (0.21, 891) <.0001 (4.11, 891) 0.043 (-2.05, 133) 0.92 (0.10, 119) 0.67 (0.43, 146)

Table 9 (continued): Texture Liking Ratings					
Model: TextureLiking = FoodType MouthBehavior MouthBehavior*FoodType					
Low starch week					
Effect	***Estimate (SE)***	***F (DF)***	***p-value***	***Comparisons***	***p-value* (t, DF)***
Food Type	Low starch food: 23 (3) High starch food: 23 (3)	0.01 (1/858)	0.92		
Mouth Behavior	Cruncher: 21 (4) Chewer: 31 (6) Sucker: 26 (7) Smoosher: 12 (5)	2.35 (3/88.3)	0.078		
*MouthBehavior*FoodType*	Low Starch Cruncher: 28 (4) High Starch Cruncher: 15 (4) Low Starch Chewer: 26 (6) High Starch Chewer: 36 (6) Low Starch Sucker: 35 (8) High Starch Sucker: 18 (8) Low Starch Smoosher: 3.6 (6) High Starch Smoosher: 21 (6)	9.75 (3/858)	<.0001	High to Low Starch Cruncher: High to Low Starch Chewer: High to Low Starch Sucker: High to Low Starch Smoosher: Within high starch food type, Smoosher to Cruncher: Within high starch food type, Smoosher to Chewer: Within high starch food type, Smoosher to Sucker:	0.0009 (-3.33, 858) 0.094 (1.68, 858) 0.047 (-1.99, 858) 0.0007 (3.41, 858) 0.38 (-0.89, 105) 0.086 (1.73, 93) 0.74 (0.33, 239)

*p-values from unadjusted pos-hoc comparisons. These are left unadjusted as many comparisons run by the analyses are not logical (example: End of high starch week to beginning of low starch week), and as these comparisons were planned a priori.
SE: standard error
Analyses were run separately for the two intervention weeks in order to avoid having to test and interpret a large number of two- and three-way interaction effects.

3.2.5 Overall Liking

Overall liking followed similar patterns to texture liking, just with a slightly different assortment of effects reaching significance. Significant effects were all driven by the interactions of mouth behavior and high vs. low starch foods. Crunchers again liked low starch foods more than high starch foods (p=0.029 high starch week p=0.035 low starch week). Chewers liked high starch foods more than low starch during the high starch week (p=0.043) but not during the low starch week (p=0.31). Smooshers liked the high starch foods more than the low starch foods (p=0.0003 high starch week and p=0.0078 low starch week). No significant differences were found for suckers. Data are shown in Figure 12.

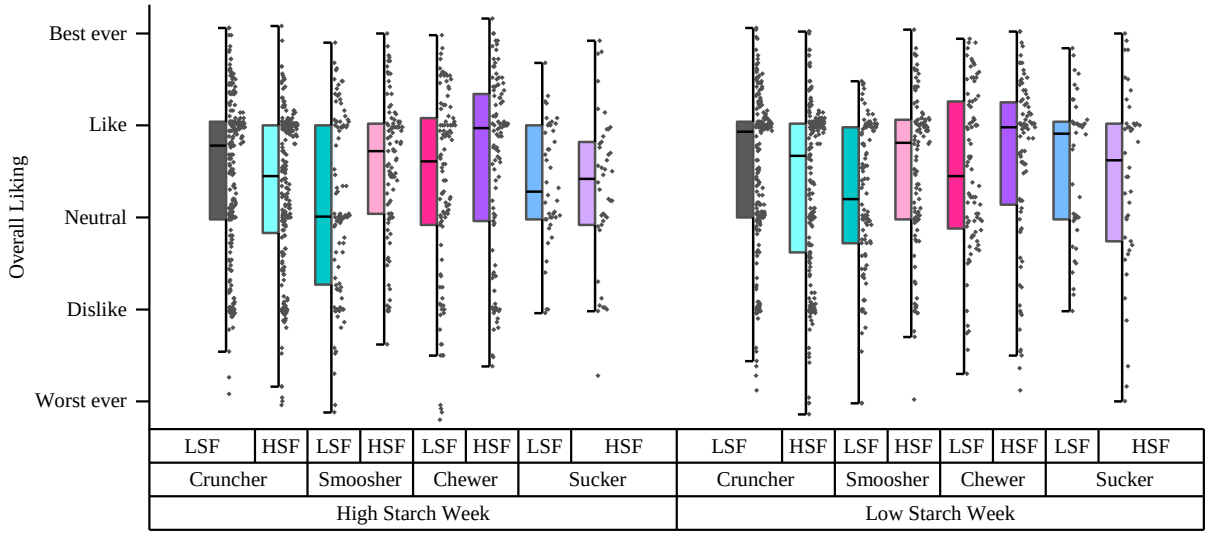

Figure 12. The effects of food type, intervention week, and mouth behavior categories on overall liking ratings. Low Starch Food is abbreviated "LSF" and High Starch Food is abbreviated "HSF". Boxes indicate the 25th to 75th percentile, the median is the horizontal line, and whiskers go from the 5th to the 95th percentile.

