AN ABSTRACT OF THE THESIS OF

Amy Balto for the degree of Master of Science in Food Science and Technology presented on May 27, 2015.
Title: The Production and Human Taste Detection of Glucose Polymers with Distinct Chain Length Ranges

Abstract approved: _____

Juyun Lim

A majority of human caloric intake is in the form of complex carbohydrates, which have been assumed to be tasteless. However, there is considerable evidence from rat and human studies to support the existence of a secondary carbohydrate taste receptor that detects glucose polymers. Psychophysical studies in our lab suggest that humans can taste maltodextrins and cooked starch after active tasting and thus support the presence of the postulated complex carbohydrate taste receptor. However, maltodextrins contain simple sugars (i.e., glucose and maltose) that activate the known sweet taste receptor, which is confounding when investigating the perception of glucose polymers. This thesis had two objectives: to produce three glucose polymer stimuli devoid of simple sugars with distinct chain length ranges and to evaluate the human taste detection of the stimuli. We developed an economical highly-modifiable methodology to produce large quantities of three compositionally distinct glucose polymer stimuli (i.e., Samples 1-3) from a corn syrup solid (CSS) starch hydrolysis product (CSS DE20). Ethanol-water differential solubility was used

to remove simple sugars and to narrow the glucose polymer chain length ranges before rotary evaporation and freeze-drying. Determined by HPLC analysis, the average percent composition of Samples 1-3 were DP3-DP8, DP9+: 26.4 ± 1.5%, 73.6 ± 1.5%; 75.5 ± 1.8%, 24.5 ± 1.8%; and 0%, 100%. The average percent yield of Samples 1-3 were: 51.20 ± 1.33%, 8.04 ± 0.37%, and 25.98 ± 2.07%, respectively. To achieve the second thesis objective, the taste detection of Samples 1-3 at 6 and 8% (w/v) was evaluated using triangle tests without and with acarbose, an α-amylase inhibitor. Based on the findings of Sclafani et al. (1987), it was hypothesized that humans can detect the glucose polymer stimuli based on their proportion of short chain glucose polymers; the relative taste detection of the test stimuli would be Sample 2 (75% DP3-8) > Sample 1 (25% DP3-8) > Sample 3 (0% DP3-8). It was hypothesized that as glucose polymer stimuli concentration increased there would be an increase in the degree of taste detection. It was hypothesized that the presence of acarbose would alter the degree of taste detection if the hydrolysis of the glucose polymer stimuli was significant. According to the calculated d' values, subjects were able to significantly discriminate Sample 1 and Sample 2 against water, but were unable to detect Sample 3. There was a significantly higher discrimination of Sample 2 at 8% (w/v) compared to 6% (w/v). However, the discrimination of Samples 1 and 3 were not different across concentrations, possibly due to a trivial increase in the number of detectable molecules from 6 to 8% (w/v). In addition, the presence of acarbose did not elicit any significant difference in the discrimination of Samples 1-3 against water. This result implies that salivaryα-amylase did not play a significant role in the glucose polymer stimuli detection likely due to an insignificant alteration

of the stimuli saccharide profile. Overall, this research produced three glucose polymer stimuli devoid of simple sugars with distinct chain length ranges and supports the existence of a postulated complex carbohydrate receptor in humans that can detect short chain glucose polymers.

The Production and Human Taste Detection of Glucose Polymers with Distinct Chain Length Ranges

by
Amy Balto

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented May 27, 2015
Commencement June 2016

Master of Science thesis of Amy Balto presented on May 27, 2015

APPROVED:

Major Professor, representing Food Science and Technology

Head of the Department of Food Science and Technology

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Amy Balto, Author

ACKNOWLEDGEMENTS

I would like to express genuine appreciation to my major advisor, Dr. Juyun Lim, for the inspiring opportunities to study sensory and food science and to pursue a Masters degree at Oregon State University. I would like to sincerely thank my co-advisor Dr. Mike Penner for sharing his endless wisdom and for all of his patience and guidance. Thanks to Dr. Elizabeth Tomasino and Dr. James Hermes for being part of my defense committee. I am especially thankful of my labmates Trina Lapis, Tyler Linscott, and Tyler Flaherty who were a pleasure to work with and provided a nice balance of sass and support. Lucy (Shu) Xiang and Tuba Karaarskan were deeply helpful and compassionate during my time here. I am grateful of the graduate students and Faculty of the Department of Food Science who have supported me through this endeavor.

I would also like to express gratitude to my family and friends who have supported me throughout her entire academic career. Special thanks to Brian Yorgey, Teresa Niebur, Christina Mielke, Annie Balto, Ellen Parkin, and Austin Lowder who were my collective rock throughout this experience.

TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

Page

LIST OF FIGURES

LIST OF TABLES

LIST OF APPENDIX FIGURES

The Production and Human Taste Detection of Glucose Polymers with Distinct Chain Length Ranges

Chapter 1: General Introduction

1.1 Taste and taste transduction mechanisms

1.1.2 The five canonical tastes

It is generally accepted that there are five distinct taste qualities (i.e., salty, sweet, umami, sour, and bitter), which are associated with nutritional benefits and/or potential toxins (Breslin and Spector, 2008). For example, taste sensations of sweetness, umami, and saltiness are related to their status as macronutrients and macrominerals, meaning that the body needs them in large quantities (Gropper and Smith, 2013). Sweetness is a signal for simple carbohydrates such as sucrose, fructose, glucose, that allow for essential metabolic functioning (Breslin and Spector, 2008). Umami signals the ingestion of proteins, which provide essential amino acids, "the building blocks of life" (Scott and Verhagen, 2000). Saltiness is essential as its associated ions are necessary for regulating body fluid movement, signaling processes, and protein channels (Scott and Verhagen, 2000). Bitter taste can warn us against ingesting harmful poisons and toxins, which are often perceived as unpleasant at high concentrations. Sourness is important in food selection to warn against spoilage and rancidity (Scott and Verhagen, 2000) and can indicate fermented foods, which can have an enriched nutrient bioavailability (Breslin and Spector, 2008).

1.1.3 Taste transduction mechanisms for the five tastes

The five tastes are elicited in the brain by neurotransmitter signals transduced after ligands bind to taste receptors or ions pass through ion channels on microvilli at the

apex of taste receptor cells (Scott and Verhagen, 2000) seen below as Figure 1.1. The various classes of GPCRs (e.g., T1Rs, T2Rs) allow for the perception of different tastes. Taste receptors are class C, G-protein-coupled receptors (GPCRs) with active sites that bind specific ligands to produce bitter, umami, and sweet tastes (Alder et al., 2000; Nelson et al., 2002; Nelson et al., 2001). Bitter ligands (e.g., caffeine, cycloheximide, and quinine) are detected by T2R receptors, which vary in number and ligand selectivity and sensitivity (Alder et al., 2000). Umami is the sensation elicited by glutamate and L-amino acids binding to the T1R1/T1R3 receptor (Nelson et al., 2002). Simple sugars, artificial sweeteners, and sweet proteins are detected by the obligate heterodimer sweet-receptor T1R2/ T1R3 (Nelson et al., 2001). Ion channels allow for the passive flow of ions (e.g., H^+, Na^+) into a taste receptor cell to elicit sour and salty tastes.

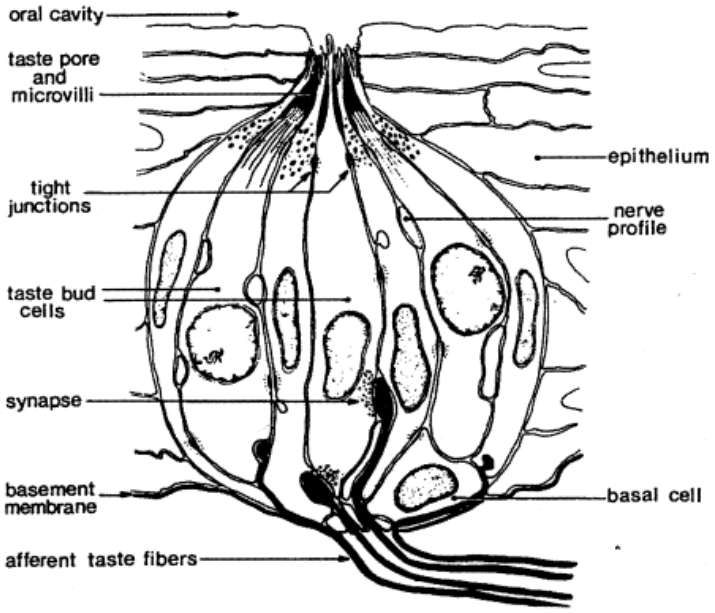

Figure 1.1. Taste bud and associated structures (Scott and Verhagen, 2000).

1.1.4 Non-canonical tastes

Taste receptor research is a relatively novel field and taste transduction mechanisms are not fully understood. For example, T1R3 is known to detect sweet compounds, but recently it was determined that the T1R3 receptor can also detect calcium ions (Tordoff et al., 2012), which are macrominerals and needed by the body in high quantities for bones, cellular processes, and muscle contractions (Gropper and Smith, 2013). Recent studies have focused on the investigation of potential fat taste receptors that could detect fatty acids. (Galindo et al., 2012). This fat taste could be beneficial as a signal for a source of caloric energy and possibly for essential fatty acids that are not produced in the body (Gropper and Smith, 2013).

Currently, for humans, it is believed that complex carbohydrates (i.e., starch, starch hydrolysis products) are tasteless (Ramirez, 1991) and that simple sugars (i.e., mono- and disaccharides) are the only form of carbohydrates that can be tasted. Raw starch is composed of insoluble granules that are thought to be unable to interact with taste receptors (Robyt, 2008). Although cooking ruptures granules and solubilizes starch (BeMiller and Whistler, 2009), it is thought that the soluble starch molecules are likely too bulky to interact with taste receptors. Studies have found that humans often find starch hydrolysis products (i.e., maltodextrins) to be less pleasant than sucrose or maltose and are described as “bland-tasting” or “tasteless” (Feign et al., 1987; Sclafani, 1987). However, the findings of several animal and human studies (see sections 1.2.1 and 1.2.2, respectively), have supported that rats and humans have a mechanism for the oral detection of complex carbohydrates. Complex carbohydrates may be detected through sensory perception mechanisms other than

taste, such as postingestive behavior and olfactory or somatosensory cues (Robyt, 2008). A major nutritional benefit of a complex carbohydrate taste would be that it signals for the consumption of a large quantity of calories (i.e., potential energy).

1.2 Complex carbohydrate sensing research

Note: the structure and terminology of complex carbohydrates discussed hereafter will be defined in section 1.4.

1.2.1 Animal studies

The preponderance of animal studies regarding complex carbohydrate sensing began with investigations into macronutrient balance (i.e., carbohydrates, protein) in the diet (i.e., food selection) of rats, which is influenced by several factors including taste, palatability, postingestive behavior, caloric value, and nutritional consequences (Hill et al., 1980). Assuming that complex carbohydrates are tasteless, Hill et al. (1980) unexpectedly found that rats consumed equal caloric values of sucrose solutions and "tasteless dextrinized starch powder" suggesting that sweet taste may not dictate carbohydrate intake. However, Hill et al. (1980) had a couple confounding factors such as the differing molecular structure between the disaccharide and polysaccharides and the physical form of the stimuli (liquid vs. solid), which were accounted for in a further study by Sclafani and Xenakis (1984) who used liquid solutions and both "bland" and sweetened (saccharin) polysaccharide stimuli (i.e., Polycose; a starch hydrolysis product and nutritional supplement). Sclafani and Xenakis (1984) found that there was a greater caloric value intake of the two Polycose stimuli compared to sucrose and the sweetened Polycose was consumed more than Polycose alone, thereby refuting the suggestion of Hill et al. (1980) that

sweetness does not influence carbohydrate intake. Importantly, Sclafani and Xenakis (1984) inferred that rats perceived the Polycose as minimally sweet as rats had an equal preference for 2% sucrose and 32% Polycose solutions, which could be attributed to the presence of glucose and maltose in Polycose. The results of Sclafani and Xenakis (1984) attributed caloric value and sweetness are factors in rats' decision of Polycose intake. However, the Sclafani and Xenakis (1984) study had a possible confounding factor as both the saccharin and Polycose stimuli contain ions (i.e., sodium, calcium), which have a distinct taste quality and macromineral status that could also influence consumption level. Nonetheless, the findings of this study led to the questions of why Polycose, a "bland" complex carbohydrate stimuli, was highly palatable for rats. In a series of experiments, Sclafani and Kirchgessner (1986) determined that, palatability is a more important driver than nutritional composition for rats' appetite for carbohydrates. The results of Sclafani and Kirchgessner negate the original thought that macronutrient balance of complex carbohydrates is an important factor for rats' food selection.