Table 10: Overall Liking Ratings					
Model: OverallLiking = FoodType MouthBehavior MouthBehavior*FoodType					
High starch week					
Effect	***Estimate (SE)***	***F (DF)***	***p-value***	***Comparisons***	***p-value* (t, DF)***
Food Type	Low starch food: 17 (4) High starch food: 22 (4)	2.87 (1/890)	0.090		
Mouth Behavior	Cruncher: 20 (4) Chewer: 22 (5) Sucker: 19 (8) Smoosher: 17 (6)	0.13 (3/94.4)	0.94		
*MouthBehavior*FoodType*	Low Starch Cruncher: 24 (4) High Starch Cruncher: 16 (4) Low Starch Chewer: 16 (6) High Starch Chewer: 28 (6) Low Starch Sucker: 20 (9) High Starch Sucker: 17 (9) Low Starch Smoosher: 6.3 (6) High Starch Smoosher: 28 (6)	7.05 (3/890)	0.0001	High to Low Starch Cruncher: High to Low Starch Chewer: High to Low Starch Sucker: High to Low Starch Smoosher: Within high starch food type, Smoosher to Cruncher: Within high starch food type, Smoosher to Chewer: Within high starch food type, Smoosher to Sucker:	0.29 (-2.19, 890) 0.043 (2.03, 890) 0.74 (-0.33, 890) 0.0003 (3.61, 890) 0.10 (-1.65, 129) 0.96 (-0.06, 116) 0.32 (1.00, 142)

Table 10 (continued): Overall Liking Ratings					
Model: OverallLiking = FoodType MouthBehavior MouthBehavior*FoodType					
Low starch week					
Effect	***Estimate (SE)***	***F (DF)***	***p-value***	***Comparisons***	***p-value* (t, DF)***
Food Type	Low starch food: 22 (4) High starch food: 24 (4)	0.25 (1/858)	0.62		
Mouth Behavior	Cruncher: 21 (4) Chewer: 29 (6) Sucker: 24 (7) Smoosher: 18 (5)	0.80 (3/84.4)	0.50		
*MouthBehavior*FoodType*	Low Starch Cruncher: 25 (4) High Starch Cruncher: 17 (4) Low Starch Chewer: 26 (7) High Starch Chewer: 33 (7) Low Starch Sucker: 27 (9) High Starch Sucker: 21 (9) Low Starch Smoosher: 10 (6) High Starch Smoosher: 26 (6)	4.37 (3/858)	0.0046	High to Low Starch Cruncher: High to Low Starch Chewer: High to Low Starch Sucker: High to Low Starch Smoosher: Within high starch food type, Smoosher to Cruncher: Within high starch food type, Smoosher to Chewer: Within high starch food type, Smoosher to Sucker:	0.035 (-2.11, 858) 0.32 (1.00, 858) 0.47 (-0.73, 858) 0.0077 (2.67, 858) 0.22 (-1.22, 104) 0.44 (0.78, 92.6) 0.62 (0.50, 231)

*p-values from unadjusted pos-hoc comparisons. These are left unadjusted as many comparisons run by the analyses are not logical (example: End of high starch week to beginning of low starch week), and as these comparisons were planned a priori.
SE: standard error
Analyses were run separately for the two intervention weeks in order to avoid having to test and interpret a large number of two- and three-way interaction effects.

3.2.6 Desire to Eat

For desire to eat, we observed some potential effects driven by an interaction of visit type (start compared to end of the week) and amount of pudding remaining/amylase activity. These patterns differed across the two intervention weeks. Desire to eat may have a trend to associate with more pudding remaining (less amylase activity) during the low starch intervention week ($p=0.082$), but during the high starch intervention week this association was reversed at the end of the week (less pudding remaining/more amylase activity association with greater desire to eat; $p=0.024$ for the interaction).

Desire to eat also showed effects that differed by the interaction of mouth behavior group and food type (high vs. low starch). Closer inspection shows these effects were found in the smooshers, which was a very small group of participants. Nonetheless, the smooshers had a lower desire to eat low starch foods ($p=0.0028$ high starch week and $p=0.0023$ low starch week). Data are shown in Figure 13.

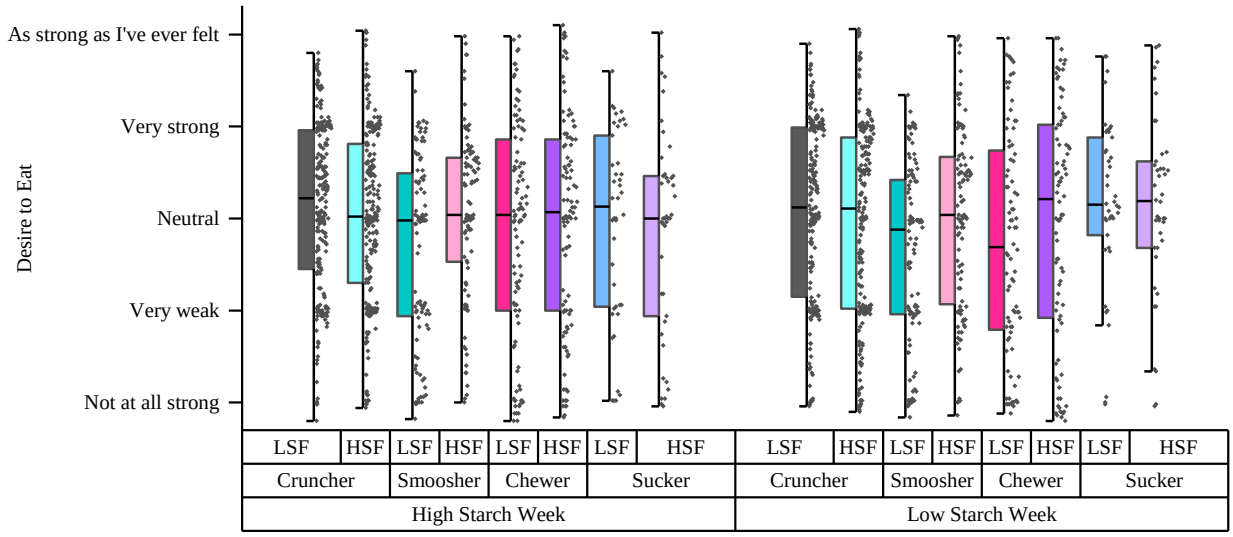

Figure 13. The effects of food type, intervention week, and mouth behavior categories on desire to eat ratings. Low Starch Food is abbreviated "LSF" and High Starch Food is abbreviated "HSF". Boxes indicate the 25th to 75th percentile, the median is the horizontal line, and whiskers go from the 5th to the 95th percentile.

Table 11: Sensory outcomes

Desire to Eat
Model: DesireToEat = Pudding40s VisitType FoodType MouthBehavior Pudding40s*VisitType MouthBehavior*FoodType

High starch week

Effect	***Estimate (SE)***	***F (DF)***	***p-value***	***Comparisons***	***p-value* (t, DF)***
Pudding40s	0.52 (1)	0.61 (1/180)	0.43		
Visit Type	Start: 2.4 (5) End: -3.4 (5)	2.10 (1/919)	0.15		
Food Type	Low Starch: -2.6 (5) High Starch: 1.6 (5)	1.46 (1/887)	0.23		
Mouth Behavior	Cruncher: 0.83 (5) Chewer: 0.016 (7) Sucker: -0.74 (10) Smoosher: -2.2 (8)	0.04 (3/148)	0.99		
*Pudding40s*VisitType*	Start to End difference in estimate: -2.8 (1.2)	5.09 (1/906)	0.024		
*MouthBehavior*FoodType*	Low Starch Cruncher: 3.5 (5)	3.55 (3/887)	0.014	High to Low Starch Cruncher:	0.21 (-1.26, 887)
	High Starch Cruncher: -1.8 (5)			High to Low Starch Chewer:	0.43 (0.79, 887)
	Low Starch Chewer: -2.4 (8)			High to Low Starch Sucker:	0.82 (-0.22, 887)
	High Starch Chewer: 2.5 (8)			High to Low Starch Smoosher:	0.0028 (3.00, 887)
	Low Starch Sucker: 0.34 (11)			Within high starch food type, Smoosher to Cruncher:	0.32 (-0.99, 160)
	High Starch Sucker: -1.8 (11)			Within high starch food type, Smoosher to Chewer:	0.65 (-0.45, 136)
	Low Starch Smoosher: -12 (8)			Within high starch food type, Smoosher to Sucker:	0.50 (0.68, 173)
	High Starch Smoosher: 7.5 (8)				