Researchers then investigated whether postingestive effects influenced driver of complex carbohydrate intake for the rat (Sclafani and Nissenbaum, 1987). The researchers Sclafani and Nissenbaum (1987) determined that it was likely that postingestive behavior was the driver of long term complex carbohydrate intake as rats increased their preference for bitter (sucrose octa acetate; SOA) Polycose solution to sucrose over time and orosensory properties (i.e., taste, odor, viscosity) were likely the drivers of initial SOA Polycose consumption. Then Vigorito and Sclafani (1987) determined that the sweetness (of glucose and maltose), viscosity, or

bitterness of SOA Polycose were not driving factors for rats that sham-feed (no digestion) the SOA Polycose solutions meaning that postingestive behavior may not mediate Polycose intake and thus, rats may be attracted to an unknown odor or taste of Polycose.

The assumption that complex carbohydrates are tasteless was reevaluated through several animal studies investigating the orosensory properties of various complex carbohydrate stimuli. Sclafani and Vigorito (1987) determined that Polycose intake was not affected when rats were unable to smell (anosmia) meaning that odor may not be a significant factor in complex carbohydrate intake. Researchers have shown that rats are able to discriminate the taste between: Polycose and starch (Ramirez, 1991); Polycose and MOS DP 3-7 (Ramirez, 1994); and MOS and simple sugars (Nissenbaum and Sclafani, 1987; Sclafani et al., 1987; Sclafani, 1998). From these studies it was inferred that there could be separate mechanisms of taste detection for simple sugars and complex carbohydrates.

There is neurological evidence to support that rats have separate taste mechanisms for perceiving Polycose and sugars (Sako et al., 1994; Treesuukol et al., 2011; Zuckerman et al., 2009). Researchers investigated the effect of a sweetness enhancer (i.e., $KCHO_3$) and two sweetness blockers (i.e., gurmarin, proteolytic enzyme Pronase E) on the rat neurological signals produced by simple sugars (i.e., glucose, maltose, sucrose, fructose) and Polycose (Sako et al., 1994), and found that the neural response to Polycose was not altered by the sweetness enhancers and blockers, which supports that there is a separate taste mechanism for Polycose and sugars. Another study found that rats lacking T1R2, T1R3, and both T1R2/T1R3

sweet taste receptors greatly consumed Polycose, but not glucose, maltose, or maltotriose (Treesukol et al., 2011), which also supports that there is a complex carbohydrate receptor distinct from the T12R/T1R3 sweet receptor that may perceive MOS and/or MPS found in Polycose.

Polycose contains a wide polymeric composition range (i.e., DP1-30+) and it was necessary to investigate the influence of glucose polymer chain length in order to determine the specific Polycose components (i.e., MOS, MPS) that could be detected possibly by a proposed secondary carbohydrate receptor (Sclafani et al., 1987). It was found that at low molar concentrations, rats had a greater intake of MOS DP3-6 compared to either glucose or maltose (Davis and Breslin, 2000). Another study determined that rats prefer various saccharide stimuli in following order: MOS DP4-8 > Polycose (DP 1-30+) = MPS DP ~43 > maltose (DP2) = maltotriose (MOS DP3) (Sclafani et al., 1987). This suggests that as glucose polymer chain length increases from DP2 to DP4-8, taste preference greatly increases, but after DP8, taste preference declines. However, the taste perception of glucose polymers of DP9+ was not specifically investigated and further research is necessary to determine at what specific DP the taste preference of glucose polymers decreases. Overall, the results of the rat studies suggests the existence of a secondary carbohydrate receptor that is defined by multiple glucose subsites.

1.2.2 Human studies

It has been assumed that humans are unable to taste complex carbohydrates (Feigin, et al., 1987; Ramirez, 1991), however, human exercise studies have shown that there is general positive influence of a complex carbohydrate (i.e., maltodextrin)

rinse on physical activity (e.g., running or cycling) performance (Carter et al., 2004; de Ataide e Silva et al., 2014). For either a placebo or carbohydrate rinse, subjects were instructed to rinse by swishing a solution around their mouth for a specified amount of time before expectorating. A recent review of eleven physiological studies investigating the effect of a carbohydrate rinse on exercise performance noted that there was great variability between the studies (de Ataide e Silva et al., 2014). However, there was a general trend of exercise performance improvement in subjects given a carbohydrate rinse versus a placebo (e.g., water, color and taste-matched solution; aspartame and saccharin solution to match sweetness of a glucose solution) (de Ataide de Silva et al., 2014). As a carbohydrate rinse did not have an effect on blood glucose levels, the researchers have concluded that the enhanced performance effect is not metabolic and there could potentially be taste receptors that influence neural pathways by activating reward sensors in the brain, thereby increasing central motivation (Carter et al., 2014). The authors of the review (de Ataide de Silva et al., 2014) agreed with the hypothesized neurological mechanisms and recognized the need for further research to investigate the mechanism of enhanced performance and activation of the brain regions.

A neurological study has used functional magnetic imaging (fMRI) to show that ingestion of maltodextrin and glucose activate overlapping and distinct areas of the brain (Chambers et al., 2009). The results showed that an 18% glucose solution significantly activated the insula/frontal operculum, an area of the medial orbitofrontal cortex (OFC), dorsolateral prefrontal cortex (DLPFC), left and right caudate (striatum), and dorsal regions of the anterior cingulate cortex. An 18%

maltodextrin solution activated the same brain regions as glucose except the left caudate and dorsal regions of the anterior cingulate cortex. In addition, the maltodextrin activated a rostral part of the anterior cingulate cortex. It is important to note that glucose and maltose are components of maltodextrin, which may allow for an overlap in brain activity. Chambers et al. (2009) speculated that because the concentration of glucose and maltose was only about 1.6% in the taste solution that it would not have significantly affected brain activity, however, this hypothesis was not been tested. Both the glucose and maltodextrin solutions elicited reward-related regions (i.e., insular/frontal operculum, striatum, OFC) of the brain (Chambers et al., 2009). Although maltodextrin was not rated as 'pleasant' as glucose, the maltodextrin solution still activated the ventral striatum, which is related to motivation (Chambers et al., 2009). Overall, Chambers et al. concluded that the differences in regions of the brain activated by glucose and maltodextrin solutions supports the presence of a secondary oral carbohydrate taste receptor possibly queued to the caloric value of maltodextrin components (Chambers et al., 2009).

Previous studies by Lapis et al. (2014, submitted) have also shown that humans can consistently taste complex carbohydrates. Lapis et al. (2014) investigated the taste perception of the commercially available maltodextrins STAR-DRI DE5, 10, and 20 and found that the responsiveness to STAR-DRI DE5 and DE10 was independent of that to simple sugars. The researchers found that response of maltodextrin STAR-DRI DE20 was correlated to sweet tastants (i.e., sucrose, glucose) likely due to the 0.7% of glucose and maltose present within the maltodextrin solution. The results of Lapis et al. (2014) suggest that whether subject

had relatively low or high salivary α-amylase activity did not dictate their ratings of the maltodextrins therefore, salivary α-amylase activity had an insignificant influence on the taste perception of the maltodextrins. Another study by Lapis et al. (submitted) has indicated that some people can consistently taste a low concentration of cooked starch after active tasting, likely due to the detection of glucose polymers produced through oral starch hydrolysis. This study also concluded that salivary α-amylase activity could influence the taste perception of cooked starch through the production of short chain glucose polymers. Although these studies support the existence of a secondary carbohydrate receptor, there are several confounding factors that need to be addressed in order to optimally evaluate the human taste detection of complex carbohydrates.

1.3 Limitations of previous work on complex carbohydrate sensing

1.3.1 Simple sugars present in starch hydrolysis products

Simple sugars (i.e., glucose and maltose), present within starch hydrolysis products, are known to be perceived by the T1R2/T1R3 sweet taste receptor and thus, interfere in the sole taste detection of the MOS and MPS. It has been shown in a previous study (Lapis et al., 2014) that for certain maltodextrins (i.e., STAR-DRI® DE5 and DE10), the concentration of glucose and maltose are below the taste detection threshold. However, there could have been an uninvestigated matrix effect (e.g., synergistic, additive) of the total saccharide profile (including hydrolysis products discussed below in section 1.3.2) on the taste detection threshold of glucose and maltose. Therefore, to investigate the proposed secondary carbohydrate receptor

without confounding simple sugars, it is necessary to develop glucose polymer stimuli devoid of simple sugars.

1.3.2 Salivary α-amylase present within the mouth

α-Amylase is an endoenzyme present in animal and human saliva that randomly hydrolyzes α-1, 4 glucosidic bonds of glucose polymers and produces maltose and MOS (Mishra et al., 2002). Maltose (as stated above) is confounding in taste studies investigating the sole detection of MOS and MPS. Although a past study in our lab (Lapis et al., 2014) concluded that the concentrations of simple sugars present in maltodextrins DE5 and DE10 were trivial and likely undetectable, it is possible that salivary α-amylase hydrolysis of the maltodextrins produced sufficient maltose to reach a detectable level. Further studies in our lab have shown that maltose and MOS are produced from the hydrolysis of starch (Lapis et al., submitted) and maltodextrins (unpublished data) within two-seconds. The maltose produced through hydrolysis could have elicited a confound taste in past studies. To investigate the sole taste detection of glucose polymer stimuli without the production of maltose or alteration of the MOS/MPS composition, it is necessary to inhibit salivary α-amylase.

1.3.2.1 Variables that influence human salivary α-amylase activity

There are numerous variables that can influence human salivary α-amylase activity such as genetics, health, and diet. Past studies in our lab have determined that there is a wide range of human salivary α-amylase activity (Lapis et al., 2014). Perry et al. (20007) found that the diploid gene copy number of the salivary amylase gene AMY1 is significantly positively correlated to human salivary α-amylase protein concentrations, which varies per person. Some health and diet factors that have been

shown to influence salivary α-amylase activity are caffeine, alcohol, prescription drugs, exercise, somatic and psychiatric diseases, and smoking (Rohleder and Nater, 2009). Salivary α-amylase level and activity typically follow a diurnal pattern and are lower in the morning, especially close to the time of awakening (Nater et al., 2007). The factors that influence salivary α-amylase activity (e.g., time of day, health, diet) will have to be considered when inhibiting a wide range of human salivary α-amylase activity.

1.3.2.2 Mechanism of human salivary α-amylase hydrolysis

Human salivary α-amylase is known to have six subsites with a specific catalytic cleavage site, seen as a black triangle between the -1 and +1 subsites in figure 1.1 (Robyt, 2005). Neither terminal glucose linkages nor α-1, 6 linkages can be cleaved by α-amylase (de Sales et al. 2012). The hydrolysis action of α-amylase on glucose polymers can produce maltose and maltooligosaccharides that contain both α-1, 4 and α-1, 6 linkages (de Sales et al., 2012).

1.3.2.3 Acarbose, an α-amylase inhibitor

Acarbose is a competitive α-amylase inhibitor that is approved for human consumption. Currently, acarbose is used as a treatment for type II diabetes patients to delay carbohydrate digestion by inhibiting pancreatic α-amylase activity thereby reducing glucose absorption (Balfour and McTavish, 1993). Seen below in Figure 1.2, acarbose is a pseudo-tetrasaccharide composed of acarviosine and maltose (Robyt, 2005). Acarbose is not absorbed by the body and is broken down in the large intestine into acarviosine and two glucose molecules. Acarbose has been used in several rat studies to assess whether acarbose could alter factors of obesity and diet motivations

(Vasselli et al., 1983) and to determine how acarbose effects carbohydrate intake (Davis et al, 1994). Acarbose was only found to have been used in one human taste study that investigated the effect of salivary α-amylase on the perceived texture and flavor of starch-based custards (Wijk et al., 2004).

The mechanism of acarbose inhibition of various amylases (i.e., bacterial, porcine, human) with differing numbers of glucose binding subsites has been investigated (Li et al., 2005; Yoon and Robyt, 2003; Robyt, 2005). Li et al (2005) has hypothesized that the reversible inhibition mechanism of human pancreatic α-amylase (five subsites) involves acarbose rearrangement prior to reversibly binding to α-amylase in a series of reactions (i.e., enzymatic hydrolysis, condensation, transglycosylation) resulting in uninhibited α-amylase and inert acarbose. The acarbose inhibition mechanism of human salivary α-amylase (six subsites) has only been studied with acarbose analogues (Yoon and Robyt, 2003; Robyt, 2005) and it has been postulated that acarbose generally inhibits α-amylases (both human and pancreatic) by binding to the glucose subsites (+1, -1) on either side of the catalytic cleavage group (Robyt, 2005). The mechanism of acarbose inhibition of salivary and pancreatic α-amylase would likely be similar as they have a high degree of similarity in amino acid sequence (Li et al., 2005).