Table 11 (continued): Sensory outcomes

Low starch week

Effect	***Estimate (SE)***	***F (DF)***	***p-value***	***Comparisons***	***p-value* (t, DF)***
Pudding40s	2.1 (1)	3.05 (1/226)	0.082		
Visit Type	Start: -0.24 (5) End: -4.8 (5)	0.00 (1/887)	0.97		
Food Type	Low Starch: -5.6 (5) High Starch: 0.48 (5)	2.58 (1/854)	0.11		
Mouth Behavior	Cruncher: 0.89 (5) Chewer: -4.7 (8) Sucker: 2.7 (10) Smoosher: -9.0 (8)	0.58 (3/113)	0.63		
*Pudding40s*VisitType*	Start to end difference in estimate: -0.70 (1.2):	0.31 (1/888)	0.58		
*MouthBehavior*FoodType*	Low Starch Cruncher: 3.9 (6) High Starch Cruncher: -2.1 (6) Low Starch Chewer: -10 (9) High Starch Chewer: 0.96 (9) Low Starch Sucker: 3.1 (11) High Starch Sucker: 2.2 (11) Low Starch Smoosher: -19 (8) High Starch Smoosher: 0.92 (8)	3.99 (3/854)	0.0078	High to Low Starch Cruncher: High to Low Starch Chewer: High to Low Starch Sucker: High to Low Starch Smoosher: Within high starch food type, Smoosher to Cruncher: Within high starch food type, Smoosher to Chewer: Within high starch food type, Smoosher to Sucker:	0.19 (-1.32, 854) 0.13 (1.53, 854) 0.93 (-0.09, 854) 0.0023 (3.06, 854) 0.75 (-0.32, 114) 1.0 (0.00, 94.4) 0.92 (-.10, 279)

*p-values from unadjusted pos-hoc comparisons. These are left unadjusted as many comparisons run by the analyses are not logical (example: End of high starch week to beginning of low starch week), and as these comparisons were planned a priori.
SE: standard error
Analyses were run separately for the two intervention weeks in order to avoid having to test and interpret a large number of two- and three-way interaction effects.

CHAPTER 4. DISCUSSION

Our original hypotheses with this study were that:

- The pudding/salivary amylase activity assay would show
    - More activity (less pudding remaining) at the end compared to beginning of the high dietary starch intervention week, but no or less change during the low dietary starch intervention week. This was not supported by our data. Instead, we saw a potential trend for less activity after the low starch intervention.
    - More activity (less pudding remaining) for people with greater baseline starch consumption compared to less baseline starch consumption. This was not supported by our data.

Secondary hypotheses we had planned to analyze with the study were:

- If amylase activity changed from the intervention, then sensory ratings would shift for the intervention foods. For example, higher starch foods would become less chewy and/or less hard. Lower starch foods would not change in sensory properties in response to amylase changes. As we did not see changes due to the interventions, these hypotheses were not supported by our data.
- Sensory ratings would differ among the mouth behavior groups, especially for overall liking and texture liking. High starch snacks (expected to be chewier) would be better liked by the chewers. These were not strongly supported by our data. Instead, most of the patterns we observed were driven by the smooshers group. The patterns were logical for the definitions of the groups, but the observation of the greatest effects for this mouth behavior group was unexpected.

Details on the actual observations from our data, and how they fit or contradict our hypotheses and prior work, are below.

4.1 Salivary Amylase

We observed a trend for less salivary amylase activity (more pudding remaining in the syringe) at the end compared to the beginning of our interventions; this was driven by the low starch intervention week more than the high starch week. So, the original hypotheses were not confirmed by our interventions. However, the pattern of results is the same for the high starch week (Figure 4) which could indicate a lack of statistical power. This trend for less salivary amylase activity at the end compared to the beginning of our intervention weeks is not consistent with our expectations. Notably, the low starch foods were harder than the high starch foods (Figure 9), which also could have influenced saliva and potentially altered amylase activity. Yet again, we would have expected this increased hardness to result in more amylase (from more chewing and thus more saliva), and we observed the opposite. Previous research has demonstrated that salivary flow is independent of chewing rate, but that chewing starchy foods like bread can increase saliva and alpha amylase secretion (Mackie & Pangborn, 1990). Research surrounding solid and complex foods, mainly breads, also demonstrates high variability of salivary amylase activity between subjects (Joubert et al., 2017). The findings from the literature also suggest that the complexity and composition of the food, in this case varying types of bread, can influence saliva uptake, salivary amylase activity, and salivary protein concentration affected by water soluble proteins released from the bread during oral processing (Joubert et al., 2017). However, other literature suggests that this effect of chewing on salivary proteins is minimal (Al-Manei et al., 2020). While our study did not analyze other salivary proteins, previous research also shows that other proteins present in saliva may influence the sensory perception of starch-based foods, as sweetness, saltiness, bitterness, and roughness ratings from this research positively correlated to protein banding of an SDS-PAGE salivary profile (Lamy et al., 2021). Future work using similar foods to our study snacks could lead to more information about the effects of solid and complex foods on salivary amylase activity. With this knowledge in mind, we are curious to see how the chewing of our study foods could have influenced salivary flow, alpha amylase secretion, and salivary protein concentration. Further research is needed to determine whether the effect of our study interventions on changes in amylase activity is real or if this effect is an artifact of our analysis, as the effects were not strong.