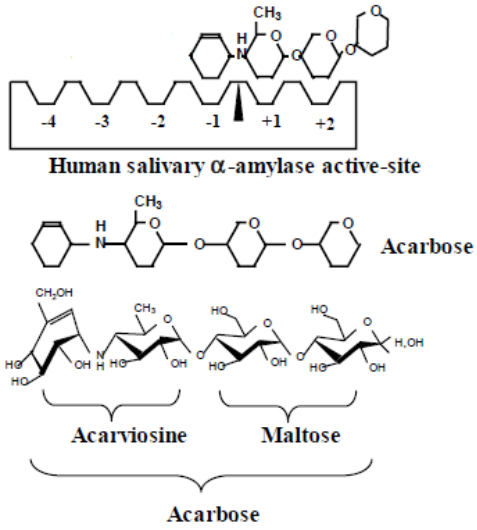

Figure 1.2 Acarbose chemical structure and proposed mechanism of HSA inhibition modified from Robyt (2005).

1.4 Complex carbohydrates

1.4.1 Starch and starch hydrolysis: structure and classification

Starch, an insoluble granule, is composed of the glucose polymers amylose and amylopectin, which contain both α-1→4 and α-1→6 linked glucose units (BeMiller, 2007) portrayed below in figure 1.3. Amylose is predominately linear with α-1→4 linked glucoses, but can also have α-1→6 branch points as well (0.3-0.5% of the total linkages; BeMiller, 2007). Amylopectin is a highly branched polymer with linear α-1→4 linkages and α-1→6 linked branches every 20-30 linearly linked glucose units (BeMiller, 2007).

Starch is often modified through enzyme and/or acid hydrolysis to produce starch hydrolysis products such as maltodextrins, and syrup solids (BeMiller and Whistler, 2009). Enzymes used for industrial starch hydrolysis are often associated with bond specificity as α-amylases preferentially cleave the α-1→4 glucosidic bonds. Glucoamylases can cleave the α-1→6 bonds and isoamylases preferentially

cleave the α-1→6 bonds (BeMiller and Whistler, 2009). During acid hydrolysis, both α-1→4 and α-1→6 bonds are cleaved (BeMiller and Whistler, 2009).

Starch hydrolysis products are composed of glucose and glucose polymers of various chain lengths: disaccharides, maltooligosaccharides (MOS), and maltopolysaccharides (MPS) (Neelam et al., 2012). MOS and MPS are generally classified by two factors, 1) the chain length of linked glucose units expressed as the degree of polymerization (DP) (Whistler, 1997) and 2) whether the glucose units are solely linked α-1→4 or if there is an α-1→6 link present. There are various DP definitions of MOS/MPS, but this thesis will use the terms "oligo" and "poly" to describe MOS and MPS having a DP3-10 and □DP10, respectively (IUPAC, 2007). MOS and MPS with at least one α-1→6 linkage are denoted by the prefix *-iso*. As seen in Figure 1.3, the disaccharides maltose (**A**) and isomaltose (**B**) both have a DP2, but are named differently because the two glucose units are linked α-1→4 and α-1→6, respectively.

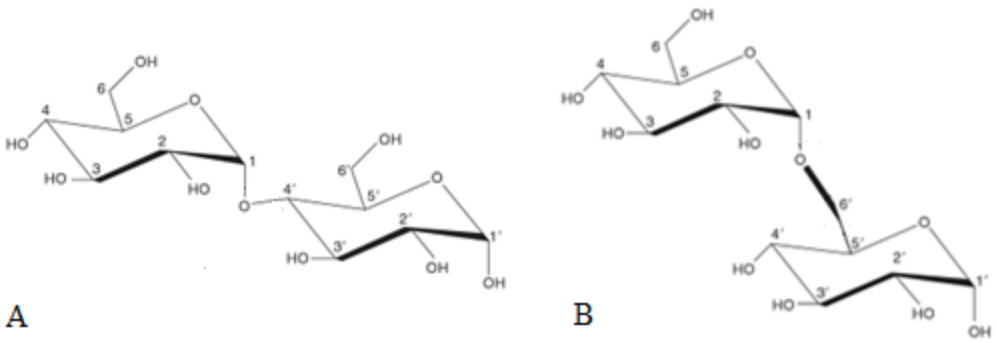

Figure 1.3 The chemical structure of (**A**) α-1→4 and (**B**) α-1→6 linked glucose units with numbered carbons (BeMiller and Whistler, 2009).

In the food industry, starch hydrolysis products are generally classified by their dextrose equivalence (DE), a measure of the total reducing sugars calculated as dextrose and expressed as a percentage of the total dry substance (Whistler, 1997).

Dextrose (i.e., anhydrous D-glucose) has a DE value of 100 (Bertolini, 2010). DE and the DP are related through the following equation:

$$DE = \frac{100}{DP}$$

Equation 1.1: The mathematical relationship between dextrose equivalence (DE) and degree of polymerization (DP).

Therefore, as there is an increase in the average DP, the DE value decreases and vice versa. As starch hydrolysis progresses, the average DP of the polymers decreases and the number of total reducing ends increases, resulting in a higher DE value (BeMiller, 2007). Although maltodextrin was the general name for partial starch hydrolysis products coined in 1879 by the German starch scientist Herzfeld (BeMiller and Whistler, 2009), it is now understood that maltodextrins and syrup solids are classified by a DE value <20 and ≥20, respectively (BeMiller and Whistler, 2009).

1.4.2 Starch and starch hydrolysis products as food sources

Starch is a major part of the human diet and is an important factor of human health. Starch, a storage form of complex carbohydrates in plants, supplies about 70-80% of human caloric intake through the consumption of cereals (e.g., corn, wheat), roots (e.g., cassava), tubers (e.g., potato) and other foods (BeMiller, 2007; Bertolini, 2010). Starch is also found in numerous processed foods that make up the western diet (e.g., breads, pastas). As stated above, complex carbohydrates (i.e., starch) intake is important factor for health especially in regards to chronic dietary diseases.

Starch, in several forms (e.g., cooked starchy-vegetation (e.g., corn, potatoes), raw powder, a cooked solubilized gel), and starch hydrolysis products are ubiquitous ingredients in the food industry. Starch and starch hydrolysis products are used in a

variety of food products (e.g., soups, dressings, confections) to influence structure/function properties like hygroscopicty, viscosity, stability, gelling, and browning (BeMiller and Whistler, 2009; Sheldrake, 2010). Sensory properties of foods such as mouth feel, appearance, and texture are also influenced by starch and starch hydrolysis products. Starch hydrolysis products can additionally influence the sweetness of food products as they contain glucose and maltose (BeMiller and Whistler, 2009; Bertolini, 2010; Sheldrake, 2010).

There have been numerous studies of the structure/function/sensory properties of starch and starch hydrolysis products (Hofmann et al., 2015; Marchal et al., 1999; Chronakis, 1998; Feigin et al., 1987). However, starch hydrolysis products vary greatly in their glucose polymer composition. Therefore, to better understand how the starch hydrolysis product composition influences its properties in food systems, it would be beneficial to reduce the compositional range of starch hydrolysis products to a narrow range of glucose polymers (i.e., MOS/MPS).

1.4.3 Refinement of starch hydrolysis products

Several fractionation techniques may be utilized to refine starch hydrolysis products, such as maltodextrin and syrup solids, into preparations with narrow MOS/MPS chain length ranges based on differences in molecular properties (e.g., chemical affinity, molecular size, electronegativity, diffusion) (Giddings, 1991; Sanz and Martinez-Castro, 2007). A combination of ultra- and nanofiltration systems have been used to remove simple sugars and isolate oligosaccharides DP3-10 (Kamada, 2002). Ion-exclusion chromatography and supercritical fluid extraction have been used in the food industry to fractionate saccharides into large quantities of purified

product (i.e., sucrose, lactose, and galactosyl oligosaccharides) (Sen et al., 2011). Charcoal column chromatography has been used to fractionate starch hydrolysis products into milligram quantities of MOS of individual DP (French, 1966; Whelan, 1953). However, in general, using the fractionation techniques stated above may not yield food grade products that can be applicable in sensory research and can be relatively complex, costly to scale up, and energy intensive.

1.4.3.1 Differential solubility

Differential solubility, using a solvent system to selectively precipitate solutes, is another fractionation technique that could be utilized to refine starch hydrolysis products for use in sensory research. There are numerous U.S. Pharmacopeia (USP)-grade organic solvents (e.g., acetone, ethanol, propylene glycol, etc.) approved as food additives/processing aids that are utilized to fractionate starch hydrolysis products (National Research Council, 2003). However, the appropriate solvent system used to fractionate starch hydrolysis products into narrow ranges of food-grade MOS/MPS, must 1) precipitate MOS/MPS, 2) readily dissolve glucose and maltose, and 3) be completely removable.

Using ethanol as a solvent for ethanol differential solubility has several benefits. The solubility trends of glucose, maltose and larger glucose polymers is well understood: generally, as ethanol concentration increases the concentration of soluble saccharides decreases (Defloor et al., 1998; Bouchard et al., 2007). Importantly, the solubility of glucose and maltose in ethanol has been documented (Alves et al., 2007). Lastly, it is important to completely remove ethanol from the refined starch

hydrolysis fractions as it has been noted to have both odor and taste qualities (Mattes and DiMeglio, 2001).

There have been numerous studies that have utilized ethanol differential solubility to refine starch hydrolysis products (Defloor et al. 1998; Frigard et al., 2000; French, 1966; Whelan, 1953). Early research focused on the combined use of charcoal columns and ethanol solubility to produce milligram quantities of singular DP compounds (French, 1966; Whelan, 1953). More recent research has precipitated MOS/MPS of similar chain length ranges with ethanol to study enzymatic degradation of amylopectin (Frigard et al., 2000). Defloor et al. (1998) has fractionated maltodextrins to narrow the chain length ranges in order to study their role in bread firming. However, there is currently no published method that use ethanol differential solubility to fractionate starch hydrolysis products into glucose polymer preparations with narrow chain length ranges devoid of simple sugars.

1.5 Thesis objectives

This thesis had two distinct objectives: 1) to produce three glucose polymer stimuli (i.e., MOS/MPS) devoid of confounding simple sugars (i.e., glucose and maltose) with distinct chain length ranges and 2) to evaluate the human taste detection of the three glucose polymer stimuli accounting for the presence of salivary α-amylase with acarbose.

Due to the lack of commercial glucose polymer preparations with narrow chain length ranges, starch hydrolysis products have been used in human studies to investigate oral digestion and taste perception of glucose polymers (see section 1.2.2 above). However, starch hydrolysis products are not optimal stimuli for such studies

as they contain simple sugars (i.e., glucose, maltose). Currently, there are not any published methods to prepare lab-scale quantities of food-grade glucose polymers with narrow chain length ranges devoid of simple sugars. In regards to sensory research, the refined glucose polymers should be: 1) food-grade and thus, safe for human consumption, 2) produced in large quantities (i.e., grams to kilograms) for use in taste studies, and 3) produced using economically-viable methods

To achieve the first objective of this thesis, we developed a method to produce glucose polymer stimuli enriched in MOS and/or MPS. This method (described in Chapter 2) utilized the selective fractionation of commercial corn syrup solids using differential solubility with water-ethanol solutions. Commercial corn syrup solid preparation STAR-DRI DE20 was chosen as the starting material because of its relatively low simple sugar content (about 7.5%) and high MOS content, about 46.6% MOS. It has been hypothesized that glucose polymers of DP4-8 are optimally perceived by the postulated secondary carbohydrate receptor (Sclafani et al., 1987). However, it is possible that the receptor could perceive MPS DP9+ as well. Therefore, it was necessary that the three glucose polymer stimuli be distinct in regards to their chain length ranges regarding both MOS and MPS.

To achieve the second objective of this thesis, a psychophysical study was performed with two sessions of triangle tasks using 6% and 8% (w/v) solutions of the three glucose polymer stimuli, without and with acarbose. An acarbose treatment was used in order to inhibit the salivary α-amylase hydrolysis of the three glucose polymer stimuli. Each session had six triangle tests, three of which containing 145.26 mg of acarbose to meet the IRB limitation of acarbose (50-200 mg per session three

times daily). It was hypothesized that there will be an effect of concentration and the presence of acarbose on the taste detection of the three glucose polymer stimuli and that subjects will be able to detect the taste of the glucose polymer stimuli with different degrees of detection based on the proportion of short chain glucose polymers. It was expected that the glucose polymer stimuli with the largest proportion of MOS DP4-8 would be perceived more easily based on the findings of Sclafani et al. (1987). Humans may be able to detect glucose polymers with a DP9+, however, their detection may be impeded due to the steric hindrance of larger molecules that may not fit within the subsites of the proposed secondary carbohydrate receptor.