We observed no patterns between prior diet and our amylase activity assay. This was not expected. We would have expected prior diets higher in starch to increase amylase activity. Prior work from our research group shows that exposure to a bitter flavanol associates with increased amylase concentration for participants who originally had relatively low flavanol intake (Davis & Running, 2021). Similar work from our lab demonstrates that amylase concentration increased after exposure to chocolate milk containing bitter flavanols (Crawford & Running, 2020). So, bitter flavanols may associate with increased concentrations of amylase, but it is important to note that these studies did not measure salivary amylase activity. Thus, as demonstrated from prior research, we know there are dietary interventions that shift amylase concentration in saliva. Bitter flavanols may inhibit amylase activity, and thus increases in amylase after a flavanol intervention could a compensatory mechanism (Crawford & Running, 2020). Consuming more flavanols may have caused saliva to adapt to produce more amylase, demonstrating a potential feedback mechanism between diet and amylase. Researchers have also seen that certain diets such as the Mediterranean diet and the low carbohydrate Ketogenic diet positively associate with salivary protein composition and higher amylase levels (Louro et al., 2021; Polito et al., 2021). Previous work has also studied the associations between salivary flow, composition, and habitual nutrient intake (Méjean et al., 2015). Researchers found a correlation between salivary amylase and consumption of simple carbohydrates (Méjean et al., 2015). This aligns with early literature that saw a positive association between salivary amylase secretion and carbohydrate intake (Squires, 1953). Nonetheless, our current work did not show any patterns connecting our starch-focused intervention with changes in amylase activity. Future work should implement more tightly controlled feeding trials to illuminate these potential relationships.

It is possible that our measurements at the beginning of the week were driven by baseline diet. Our analysis of dietary factors showed no relationship with the salivary amylase/pudding assay, but we had relatively few participants whose dietary reporting was plausible. Rather than the starch itself increasing amylase activity for the low starch week, it could be protein or fat content from the low starch week decreasing the activity, as lower starch diets tend to be higher in fat or protein (Kelly et al., 2019). Additionally, processed foods and foods containing high sugar and high fat contents tend to be softer, more plasticizing, and may even increase eating rate (Forde, et. al., 2020). We do not know the influence the prevalence of these types of foods in baseline diet of our participants, due to the low number of plausible dietary reporters. Future

studies should re-test the assay and control diet more directly, while also incorporating measures to assess food texture.

Much of the research surrounding salivary amylase and its relationship to food texture has been conducted with liquids or semisolids. Previous studies have demonstrated that alpha amylase activity varies strongly within subjects, and that this variation in activity causes changes in the perceived thickness, creaminess, and mouthfeel of starch containing foods (de Wijk et al., 2004; Heinzerling et al., 2008). Similar work has studied oral sensation and structural breakdown of hydrocolloid-thickened starch systems, and has determined that different characteristics of hydrocolloids (e.g. xanthan gum, lambda-carrageenan, and carboxymethylcellulose) can be responsible for differences in perceived smoothness and creaminess of the semisolids (Laguna et al., 2020). Little data are available on complex, hard foods, and salivary amylase.

Additionally, greater salivary flow associated with greater amylase activity in our study. This finding is not necessarily inconsistent with prior work. Typically, studies show a lower concentration of salivary protein with greater salivary flow, but volume is held constant in most of these studies. In our project, we added all the accumulated saliva to the pudding cup (rather than, for example, adding 1mL of each participants' saliva to their pudding cup). We did this to better reflect the natural eating environment, where all participants' saliva would mix with the food. Thus, while a person's salivary proteins, and thus amylase, may have been more dilute with higher salivary flow, a greater flow means more volume of saliva would have been added to the pudding, which could offset the lower concentration. In other words, adding a large amount of a dilute enzyme can result in greater activity than adding a small amount of a concentrated enzyme—it all depends on the total volume added. Prior research demonstrates that as the duration of salivary collection increased, saliva volume increased while salivary alpha amylase activity decreased (Beltzer et al., 2010). Again, this points to dilution of the amylase with longer collection times. Other research has shown this dilution effect as well, where measured salivary alpha amylase activity levels decrease in response to increased salivary secretion (Kugler et al., 1992). The literature suggests that even though amylase may be diluted with greater flow rates, this does not mean that the absolute quantity of proteins and amylase differ. The way in which saliva is collected from participants has also been discussed in the literature, and findings suggest that the method of salivary collection (e.g., passive drool, cotton, sponges, filter paper, etc.) may