Submitted to *Food Chemistry*

Chapter 2: On the use of differential solubility in aqueous ethanol solutions to narrow the DP range of food-grade starch hydrolysis products

Amy S. Balto and authors

Department of Food Science and Technology, Oregon State University, Corvallis, OR

CONTRIBUTION OF AUTHORS

Dr. Juyun Lim provided the concept of the project and sponsored the work. Dr. Michael Penner was involved in the experimental design/data interpretation and provided access to an HPLC system. Trina Lapis and Rachel Silver performed the BCA assay and subsequent data analyses. Drew Ferreira collected NMR data and assisted in the data analysis. Dr. Christopher Beaudry sponsored NMR analyses and provided a high-pressure pump for rotary evaporation.

Correspondence to be sent to: Juyun Lim, Ph.D. and Michael Penner Ph.D.
Department of Food Science and Technology,
Oregon State University,
100 Wiegand Hall, Corvallis, OR, 97331, USA
Email: juyun.lim@oregonstate.edu, mike.penner@oregonstate.edu
Phone: +1-541-737-6507, +1-541-737-6513

Key words: starch hydrolysis products, corn syrup solids, maltooligosaccharides, maltopolysaccharides, ethanol-fractionation, dispersity

Running Head: Maltodextrin ethanol fractionation

2.1 Abstract

Considerable research is focused on understanding the functionality of starch hydrolysis products (SHP) consisting of glucose, maltose, maltooligosaccharides (MOS), and maltopolysaccharides (MPS). A confounding factor in this research is the high molecular dispersity of commercially available SHP. The study presented herein characterizes a flexible fractionation approach for lowering the dispersity of such products. This was accomplished by fractionating a corn syrup solids (CSS) preparation based on the differential solubility of its component saccharides in aqueous-ethanol solutions. Products obtained from selected fractionations were characterized with respect to degree of polymerization (DP; liquid chromatography), dextrose equivalency (reducing sugar assays), and prevalence of branching (NMR). Glucose and maltose were preferentially removed from CSS using high (≥90%) ethanol extractants. Preparations with relatively narrow ranges of MOS, lower DP MPS, and higher DP MPS were obtained through repetitive 70%-ethanol extractions. Linear, as opposed to branched, MOS and MPS were preferentially extracted under all conditions tested.

2.2. Introduction

Starch hydrolysis products, including maltodextrins, corn syrup solids, high fructose corn syrups, glucose syrups, and cyclodextrins, have been commercially available for use as food ingredients for many years. Of these, maltodextrins (MD) and corn syrup solids (CSS) are primarily composed of glucose and glucose polymers [i.e., disaccharides, maltooligosaccharides (MOS), and maltopolysaccharides (MPS)] (Damodaran, Parkin, & Fennema, 2008). MOS and MPS are typically classified

based on two factors, (a) their chain length expressed as degree of polymerization (DP) and (b) whether or not the molecules contain α-1, 6 linkages (Whistler & BeMiller 1997). The IUPAC suggestion with respect to the nomenclature of polymers with repeating units, as is the case with MOS and MPS, is to use the term "oligo" for those polymers with DP 3-10. Therefore, in this paper MOS and MPS are defined as having DP3-10 and DP>10, respectively. Commercial MD and CSS are differentiated based on their dextrose equivalency (DE), where DE is the reducing power of the product as a percentage of the reducing power of an equivalent weight of glucose. CSS have DE values equal to or greater than 20; MD have DE values less than 20. DE values of products are inversely related to the number-average DP of the component glucose polymers.

The structural, functional, and nutritional properties of commercial CSS/MD preparations have been studied extensively (for general reviews see Hofmann, Van Buul, & Brouns, 2015; Marchal, Beeftink, & Tramper, 1999; Chronakis, 1998). This includes studies pertaining to their use in fat replacement formulations (Hadnadev et al., 2014; Psimouli & Oreopoulou, 2013), thickener applications (Lakshminarayan, Rathinam, & KrishnaRau 2006; Avaltroni, Bouquerand, & Normand,2004; Wang & Wang, 2000), bulking agent applications (Shah, Jones, & Vasiljevic, 2010), emulsion stabilization (Dokic-Baucal, Dokic, & Jakovljevic, 2004), gelation (Loret, Meunier, Frith, & Fryer, 2004; McPherson & Seib, 1997), flavor encapsulation (Madene, Jacquot, Scher, & Desobry, 2006), applications as drying aids (Werner, Fanshawe, Paterson, Jones, & Pearce, 2006), their use in infant and clinical nutrition (Braquehais & Cava, 2011), and as a starting material for the production of novel dietary fibers

(Leemhuis et al., 2014). The vast majority of such studies compare the performance of commercially available CSS/MD preparations based solely on their DE values. This is bothersome as CSS/MD preparations of equivalent DE may have significantly different DP profiles and it is the DP profile that is likely to dictate functionality (White Jr., Hudson, & Adamson, 2003). An approach to improving the interpretability of such studies is to use CSS/MD preparations having relatively narrow, well-defined DP profiles. This approach would also be beneficial in sensory studies investigating the taste properties of CSS/MD preparations. In such cases, it would be particularly important to remove the simple sugars from CSS/MD preparations since they, in particular, evoke sweet taste (Lapis, Penner, & Lim, 2014; Turner, Byblow, Stinear, & Gant, 2014; Hettinger, Frank, & Meyers, 1996).

A number of fractionation techniques can be applied to the task of narrowing the DP range of CSS/MD preparations. These separation techniques, which are based on differences in molecular size, ion interactions, hydrophobicity, solubility, etc., are analogous to those used in carbohydrate analyses (Sanz & Martínez-Castro, 2007). The techniques best suited for CSS/MD fractionation are expected to be dependent, at least to some extent, on how the fractionated CSS/MD are to be used. CSS/MD-based studies in the food sciences often attempt to correlate the physicochemical properties of CSS/MD-containing products with the sensory attributes of those products. With this in mind, the fractionation technique used to modify the DP profile of a CSS/MD preparation (1) should be capable of producing relatively large amounts of material such that functional and sensory tests can be performed (i.e. tens to hundreds of grams of refined CSS/MD preparations are likely to be required), (2) the resulting

final products must be food grade, and (3) the methods used cannot be prohibitively expensive when working at the scale necessary for functional/sensory studies. Furthermore, it would be beneficial if the glucose and maltose content of the fractionated MOS/MPS preparations was minimized since the sweetness associated with these sugars may confound the preparations' other sensory properties (Blanchard & Katz, 2006; Feigin, Sclafani, & Sunday, 1987) . Fractionation schemes based on the differential solubility of MOS/MPS in ethanol/water mixtures are capable of meeting all of the above criteria. A further benefit of such schemes is the antimicrobial nature of ethanol.

The general relationship between the DP of MOS/MPS and their relative solubility in ethanol/water mixtures is well established. In general, MOS/MPS decrease in solubility with increasing ethanol concentrations and for any given ethanol concentration the higher the DP of the MOS/MPS the lower its solubility (Defloor, Vandenreyken, Grobet, & Delcour, 1998; Bouchard, Hofland, & Witkamp, 2007). These relationships have been exploited in cases where the DP of MOS/MPS is relevant to data interpretation. For example, Robyt & French (1967) used ethanol precipitation (final concentration 66% w/v) to separate larger MPS (average DP $\geq$20) from smaller MOS/MPS (DP $\leq$12) while studying the action-pattern of amylase-catalyzed amylose hydrolysis. Frigård, Andersson, & Åman (2002) used a similar approach, precipitating MOS/MPS with sequential additions of ethanol (ethanol concentrations from 20 – 80% w/v), to study the enzymatic digestion of amylopectins. Gelders, Bijnens, Loosveld, Vidts, & Delcour (2003) also used stepwise increases in ethanol content (10% w/v increments) to obtain MOS/MPS of

similar DP for subsequent chromatographic analyses. The amounts of MOS/MPS produced in each of these studies were on the analytical scale, typically milligrams. Fractional precipitation with ethanol has also been used on the preparative scale, such as for the separation of amylose and amylopectin from starch dispersions/solutions (Patil, Somvanshi, Gupte, & Kale, 1974) and for the partial fractionation of MD preparations in an investigation of their role in bread firming (Defloor et al., 1998).

The present paper describes an ethanol-based fractionation approach for use with commercially available CSS/MD products that results in food-grade MOS/MPS preparations having relatively narrow DP profiles. The approach is an extension of that presented by Defloor et al. (1998) in which they used single ethanol precipitations/extractions to narrow the DP profile of commercial MD preparations. Their approach was successful in that the average DP of the MOS/MPS preparations shifted relative to that of the starting material; the associated standard deviations describing DP dispersity decreased but the actual DP-ranges of the different MOS/MPS preparations remained large. This result is undoubtedly due to the use of single ethanol extractions for fractionations. Equilibrium considerations based on component saccharide solubilities suggest that multiple precipitations/extractions will significantly improve the DP character of the resulting MOS/MPS preparations. That is the approach outlined in this work to obtain relatively large amounts of food-grade solvent-free MOS/MPS preparations of relatively narrow DP range containing minimal amounts of glucose and maltose.

2.3 Materials and Method

2.3.1 Materials:

Corn syrup solids (CSS): STARDRI® DE20, kindly provided by Tate & Lyle Ingredients Americas (Decatur, IL).

Carbohydrate standards: glucose and maltose (Sigma Aldrich Corporation, St. Louis, MO); maltotriose, maltotetraose, and maltooctaose (Carbosynth Limited, UK); maltopentaose, maltohexaose, and maltoheptaose (TCI America, Portland, OR).

Reagents: ACS-grade anthrone (99%, Alfa Aesar, Ward Hill, MA); bicinchoninic acid sodium salt (BCA; Pierce Chemical Co., Rockford, IL); cupric sulfate pentahydrate (Sigma Aldrich Corporation, St. Louis, MO)

Solvents: ACS/USP-grade ethanol (100%, Pharmco Aaper, Shelbyville, KT); deuterium oxide (99.96%, Cambridge Isotope Laboratories, Tewksbury, MA); deionized water for aqueous solutions and HPLC analyses (18.2 Ω, produced using a Millipore Direct-Q® 5 UV-R water purification system).

2.3.2 Methods

2.3.2.1 MOS/MPS Sample Preparation Procedure - In the following text the term "washed" is used in reference to components recovered from the solid phase following centrifugation of a liquid/solid two phase system; the term "extracted" is used in reference to components recovered from the liquid phase following centrifugation of a liquid/solid two phase system.

Fractionation step 1

A 50% (w/v) CSS in water mixture was prepared by adding 75 g CSS to a 150 mL volumetric flask to which DI water was added to volume; stirring was continued until a translucent solution was obtained. The solution was then split into thirds, 50 mL each, in three separate beakers. To each beaker was added 450 mL 100% ethanol;

ethanol addition resulted in immediate formation of a white opaque suspension and visible precipitate. The opaque suspension was stirred for 5 minutes at 300 rpm, the liquid phase was then transferred to 250 mL HDPE bottles and centrifuged for 15 minutes at 10,000 rpm. The resulting clear supernatant was decanted and saved for analysis. The washed white pellet was returned to the original beaker containing remnant precipitated solids; the combined solids in the beaker, at this point, had thus been washed once with 90% ethanol. To the once-washed solids in each beaker was added 50 mL of DI water, with stirring, to again produce a clear solution. Ethanol, 450 mL, was again added to the solution, followed by mixing, centrifugation, and decantation as previously described. The solids at this point were twice-washed with 90% ethanol. This overall process was repeated four more times. The recovered solids had thus been six-times washed with 90% ethanol, all done at ambient temperature, *i.e.,* 18-21 °C. Each wash consisted of first dissolution of the solids in water, then precipitation by the addition of ethanol. The six-time 90% ethanol-washed solids, contained in the three beakers, was either dried for use directly or further processed as described below (see *"Fractionation step 2"*). When drying directly, the solids in each beaker were first dissolved in 50 ml DI water and then combined in one 1000 ml round bottom flask. Residual solvent was removed by repeated solvent-exchanges; the resulting solvent-free viscous aqueous solution was then freeze-dried as described below (see *Solvent removal and drying*). The resulting solid preparation is hereafter referred to as 90% ethanol-insoluble corn syrup solids (90EI-CSS).