influence the amount of salivary proteins and amylase collected from participants (Granger et al., 2007). Researchers found that collection methods like passive drool allow for several advantages. With passive drool, researchers can collect large sample volumes, reduce the influence of other collection substances like cotton or sponge, can assay for multiple markers, and can freeze the sample without collection substance interference (Granger et al., 2007). However, collection methods using cotton, sponges, and filter paper may limit the volume they can collect and the clarity of the samples. Future studies interested in saliva, salivary amylase, and sensory perception could consider various salivary collection methods to test for potential differences in salivary amylase activity. Ultimately, salivary flow rate captures a moment in time from the participant; in the mouth, saliva is constantly flowing in and being swallowed, fluxing the volume of saliva and its proteins. All these issues could influence relationships of chemical kinetics of an enzyme (amylase) and its substrate (starch).

Medication use associated with less amylase activity. We checked whether this pattern was confounded by saliva volume, as typically medication use is associated with dry mouth (Bardow et al., 2001). However, if anything, the participants in our study who reported using medications generated more saliva, not less. While we would hypothesize that people on medication would have less amylase activity, which is indeed what we saw, we would have expected this effect to be mediated through people on medication having lower salivary flow and less saliva volume. However, this was not the case in our data. Thus, at least in our study, medication usage and the effect of saliva volume are not inherently confounded. Usually, medication usage reduces salivary flow, so this finding of medication use associating with less amylase activity cannot be explained through salivary flow as the flow was greater for those who used mediations. This indicates the effects of medication use on salivary amylase activity may be more complex than a simple relationship with salivary flow or volume, which is worth further investigation.

We found a potential pattern between less salivary amylase activity and a greater desire to eat the study foods during the low starch intervention week. This finding was unexpected, as we would expect desire to eat to increase salivary flow (Meule & Hormes, 2015) and mouthwatering, which in turn would equate to more salivary amylase activity. However, this is not what we saw, and such unexpected finding warrants further investigation between amylase activity and desire to eat. The concept of mouthwatering is disputed in salivary research today,

and the idea that human salivary flow can be conditioned is controversial. However, data from Kershaw & Running demonstrate that conditioning salivary flow is possible, but that the duration that this conditioning lasts requires more investigation (Kershaw & Running, 2018). Interestingly, we only saw this pattern of greater desire to eat associating with lower salivary amylase activity for the low starch week. It is possible that participants' desire to eat was influenced by their lowered amylase activity, and that foods lower in starch were more desirable because they required less salivary amylase to orally break down. However, the effect we saw between salivary amylase activity and desire to eat was not particularly strong or consistent, so findings should be interpreted with caution.

4.2 Sensory Outcomes

4.2.1 Sweetness

Regarding sweetness, we observed a trend that failing the taste acuity test resulted in lesser sweet taste intensity, but only when observing data from the high starch intervention week (Figure 5). The patterns we observed appear to be the same in the low starch intervention week, so this may be an issue of statistical power as not many people failed the test. Notably, participants who passed versus failed the taste acuity test were not always the same. However, we note that any participant who ever failed the taste acuity test always failed first on visit 1. If they failed a second time, they failed on visit 1 and 2; and the pattern continued for visits 3 and failing 3 times and visit 4 and failing 4 times (only 1 participant failed all 4 times). This could indicate that these participants were learning the taste acuity test and becoming better over time. Alternatively, this pattern could indicate that these people had some taste loss and then regained taste function over the course of the experiment. It is also possible that these participants had COVID19 associated taste loss prior to participation in the experiment, and recovered some taste sensation over the course of the 4-week study (Parente-Arias et al., 2021; Printza et al., 2021; Reiter et al., 2020). Future studies should implement questionnaires about recent contraction of COVID19 to help illuminate patterns between taste or smell dysfunction and sensory ratings. Interestingly, the effect of taste acuity only impacted the attribute of sweetness, which makes sense because sweetness is the only attribute directly related to taste that we studied in the sensory testing. We propose that this observation is another indicator that the effect of failing the taste acuity test on reduced sweetness sensation is likely real, rather than just the participants

learning how to do the taste acuity test over time. If the effect were a learning phenomenon with no actual taste component, we would not expect to see any association between failing the taste acuity test and only sweetness intensity.

We observed a significant effect of food type on sweetness ratings during both the low and high starch intervention weeks. Our study foods consisted of oat and nut-based granola bars, candies, cookies, and pretzels. Inherently, these foods have varying levels of sweetness. Taking a closer look at how these foods were grouped into low starch and high starch categories, it is evident that while both categories had equal amounts of similarly sweetened granola bars, the low starch food category had two candies, Hershey Kisses and Skittles (highest ratings of sweetness for the foods), while the high starch food category contained the salty Snyder's of Hanover Mini Pretzel Twists (lowest ratings of sweetness for the foods). This grouping of foods is likely driving the difference in sweetness between the low and high starch foods, as seen in Figure 7.