Fractionation step 2

The six-time 90% ethanol-washed solids (90EI-CSS; contained in three beakers as a result of *Fractionation step 1*) were again dissolved in 50 ml water, then 117 mL 100% ethanol was added to give a 70% ethanol suspension. After stirring at 200 rpm for 5 minutes the readily-decanted off-white 70% ethanol suspension was centrifuged at 10,000 rpm for 15 minutes. The clear supernatant was decanted into a round bottom flask, ethanol was removed as described below, and the resulting aqueous solution was freeze-dried resulting in a preparation hereafter referred to as 90% ethanol-insoluble/70% ethanol-soluble corn syrup solids (90EI/70ES-CSS). The insoluble solids resulting from the first 70% ethanol wash were subsequently washed five more times by first dissolving the solids in 50 mL DI water, adding ethanol to a final concentration of 70%, stirring, centrifugation, and decantation of the liquid phase in a manner analogous to that described for "*Fractionation step 1*." The five 70% ethanol extracts were discarded; *i.e.,* only the initial 70% ethanol extract was used to make 90EI/70ES-CSS. The resulting six-times 70% ethanol-washed solids were processed to remove ethanol and freeze dried as described below; this preparation is hereafter referred to as 90% ethanol-insoluble/70% ethanol-insoluble corn syrup solids (90EI/70EI-CSS).

The entire fractionation scheme used to prepare the three MOS/MPS preparations (i.e., 90EI-CSS, 90EI/70ES-CSS and 90EI/70EI-CSS) is depicted in Figure 2.1.

Solvent removal and drying

Ethanol was removed from all preparations using a rotary evaporator (Büchi Rotovapor R-205, Büchi Labortechnik AG) equipped with a 60 °C water bath (Buchi

B-490) and a high vacuum pump (Chemglass Scientific Apparatus/10 Torr). Complete ethanol removal required several solvent-displacement steps as follows; initial solvent removal was done by rotary evaporation for 10 minutes at 120 rpm (at this point samples were highly viscous liquids), 100 mL of DI water was then added to the sample with mixing, rotary evaporation was again done for approximately 10 minutes. This solvent-displacement process, i.e. adding DI water followed by evaporation, was repeated twice more (i.e., three solvent exchanges following initial solvent removal). 90EI/70ES-CSS, due to its greater solvent content, required rotary evaporation for 20 minutes for initial solvent removal. In all cases, complete ethanol removal was assessed using proton NMR (see below). Final ethanol-free samples, as viscous aqueous solutions, were then frozen at -12°C (-10 °F) and subsequently dried by lyophilization in a VirTis CONSOL 4.5 freeze dryer.

2.3.2.2 Total carbohydrate assay

The total carbohydrate content of each MOS/MPS preparation was determined by the spectrophotometric anthrone/sulfuric acid assay as described by Brooks & Griffin (1987). In the standard protocol, 3 ml anthrone reagent (0.1% (w/v) in 12.4 M sulfuric acid) was added to 25 μL of a carbohydrate-containing solution in appropriately sized test tubes; tubes were immediately topped with glass marbles to prevent evaporation and immersed in boiling water for 5 minutes. Tubes were then removed and quickly submerged in ice water for 15 minutes, after which absorbance was read at 630 nm. Calibration curves were prepared using solutions containing 0-3.0 mg/mL glucose (0-75 μg glucose per assay mixture; data presented in the Appendix). Calibration curve-derived total carbohydrate values for MOS/MPS

samples were multiplied by 0.90 to adjust for the water of hydrolysis. Reported carbohydrate values are on a dry-weight basis; moisture contents having been determined by oven drying at 105 ℃ for 24 hours.

2.3.2.3 Reducing Sugar assay

Reducing ends were quantified using the bicinchoninic acid/copper-based assay as described by Kongruang, Joo Han, Breton, & Penner (2004). One-milliliter of a carbohydrate-containing solution was mixed with 1 mL BCA working reagent (prepared as in Garcia, Johnston, Whitaker, & Shoemaker, 1993) in glass tubes which were then capped with glass marbles and incubated at 80°C for 30 minutes. Tubes were then cooled to room temperature and the absorbance was measured at 560 nm. Calibration curves were prepared with solutions containing maltose (0, 5, 15, 30, 45, 60, 75 μM). Assays were done in triplicate.

2.3.2.4 High Performance Liquid Chromatography (HPLC)

Saccharide profiles were determined using a Prominence UFLC-HPLC system (Shimadzu, Columbia, MD) equipped with a system controller (CMB-20A), degasser (DGU-20A), solvent delivery module (LC-20AD), autosampler (SIL-10A), column oven (CT20-A), and evaporative light scattering detector (ELSD-LT II). Samples were separated on combined Ag^{2+} polystyrene ion-exchange guard and analytical columns (Supelcogel, Hercules, CA) using DI water as the mobile phase. The mobile phase flow rate was 0.20 mL per minute; the column temperature was kept at 80°C. Simple sugar (*i.e.*, DP1-2) and MOS concentrations (*i.e.*, DP3-8) were calculated from external standard curves prepared using commercially available standards for MOS DP 1-8. Integration was done using LCsolution computer software (Shimadzu,

Kyoto, Japan). Calibration standards for MOS/MPS DP>8 were not commercially available and the resolution of these saccharides was not sufficient for quantification.

2.3.2.5 High Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD)

HPAEC-PAD analyses were done using a Dionex modular chromatograph system (Dionex, Sunnyvale, CA, USA) equipped with a gradient pump (GP50), autosampler (AS3500), column container (LC30) kept at 25 °C, and an electrochemical detector (ED40) containing a quad potential and disposable Au electrode. Samples were dissolved in 100 mM NaOH prior to their injection (10 μL) into the chromatograph for separation using a linear gradient elution with a CarboPac PA-200 column (4 x 250 mm)/CarboPac PA-200 guard column (3 x 50 mm). The mobile phase, at a flow rate of 0.4 mL/min, was developed from eluent A (100 mM sodium hydroxide) and eluent B (100 mM sodium hydroxide containing 500 mM sodium acetate) such that the composition of the mobile phase at times 0, 30, 40, and 45 minutes were (%A-%B) 98-2, 60-40, 0-100, and 98-2, respectively. Dionex Peaknet software version 5.21 was used for data analysis.

2.3.2.6 Nuclear Magnetic Resonance (NMR)

NMR analyses of samples in an aqueous solution were used to verify the absence of ethanol in the MOS/MPS preparations (Gottlieb, Kotlyar, & Nudelman, 1997). The samples were lyophilized and again analyzed by NMR to determine the relative amounts of (1→4) and (1→6) linkages (Nilsson, Bergquist, Nilsson, & Gorton, 1996). Prevalence of bond linkage was determined by integration of the peak areas for the α-(1→4) (5.305-5.395 ppm) and α-(1→6) (4.881-4.924 ppm) signals. The α-(1→4)/ (1→6) ratios were calculated and are tabulated in Table 1. A Bruker

AVIII 700 MHz 2-channel spectrometer with a 5mm dual carbon (DCH) cryoprobe with a z-axis gradient and a Bruker AVI 400 MHz 2-channel spectrometer with a 5mm Broad Band Observe with Fluorine (BBO-F) probe with z-axis gradient was used to analyze samples at room temperature dissolved in D_2O. Topspin 2.1 computer software was used to acquire spectra (spectra presented as Figures 2 and 3 in the Appendix).

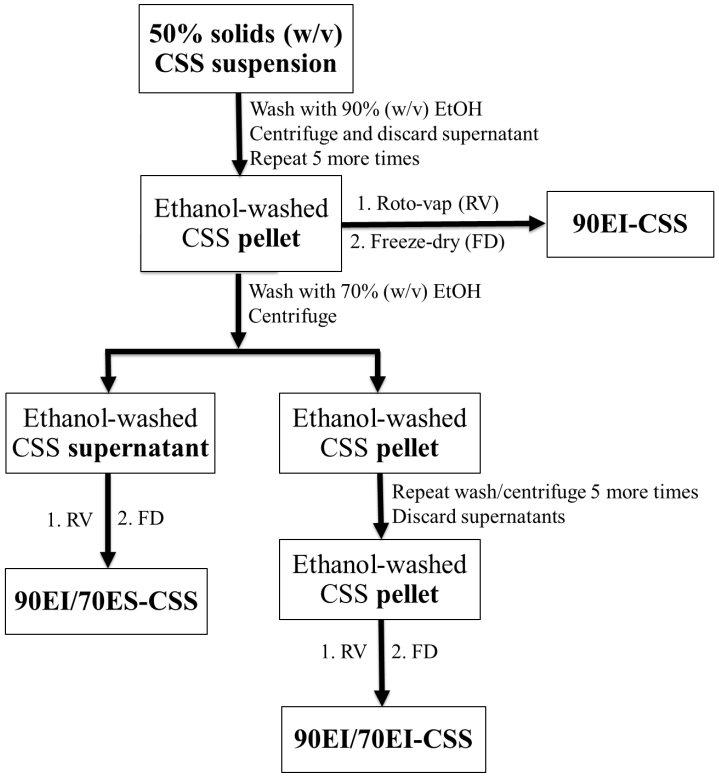

Figure 2.1. A diagram for aqueous-ethanol solubility-based fractionation of corn syrup solids (CSS). RV/FD, rotary evaporation with repeated solvent exchange (3x water) followed by freeze-drying; 90EI-CSS, 90% ethanol-insoluble solids-enriched CSS; 90EI/70ES-CSS, 70% ethanol-soluble solids-enriched 90EI-CSS; 90EI/70EI-CSS, 70% ethanol-insoluble-enriched 90EI-CSS.

2.4 Results and Discussion

The aim of the presented work was to develop a simple approach to obtain food-grade MOS/MPS preparations containing minimal amounts of glucose and maltose and having relatively narrow DP ranges. The approach was to be relatively

inexpensive and applicable to the preparation of tens-to-hundreds of grams of material, as is often needed for structure/function/sensory studies in the food and nutritional sciences. The outcome of this work is a rather simple procedure to fractionate CSS/MD preparations based on the differential solubility of the saccharide components in aqueous-ethanol solutions, as presented in Figure 2.1. The following text provides the details underlying this fractionation approach along with commentary on modifications for altering the nature of the resulting MOS/MPS preparations; characterizations of representative fractionated MOS/MPS preparations are included.

2.4.1 Qualitative studies of MOS/MPS Solubility in aqueous ethanol solutions

Initial work focused on qualitative estimates of the relative solubility of glucose, maltose, MOS and MPS in aqueous ethanol solutions containing ≥50% ethanol. This was done by chromatographic analyses of the composition of the extracts obtained from liquid-solid extractions of the CSS starting material with aqueous-ethanol mixtures differing in ethanol content. Each experiment required first dissolving the CSS preparation in water followed by the addition of the appropriate amount of ethanol; the initial dissolution in water was required due to the clumping of CSS solids when directly exposed to ≥50% ethanol solutions. Differences in the nature of the precipitates formed in different ethanol concentrations were obvious. The 90% ethanol extract of CSS formed a white opaque colloidal suspension immediately upon addition of ethanol; whereas addition of 70% ethanol to the 90% ethanol-washed CSS rapidly formed a translucent gel at the bottom of the aqueous-ethanol liquid phase. HPLC analyses of the different extracts (i.e., liquid phases)

provided information on the relative extractability of the different CSS components. As anticipated, the solubility of all components decreased with increasing ethanol content and, in general, the size of the components was inversely related to their extent of extraction into the different aqueous-ethanol solutions. Relatively simple break points were observed with regard to the extraction of MOS and MPS: (a) extracts containing ≥ 90% ethanol contained appreciable amounts of glucose, maltose and MOS of DP 3-7 (MOS_{DP3-7}), *i.e.* MOS/MPS with DP ≥ 8 were not detected in chromatographic analyses of these extracts and (b) extracts containing ≤ 70% ethanol contained, along with the lower DP components, appreciable amounts of higher DP MOS and some MPS. With respect to glucose, maltose, and MOS_{DP3-7}, amounts recovered in aqueous ethanol extracts containing ≥ 70% ethanol decreased as ethanol concentrations increased from 70 to 95%.

2.4.3 MOS/MPS fractionation

The two-step fractionation scheme depicted in Figure 2.1 is based on the observations noted in the preceding paragraph. An initial 90% ethanol fractionation step was chosen to remove glucose and maltose from the original CSS preparation based on the noted insolubility of MOS/MPS with DP ≥8, the sufficiently low solubility of MOS_{DP3-7}, and the reasonable solubility of glucose and maltose at this ethanol concentration. An alternative initial fractionation step using 95% ethanol was considered because it would likely improve the recovery of MOS and MPS in subsequent steps, but the lower solubility of glucose and maltose in 95% ethanol meant additional extractions were required for their removal and this, in turn, increased both reagent cost and time of preparation. Thus, the first fractionation step,

the principle aim of which was to remove glucose and maltose from the CSS starting material, was achieved through sequential extractions with 90% ethanol. The number of extractions required for glucose and maltose removal was determined from HPLC analyses of successive extracts. Figure 2.2 ("a" and "b") depicts chromatograms characterizing the extracts from the first and sixth 90% ethanol extractions. The absence of glucose and the trace remaining maltose in the sixth extract points to the sufficiency of six extractions; the presence of MOS_{DP3-7} in the sixth extract demonstrates the detrimental effect of further unnecessary extractions on MOS_{DP3-7} yields. The result of the first fractionation step, which consists of six 90% ethanol extractions of the CSS starting material, is an MOS/MPS preparation effectively free of glucose and maltose and containing substantially reduced amounts of the lower DP MOS. The descriptor "effectively free" or "free" is used herein to indicate that a component cannot be detected using the HPLC system employed for these analyses (detection limit of approximately 0.01 mg per mL extractant). Instrumentation with lower detection limits are likely to show the presence of these components (see HPAEC-PAD chromatograms of Figure 2.4). As noted in the "Methods" section, the preparation resulting from the first fractionation is herein referred to as 90% ethanol-insoluble CSS (90EI-CSS). The name is appropriate from the standpoint that the preparation is the insoluble phase remaining after six 90% ethanol washes, but it is a misnomer in the sense that some of the lower DP MOS contained in that preparation would partition into the liquid phase if yet another 90% ethanol wash were done (as depicted in Figure 2.2b).

The second fractionation step was designed to enrich the MOS/MPS preparation resulting from the first fractionation (90EI-CSS) with respect to MOS and to prepare a higher DP fraction that was essentially free of the lower DP MOS. This was accomplished by doing sequential 70% ethanol extractions/washes (see "Methods"). The liquid phase resulting from the first 70% ethanol extraction provided the MOS-enriched sample (referred to as 90% ethanol-insoluble/70% ethanol-soluble CSS; 90EI/70ES-CSS). Only the first extract was used to obtain 90EI/70ES-CSS because the extent of MOS enrichment decreased with each subsequent extraction (as depicted in Figure 2.2c). Thus, maximum enrichment of MOS is achieved by using only the first extract of 90EI/70ES-CSS; the tradeoff in using only the first extract is a reduced yield. The second goal of the 70% ethanol fractionation step was to prepare an MPS-enriched preparation having minimal amounts of the lower DP MOS. This was achieved by doing six successive 70% ethanol extractions of 90EI-CSS (the first extract is used to prepare 90EI/70ES-CSS as just discussed, the following five extracts containing lower DP MOS were discarded). The efficacy of using six washes to reduce MOS content and enrich MPS content is illustrated in Figure 2.2 ("c" and "d"); the second extract is shown to contain considerable $MOS_{DP\ 5\text{-}8}$, whereas there are only minimal amounts of $MOS_{DP7\text{-}8}$ in the sixth extract. The six-time 70% ethanol-washed solids (referred to as 90% ethanol-insoluble/70% ethanol-insoluble CSS; 90EI/70EI-CSS) is thus highly enriched in MPS.

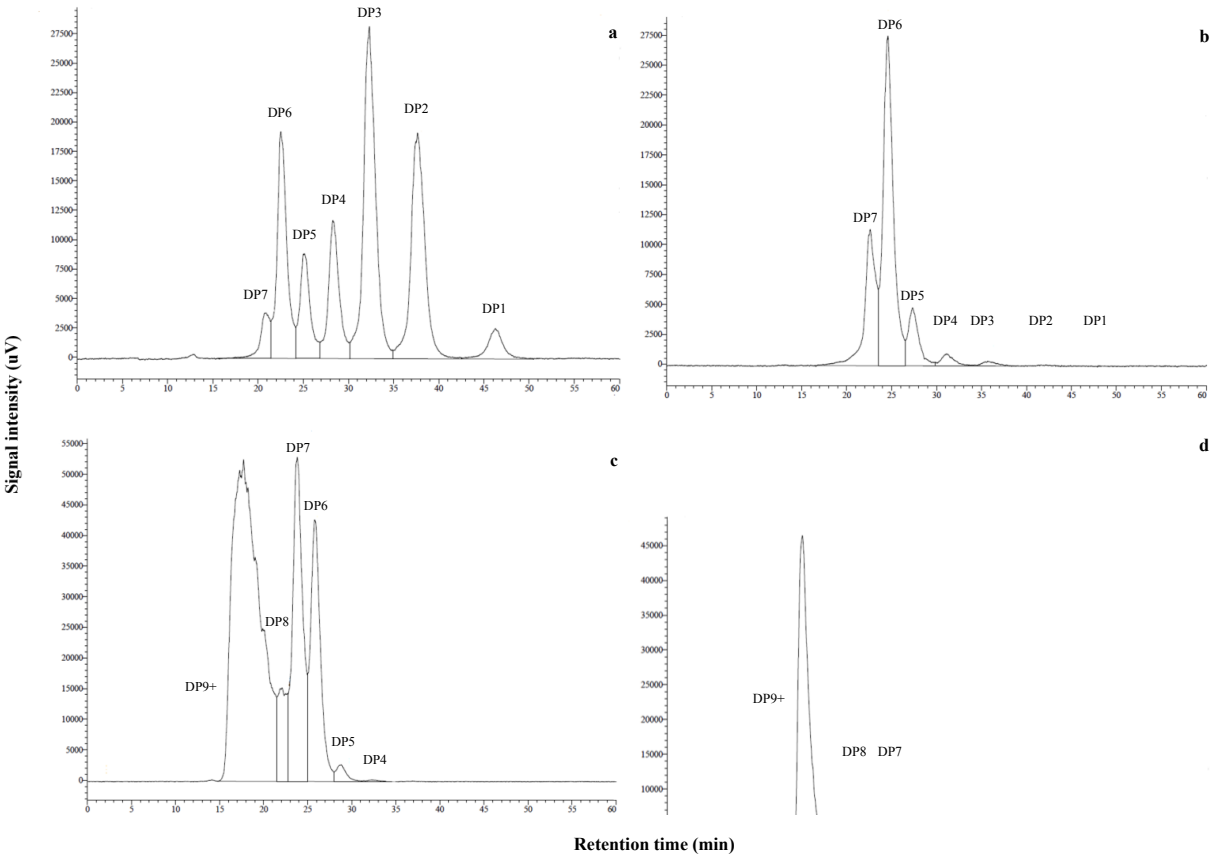

Figure 2.2 Representative chromatograms from ion-mediated liquid chromatography using an evaporative light scattering detector to depict the saccharide character of extracts noted in Figure 1: (**a**) first 90% ethanol extract of CSS; (**b**) sixth 90% ethanol extract of CSS; (**c**) second 70% ethanol extract of 90EI-CSS, (**d**) sixth 70% ethanol extract of 90EI-CSS. Acronyms are as defined in Figure 2.1; DP = degree of polymerization.

The complete process for the fractionation of commercially available CSS/MD preparations, as depicted in Figure 2.1 and described above, results in three MOS/MPS preparations: 90EI-CSS, 90EI/70ES-CSS, and 90EI/70EI-CSS. Average yields for each of the preparations, as percent of the original CSS, were as follows: 90EI-CSS, 51.2 ± 1.3%; 90EI/70ES-CSS, 8.0 ± 0.37%; 90EI/70EI-CSS is 25.9 ± 2.1%. Yield data are based on four replicates from two different experiments. Relative yields are as expected, with the highest being associated with the 90EI-CSS preparation and the lowest with the 90EI/70ES-CSS preparation. Some of the yields

are underwhelming, but that is the nature of the chosen fractionation method. The method requires multiple extractions; the bulk of the extracted solids are discarded in order to obtain preparations having relatively narrow DP ranges. It is important to recognize that even the lowest yield does not nullify the applicability of the overall method since both the starting material and the fractionating solvent are relatively inexpensive.

2.4.4 Characterization of fractionated MOS/MPS preparations

The general characteristics of the three preparations, 90EI-CSS, 90EI/70ES-CSS, and 90EI/70EI-CSS, are summarized in Table 2.1. The anthrone/H_2SO_4 assay-based carbohydrate content of each preparation was greater than 95%; which is an increase relative to the CSS starting material. Relative numbers of reducing ends per unit mass trended as expected based on the solubility of the preparations in aqueous ethanol. The least soluble preparation (90EI/70EI-CSS) had the lowest number of reducing ends per unit mass and correspondingly, its MOS/MPS composition has the highest number-average DP; this also dictates that 90EI/70EI-CSS has the lowest DE value. All preparations had lower DE values than the starting material, which was expected based on the first fractionation step removing the lowest molecular weight components. The prevalence of branching for the different preparations is reflected in the (1→4)/ (1→6) ratios obtained from NMR spectra (data Table 2.1, Figure 3 in the Appendix). The extent of branching in the different preparations bracket that found for corn starch ((1→4)/ (1→6) of ~20; Li et al., 2014). The higher (1→4)/ (1→6) ratio for CSS compared to 90EI-CSS and of 90EI/70ES-CSS compared to 90EI/70EI-

CSS reflects the preferential extraction of linear (1→4) MOS components into the aqueous ethanol phase.

Table 2.1. Chemical Characterization of CSS and MOS/MPS Preparations[a]

Preparation[b]	Percent Carbohydrate[c, d] (%)	mmoles reducing ends per gram[e]	Number-average DP[f]	Dextrose Equivalent (DE)[g]	Linkage Prevalence (1→4):(1→6)[c,g]
CSS	92.8 ± 0.52	1.09 ± 0.08	5.1	19.6	15.2:1
90EI-CSS	95.5 ± 1.28	0.44± 0.03	12.7	7.9	7.7:1
90EI/70ES-CSS	95.7 ± 0.94	0.94± 0.06	5.9	16.9	24.9:1
90EI/70EI-CSS	99.0 ± 0.81	0.14± 0.01	40.0	2.5	5.9:1

[a] CSS = Corn Syrup Solids, MOS = maltooligosaccharides, MPS = maltopolysaccharides
[b] Acronyms denoting sample preparations are as defined in Figure 2.1
[c] Values are means ± SD (where applicable) expressed on a dry weight basis
[d] Determined as "total carbohydrate" using the anthrone/H_2SO_4-assay with glucose as standard
[e] Determined using Cu/bicinchoninic acid-assay with maltose as standard
[f] DP = degree of polymerization; derived from moles reducing ends per gram
[g] Determined from ^{1}H NMR spectra

Quantitative values for the glucose, maltose and MOS_{DP3-8} content of each preparation are given in Table 2.2; the corresponding chromatograms from ion-mediated liquid chromatography with evaporative light scattering detection are depicted in Figure 2.3. Values for MOS/MPS having DP ≥9 are combined due to the extent of resolution of this analytical system and because standards for MOS/MPS having DP ≥9 are not commercially available. Amounts of glucose and maltose in each preparation were below the detection limit of the system (corresponds to levels < 0.1%); note from Table 2.2 that the CSS starting material is ~ 7.5% in combined glucose and maltose. The removal of glucose and maltose from each preparation is important with respect to keeping these primary "sweet saccharides" at negligible levels in studies with sensory/taste applications. Relative to the CSS starting material: 90EI-CSS was enriched in the higher DP MOS and MPS, 90EI/70ES-CSS was

enriched with respect to MOS and was effectively devoid of the highest MPS (the latter conclusion is based on data of Figure 2.4, see below), and 90EI/70EI-CSS was enriched in MPS and effectively free of the lower DP MOS.