4.2.2. Hardness

For hardness, participants thought the foods were harder after the high starch intervention week but not after the low starch week as seen in Figure 8. Notably, the low starch foods were rated as harder in general (see Figure 9). So, foods were perceived as harder after an intervention week of eating the softer, high starch foods. We found this interesting, especially as harder foods are often less "processed" (Bolhuis et al., 2014; Bolhuis & Forde, 2020; Mei Wee et al., 2018). We would expect our participants had a fairly "processed" or "Western" diet, which would be more like the softer, higher starch foods. However, due to the high number of implausible dietary reporters, and the lack of a dietary assessment tool for diet "hardness," we cannot confirm this hypothesis. If an assessment for dietary "hardness" could be developed, that might give us more insight into whether we observed the effect after the softer, higher starch week because it was a change from our participants' regular diet, or whether something else is driving this shift in sensory perception during the softer higher starch week compared to no effect during the harder, lower starch week.

The effect of mouth behavior on hardness ratings was driven by participants categorized as "smooshers," who perceived the high starch foods to be much harder. Again, the mouth behavior groups are defined by their preferred method of orally processing food. Crunchers and chewers

prefer to use their teeth to break down foods. Crunchers often use a strong initial bite to break the food, while chewers like to chew multiple times before swallowing. Suckers and smooshers prefer to use their tongue and palate or roof of the mouth to process foods. Suckers like hard foods that can be sucked on for a while, and smooshers like soft foods that can spread easily in the mouth and stay there for a while before swallowing (Jeltema et al., 2015, 2016, 2020). With these defining characteristics in mind, this means that the foods that many of our participants thought were softer in general (the high starch foods) were not as soft to the smooshers. Smooshers were a very small category, however, so results should be interpreted with caution. However, the patterns we observed are logical. In conjunction with the defined mouth behavior groups, we would expect people who prefer to smoosh foods in their mouth to rate things as harder compared to those who are chewing or crunching the same foods, as using the soft oral tissue to compress a food ("smoosh" it) may make the food seem harder than using the hard oral tissues to chew or crunch the food (Jeltema et al., 2015, 2016, 2020). Notably, the distribution of participants into the four mouth behavior groups is consistent with previous work that has used the typing tool; we saw the majority of our participants categorized as crunchers or chewers, while fewer participants were categorized as smooshers or suckers. (Jeltema et al., 2015; Zhou et al., 2021). In the future, we would like to further investigate mouth behavior using recorded chewing. Previous research has utilized recorded chewing and mouth behavior groupings to identify potential links between chewing styles and jaw movements (Wilson et al., 2018). The researchers found that when subjects were presented with foods that could be orally processed in various fashions, the subjects used chewing styles and jaw movements that associate with their different mouth behavior groups (Wilson et al., 2018). Similarly, we would record individuals while they chew different foods, such as almonds and chocolates, so that we could gather additional information on their jaw movements, chewing styles, and mouth behavior groups. We would use software to analyze the pattern that they chew to determine more sensory and oral processing information. These studies would help to gather more data on individual oral processing preference and may also have implications for food choice behavior.

4.2.3 Chewiness

No clear patterns were observed for chewiness. We found it interesting that we were able to observe patterns within the hardness ratings but not the chewiness ratings. We suspect that the

concept of hardness is slightly easier to grasp or more consistently defined for our untrained participants. They may have been able to use "hardness" more consistently than they would use chewiness to describe sensory attributes of foods. The connotations associated with hardness are particularly consistent, and there is little confusion surrounding its use as a sensory descriptor (Mei Wee et al., 2018). However, chewiness could indicate either a gummy or taffy consistency, or it could mean that the food requires many chews to break it down (Mei Wee et al., 2018). This discrepancy could have influenced how our participants rated foods for chewiness, resulting in no significant patterns. Perhaps further elaboration on the definitions of hardness and chewiness was needed within our sensory surveys, and future work using similar sensory attributes could test this theory.