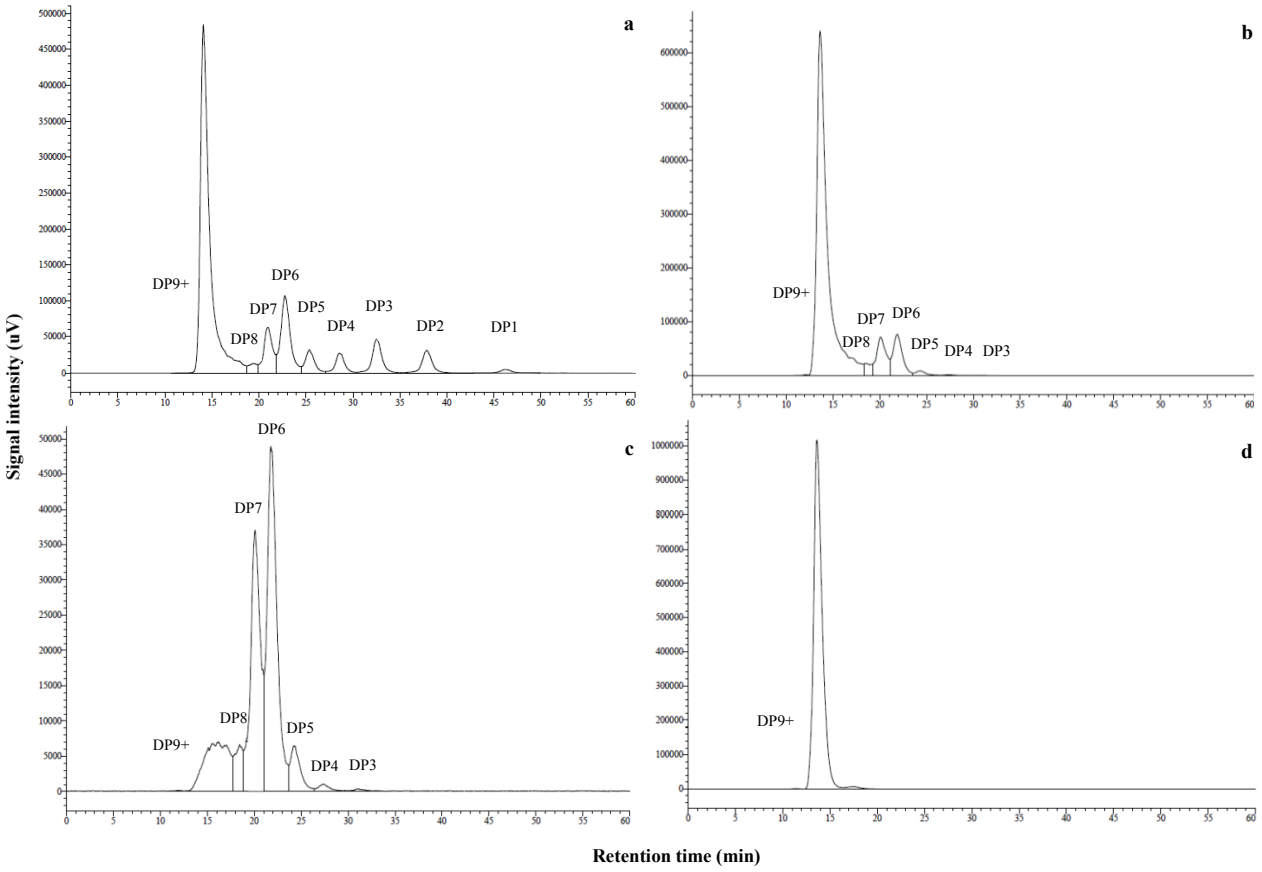

Figure 2.3 Representative chromatograms from ion-mediated liquid chromatography using an evaporative light scattering detector to depict the saccharide character of (**a**) CSS, (**b**) 90EI-CSS, (**c**) 90EI/70ES-CSS, and (**d**) 90EI/70EI-CSS. Acronyms are as defined in Figure 2.1; DP = degree of polymerization.

Table 2.2 Percent saccharide composition of CSS and MOS/MPS preparations[a]

	CSS	90EI-CSS[b]	90EI/70ES-CSS[b]	90EI/70EI-CSS[b]
DP1[c]	1.9 ± 0.0	ND[d]	ND	ND
DP2	5.6 ± 0.2	ND	ND	ND
DP3	8.6 ± 0.4	1.4 ± 0.2	4.0 ± 0.3	ND
DP4	5.1 ± 0.1	1.4 ± 0.1	4.4 ± 0.3	ND
DP5	5.7 ± 0.2	2.2 ± 0.2	7.6 ± 0.3	ND
DP6	15.6 ± 0.4	9.0 ± 0.8	28.2 ± 1.5	ND
DP7	7.5 ± 0.2	8.0 ± 0.2	20.5 ± 1.1	ND
DP8	4.2 ± 0.2	4.3 ± 0.2	10.3 ± 0.5	ND

DP1-2	7.5 ± 0.2	ND	ND	ND
DP3-8	46.6 ± 1.6	26.4 ± 1.5	75.5 ± 1.8	ND
DP9+	45.9 ± 1.8	73.6 ± 1.5	24.5 ± 1.8	100

[a] CSS = Corn Syrup Solids, MOS = maltooligosaccharides, MPS = maltopolysaccharides; all values are average ± SD of four replicates
[b] Acronyms denoting sample preparations are as defined in Figure 2.1
[c] DP = degree of polymerization; # = number of glucose units, "9+" indicates glucose polymers with ≥9 glucose units
[d] ND = Not Detected

Qualitative profiles of the saccharide component content of each preparation were obtained using anion-exchange liquid chromatography with pulsed amperometric detection (Figure 2.4). The resolution allows visualization of higher DP MOS and MPS components through DP 25; following this is a broad peak (retention time ~35-43 minutes) for the unresolved higher DP MPS. The four chromatograms nicely illustrate the disparity in DP content for the different preparations. Chromatograms "a" and "b" illustrate the preferential extraction of glucose, maltose, maltotriose, and maltotetraose through the initial 90% ethanol fractionation step. Chromatograms "c" and "d" illustrate the impact of the subsequent 70% ethanol fractionation step; preparation 90EI/70ES-CSS is shown to be devoid of the higher DP MPS which, due to their low solubility in 70% ethanol, have been concentrated in 90EI/70EI-CSS. Chromatograms from anion-exchange liquid chromatography were not used for MOS quantification due to difficulties in obtaining reliable detector response factors for all MOS (Koch, Andersson, & Amman, 1998).

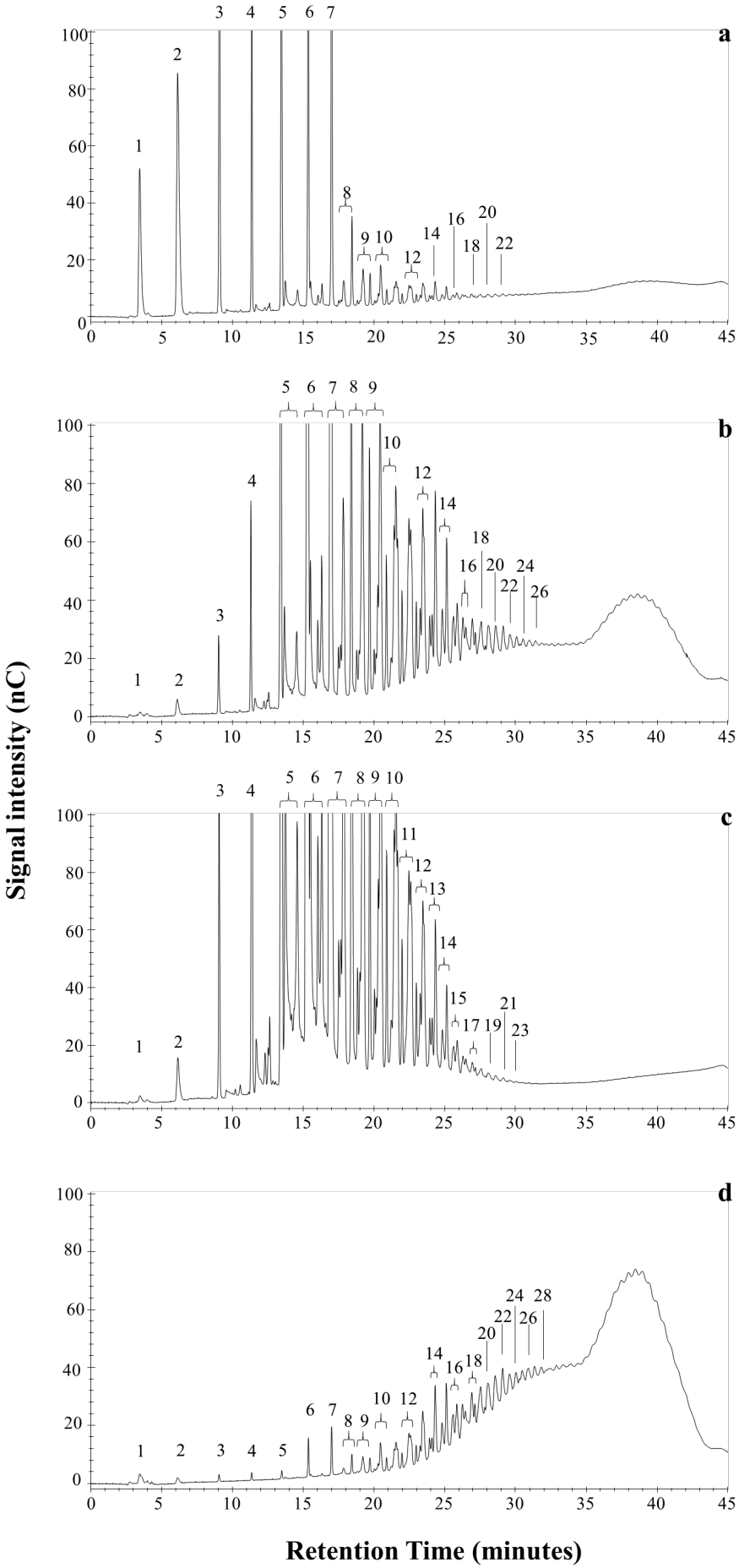
a
b
c
d
Signal intensity (nC)
Retention Time (minutes)

Figure 2.4 Representative chromatograms from anion-exchange liquid chromatography using a pulsed amperometric detector depicting the saccharide character of (**a**) CSS, (**b**) 90EI-CSS, (**c**) 90EI/70ES-CSS, and (**d**) 90EI/70EI-CSS. Acronyms are as defined in Figure 2.1; peak integers correspond to DP values.

The fractionation approach used to obtain 90EI-CSS, 90EI/70ES-CSS and 90EI/70EI-CSS is similar to that used recently by Sen, Gosling, & Stevens (2011) to selectively enrich galactosyl oligosaccharide preparations. The starting materials in the Sen et al. study and the present one differ considerably in that starch hydrolysis products contain primarily (1→4) linked α-D-glucopyranosyl units with some (1→6) branching while the galactosyl oligosaccharides of Sen et al.'s study are known to have much greater heterogeneity (Gosling, Stevens, Barber, Kentish, & Gras, 2010). The trends established in the two studies are similar, although in the present work both the initial and final monosaccharide/disaccharide content of the preparations was significantly lower.

2.5 Concluding Comments

The work presented herein outlines a rather simple approach to obtain food-grade MOS/MPS preparations having relatively narrow DP ranges. The approach is expected to be generally transferable with respect to the aqueous-ethanol solubility of components common to commercially available CSS and MD preparations. The presented work was based on a CSS starting material; the relative yields and DP profiles obtained in this work reflect that starting material. If one were to use a low-DE MD preparation as the starting material, for example, then yields from the presented ethanol fractionation scheme are expected to be higher for the higher DP MPS-containing preparations and lower for the predominantly MOS-containing preparations. Altering ethanol concentrations and/or using different food-grade

solvents for the fractionation steps will likewise change yields and DP profiles of the resulting preparations. Additional processing steps may also be included in order to adapt the method to different needs. For example, selective hydrolysis of the α-(1→6) linkages (Wang & Wang, 2000; Koch et al., 1998) may be used to enhance the linear (1→4) MOS/MPS content of preparations. Clearly, the approach characterized in this work can be readily adapted to meet different objectives; the data provided herein is expected to provide a fundamental basis upon which to make such adaptations.

Acknowledgements

This study was supported by Formula Grant no. 2014-31100-06041 from the USDA National Institute of Food and Agriculture. The authors wish to thank Dr. Tomasino for the use of her rotary evaporator. The authors would also like to thank Tate & Lyle Ingredients Americas (Decatur, IL) for providing the corn syrup solids sample.

Chapter 3: The human taste detection of three glucose polymer stimuli with distinct chain length ranges without and with acarbose, an α-amylase inhibitor

1. Introduction

While it has been assumed that humans are unable to taste complex carbohydrates (Feigin et al., 1987), exercise and psychophysical studies have suggested that humans can perceive complex carbohydrates, possibly through taste. Exercise studies have found that maltodextrin rinses can improve physical performance (Carter et al., 2004, de Ataide e Silva et al., 2014). Chamber et al. (2009) measured regions of brain activity and the results support that maltodextrin perception is likely reward-related and that there may be an oral receptor for complex carbohydrates. Recent studies in our lab have found that people can taste maltodextrins (Lapis et al., 2014) independent of simple sugars and can taste components of cooked starch (Lapis et al., submitted). The responsiveness to cooked starch was likely due to taste detection of glucose polymers produced through oral starch hydrolysis. In Lapis et al. (2014; submitted), subjects wore nose clips to omit any olfactory input and expectorated the complex carbohydrate stimuli to mitigate any postingestive behavior. However, the complex carbohydrate stimuli used in these human studies contain glucose and maltose and have a wide compositional range of glucose polymers. Thus, the objective of this study was to investigate the glucose polymer DP range devoid of glucose and maltose that humans can detect through taste using the three glucose polymer stimuli produced in Balto et al. (submitted) renamed Samples 1-3.