4.2.4 Texture and Overall Liking

For texture liking and overall liking, the different mouth behavior groups liked the high and low starch foods differently in ways that generally align with the definitions of the mouth behavior categories. While our work was not designed to validate the mouth behavior groups, the data here are consistent with how we would expect those preferred mouth behaviors to align with liking for the foods in our study. The harder, low starch foods were liked more (especially for texture) by the crunchers. We saw no significant differences in texture liking of the food types from chewers, again aligning with how chewers are defined (Jeltema et al., 2015, 2016, 2020); they enjoy foods that take many chews to break down, which applies to both the low and high starch foods. Overall liking demonstrated that chewers liked the softer, high starch foods more during the high starch intervention week. Suckers liked the texture of low starch foods more during the low starch week, but no significant patterns were found for texture liking during the high starch week, or for overall liking either intervention week. Smooshers liked the texture of the high starch foods more than the low starch foods, and overall, they liked the high starch foods more for both intervention weeks. This makes sense because a lot of our high starch foods are softer and thus more "smooshable". The patterns observed here align with previous research from Jeltema and Beckley, whose validation of the mouth behavior tool demonstrates that crunchers and chewers are more likely to prefer harder foods, while suckers and smooshers are more likely to prefer softer foods, and that smooshers like to orally soften foods before eating (Jeltema et al., 2015, 2016, 2020).

4.2.5 Desire to Eat

Smooshers had lower desire to eat the low starch foods compared to high starch foods. This makes sense, considering the low starch foods were harder and less liked by the smooshers. Again, the small size of the smooshers group must be taken into consideration when evaluating these findings. Furthermore, none of the intervention foods we selected for the study are particularly "smooshable," aside from the pudding used in the assay (which we never used to gather sensory data). So, our participants' desire to eat ratings may correlate with their feelings towards the intervention foods after eating these foods for a weeklong intervention. Nonetheless, the data we see for desire to eat again seems to align with the logical concepts of the mouth behavior groups, as defined in prior literature (Jeltema et al., 2015, 2016, 2020), with the smooshers having greater desire to eat the less hard, higher starch foods.

4.3 Conclusions and Limitations

We do not see convincing evidence that our high/low starch interventions consistently altered salivary amylase. This may be due to a true lack of effect, to a lack of power, or due to the complex nature of the foods we selected for the study. Our intervention foods were inherently confounded with other properties of texture and macronutrient content. As noted, the high starch foods were less hard than the low starch foods. The low starch diet was made up of nut bars and other hard-textured foods, while the high starch diet used many varieties of oat-based granola bars. Thus, the chewing required for each would be different in intensity and chewing style. After observing all our data, we suspect that any potential differences in our salivary amylase assay may be explained by the hardness of our interventions—especially as chewing itself stimulates saliva (Mackie & Pangborn, 1990; Ono et al., 2007). This should be tested in future work. Macronutrient content of our intervention foods may have confounded the data as well. In conjunction with our original hypothesis, we expected a higher starch diet to yield more salivary amylase activity, though interestingly we saw this pattern with the low starch week and no effect for the high starch week. Rather than the starch itself increasing amylase activity for the low starch week, it could be protein or fat content from the low starch week decreasing the activity, as lower starch diets tend to be higher in fat or protein (Kelly et al., 2019).

Our decision to supplement the participants' diet with our intervention foods without controlling their diets could have confounded the results as well. While we were able to account

for the provided intervention foods that our participants were consuming, we did not change their typical diet outside of the added intervention snacks. Aside from our 3-day dietary recall prior to the intervention weeks, we did not survey participants on their daily intake during the intervention weeks. We allowed participants the option of either substituting our snacks as part of their normal dietary patterns or supplementing their daily intake with our provided foods. Many of our foods could be used as meal (particularly breakfast) or snack replacers, though we did not require participants to do so. Thus, future studies should consider the impact that substitution versus supplementation of study foods has on salivary amylase activity and sensory outcomes and should implement dietary records or recalls during intervention weeks to gauge intake aside from study foods.

Overall, the patterns we observed for the mouth behavior groups give evidence to help validate the Jeltema/Beckley Mouth Behavior Typing Tool. However, we had many participants categorized as crunchers and chewers, while few participants fell into the smoosher and sucker categories. This finding is consistent with previous literature that has grouped participants using the mouth behavior tool (Kim & Vickers, 2020; Zhou et al., 2021). Additionally, participants completed the mouth behavior survey at every research visit session, so we found that there was not always a consistent group selection from visit to visit. This inconsistency from week to week should be taken into account when interpreting the findings, and future work should consider the impact of multiple mouth behavior assessments during the course of an intervention.

Ultimately, while our original hypotheses were not confirmed by the experiment, we believe the pudding/salivary amylase activity assay would be valuable to continue to evaluate in different settings. Our findings on mouth behavior and sensory outcomes help to validate the use of the mouth behavior tool for future work. However, future studies should further investigate the precise nature of the relationships between mouth behavior groups and sensory ratings, using a new selection of low and high starch foods and more tightly controlling the intake of starch from the entire diet.

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APPENDIX

Please refer to the html file for the final code used in data analysis.