To investigate the taste detection of the three glucose polymer stimuli, it was necessary to preserve their compositions by inhibiting salivary α-amylase present in the mouth, which hydrolyzes glucose polymers within three seconds into maltose, MOS, and MPS (unpublished data). Humans have a wide range of salivary α-amylase activity it has been determined that high α-amylase activity has a higher hydrolysis rate and produced more terminal hydrolysis products (i.e., maltose and maltotriose) whereas the saliva with low to medium α-amylase activity had a relatively lower hydrolysis rate and produced less maltose and maltotriose (Lapis et al., 2014; Lapis et al., submitted). Thus, an unpublished study in our lab developed an *in vitro* assay to measure the effectiveness of acarbose (an α-amylase inhibitor approved for human consumption) to inhibit a wide range of salivary α-amylase activity. Employing conditions representative of a psychophysical taste study (i.e., 3-sec tasting time, incubation at body temperature, average saliva resting volume), we determine that 5 mM acarbose effectively inhibits a range of salivary α-amylase activity and preserves the glucose polymer stimuli composition.

This present study was designed to evaluate the human taste detection of three glucose polymer stimuli with distinct chain length ranges devoid of glucose and maltose. Acarbose was used to inhibit a wide range of salivary α-amylase activity and a non-acarbose treatment was used as a control treatment representative of normal conditions in the mouth. Based on the findings of Sclafani et al. (1987), we hypothesized that Samples 1-3 (meaning 90EI-CSS, 90EI/70ES-CSS and 90EI/70EI-CSS) will be detected to different degrees based on the proportion of short chain glucose polymers present within each sample. Based on the proportions of short chain

glucose polymers, we expected that the relative perception of the taste stimuli would be: Sample 2 (75% DP3-8) > Sample 1 (25% DP3-8) > Sample 3 (0% DP3-8). We also hypothesized that there will be an effect of concentration and the presence of acarbose on the taste detection of Samples 1-3. We expected that taste detection would be greater at a higher percent concentration of the glucose polymer stimuli and that the perception of Samples 1-3 without acarbose (with α-amylase) may be higher than with acarbose (inhibited α-amylase) if the hydrolysis products are at a detectable level.

2. Materials and Methods

2.1 Subjects

A total of 22 subjects (11 M, 11 F) between 18 to 45 years of age (mean = 25 years old) were recruited from the Oregon State University campus and surrounding areas. Individuals who were interested in participating in this taste perception study were asked to fill out a screening questionnaire composed questions about general health. Subject inclusion criteria were individuals who are: 1) non-smokers; 2) not pregnant; 3) non diabetic; 4) not taking any prescription medication; 5) free from taste deficit or other oral disorders; and 6) without a history of food allergy. Those who met all of the above criteria were invited to participate in the study and were further asked to observe with the following restrictions prior to testing sessions: 1) no dental work within 48 hrs; 2) no alcohol consumption within 12 hrs; 3) no consumption of foods and beverages that are acidic or caffeinated and/or contain dairy within 4 hrs; 4) no consumption of food or beverage of any kind except water within 1 hr; 5) no use of any menthol-containing products (e.g., toothpaste,

mouthwash, and chewing gums) within 1 hr prior to the scheduled sessions. In order to avoid deviations from normal α-amylase activity, subjects were also asked not to engage in physically demanding activity an hour before the test sessions. The Oregon State University Institutional Review Board approved the experimental protocol. Subjects gave written informed consent and were paid to participate.

2.2. Stimuli

Three glucose polymer stimuli were prepared as described in Chapter 2. Preliminary testing showed that 6 and 8% (w/v) glucose polymer solutions were difficult to distinguish from deionized water and that 5 mM acarbose does not have a taste as it was not discriminable from deionized water. 6% and 8% (w/v) aqueous solutions were prepared with and without 5 mM acarbose (3.23 mg/mL sample) and stored in the refrigerator at 4-6 °C maximally for five days. Deionized water was also prepared with 5 mM acarbose as blank samples within the sensory tests for Samples 1-3 with acarbose. All stimuli were allowed to come up to room temperature (20-22 °C) before testing sessions.

2.3. Experimental protocol

Each subject participated in two sessions on two separate days counterbalancing the stimuli concentration of each session across subjects: one session with 6% (w/v) glucose polymer stimuli solutions and one session with 8% (w/v). For each session, subjects performed six sets of triangle tests split into three sets for each treatment (i.e., without and with acarbose). The stimuli presentation order was randomized across concentrations and acarbose treatment sets. Salivary α-amylase level and activity typically follow a diurnal pattern and are lower in the

morning, especially close to the time of awakening (Nater et al., 2007). Subjects were therefore only tested from 8:00 am to 12:00 pm. All testing was conducted on a one-on-one basis in a psychophysical testing room. Subjects were asked to follow the same restrictions in both sessions (see Subjects section above).

2.3.1. Psychophysical procedure

Before the test session, subjects were verbally instructed on the task they were asked to perform. Within each acarbose treatment sets, subjects were given one sample set at a time consisting of 1 target sample and 2 blank samples. Their task was to taste 5 mL of each sample for 3 seconds in a sequence following a sip-and-spit procedure, and to report which of the 3 samples was different by circling the 3-digit code of the corresponding sample on the provided ballot. Subjects' tasting time was timed for three seconds after which subject's immediately expectorated in order to control the time that the glucose polymer stimuli interacted with either inhibited or uninhibited salivary α-amylase. It has been noted in our past studies (Lapis et al., 2014) that the perception of commercial maltodextrin is influenced by olfactory queues, which is consistent with the findings of Hettinger et al. (1996), while it is unknown whether the test stimuli have an odor quality or not. To eliminate any possible olfactory input, subjects were asked to wear nose clips while tasting the samples. Subjects were allowed to take off the nose clips after indicating which sample that was different on the ballot. Subjects were asked to rinse their mouth once with 37°C reverse osmosis water in between the three samples. Subjects were also asked to rinse their mouths in between each set of samples three times during a 1-minute break. Subjects were given a 3-minute break between acarbose treatments.

The presentation order of the glucose polymer stimuli was randomized within acarbose treatment blocks (i.e., without and with acarbose) and counterbalanced across subjects and sessions.

2.4 Data analysis

The number of correct answers from subjects was counted for each of the three test stimuli at 6 and 8% without and with acarbose and converted to d prime (d') values (Bi et al., 1997). The d' analysis (Bi et al. 1997) was used to determine whether the subjects were able to significantly (p-value < 0.05) discriminate the test samples from the blank. The d' significance test (Bi et al. 1997) was used to determine if there was a significant difference in d' values for each glucose polymer stimuli across concentrations and acarbose treatments.

3. Results

Seen in Table 1, subjects' discriminability of the glucose polymer stimuli from water, portrayed as d' values. For all concentration and acarbose treatments, the degree of detection for Samples 1 ($p < 0.001$) and 2 ($p < 0.0001$) were significant, but not Sample 3 ($p > 0.05$). There was only a significant effect of concentration for Sample 2 as the degree of discrimination for the 6% test stimuli was significantly higher than the 8% test stimuli ($p < 0.05$). Acarbose had an insignificant effect on the degree of discrimination for all test stimuli ($p > 0.05$). Comparing the degree of discrimination for the glucose polymer stimuli, the d' values of Sample 2 were significantly higher than those of Sample 1 across concentrations and acarbose treatments except for the test stimuli at 6% without acarbose.

Table 3.1 d' values from triangle discrimination tasks for 6 and 8% aqueous Sample 1-3 solutions without and with 5 mM acarbose (n=22)

		Sample 1	Sample 2	Sample 3
6%	Without acarbose	1.93*	3.26**	0.00
	With acarbose	1.70*	3.26**	0.00
8%	Without acarbose	2.40*	4.90**	0.58
	With acarbose	1.93*	4.90**	0.00

*p-value < 0.001, **p-value < 0.0001

4. Discussion

4.1. Taste detection of three glucose polymer stimuli with distinct chain length ranges

The results of this study shows that humans are able to detect glucose polymers through taste. The detection of Samples 1 and 2 supports the postulation that shorter chain glucose polymers are detectable. The average percent composition of Samples 1-3 were DP3-DP8, DP9+: 26.4 ± 1.5%, 73.6 ± 1.5%; 75.5 ± 1.8%, 24.5 ± 1.8%; and 0%, 100%, respectively. Sample 2 was discriminated from water to a greater degree than Sample 1 except for the 6% (w/v) without acarbose treatment. Sample 3 was not significantly detected across concentrations or treatments, which may infer that long chained glucose polymers (average ~DP40) are not detectable. Overall, these findings support the hypothesis that humans can detect glucose polymers through taste and may optimally detect short chain glucose polymers, an unconfirmed postulation from Lapis et al. (2014; submitted).

The taste detection mechanism of glucose polymers is unknown and could involve multiple taste receptors. Although it has been proposed by Sclafani et al. (1987) that there could be a complex carbohydrate taste receptor distinct from the

sweet taste receptor, it is unknown whether short chain MOS (i.e., maltotriose) in the glucose polymer stimuli could be detected by either or both receptors. Sclafani et al. (1987) determined that rats prefer maltotriose similarly to maltose. Treesukol et al. (2011) found that maltotriose intake is reduced in T1R2, T1R3, and T1R2/T1R3 knockout mice, which indicates that maltotriose is possibly detected by the sweet receptors. However, whether maltotriose or larger DP MOS could activate the sweet taste receptor in humans is presently unknown. Also, if there is a complex carbohydrate receptor distinct from the sweet taste receptor that has an active site for multiple glucose subunits it is likely that maltose and maltotriose could also fit within its active site and elicit a taste.

Although the results of this study show that humans can taste glucose polymers it is presently erroneous to compare the degree of taste detection of the three glucose polymer stimuli. This psychophysical study was performed with aqueous solutions of the glucose polymer stimuli equal in weight percentage, which did not take into account the difference in molarity of the glucose polymers. There are differences in number of molecules in the three glucose polymer stimuli, which means that in this study there were differences in the number of molecules between the three glucose polymer stimuli solutions. For example, Sample 3 is composed of primarily long-chained high molecular weight glucose polymers and would thus have a lower total number of molecules in a 6% or 8% (w/v) solution compared to Sample 2, which is primarily composed of short-chain low molecular weight glucose polymers. Therefore, this research may not be truly representative of the genuine Sample 1-3 human taste detection. It is possible that the lowered detection of Sample

1 compared to Sample 2 and insignificant detection of Sample 3 was due to a difference in molarity of the solutions. Future studies in our lab evaluate the human taste detection of Samples 1-3 on an equimolar basis.

4.2. Effect of α-amylase on the taste detection of the glucose polymer stimuli

It was found that acarbose did not influence the taste detection of the three glucose polymer stimuli. Only Sample 1 had differences in the degree of taste detection between acarbose and without acarbose containing test stimuli. This noted increase in the taste detection of Sample 1 was likely due the production of hydrolysis products by uninhibited α-amylase, which could have allowed for additional detection compared to the test stimuli with inhibited α-amylase. An unpublished study in our lab determined that salivary α-amylase can hydrolyze the glucose polymer stimuli in 3 seconds, however, the hydrolysis products were consistently minimal for the low and medium α-amylase activity and there was only a significant amount of hydrolysis products for the saliva with high salivary α-amylase activity (~96^{th} percentile of the population). In this study, it is unknown which of the glucose polymer hydrolysis products (i.e., maltose, MOS, MPS) was responsible for the insignificant increase in taste detection for Sample 1.

There was no difference in the degree of Sample 2 or Sample 3 detection across the without and with acarbose treatments possibly due to the insignificant production of hydrolysis products to allow for detection of Sample 3 and additional detection of Sample 2. As only a small percentage of the population produces a large amount of hydrolysis products in three seconds, it is likely that the composition of Sample 2 and 3 was not significantly altered through salivary hydrolysis.

The salivary α-amylase activity of each subject was not measured in this study as past studies in our lab have concluded that salivary α-amylase does not play a significant role in the taste perception of some glucose polymer stimuli (Lapis et al., 2014), but it was necessary to use acarbose to inhibit salivary α-amylase in order to preserve the glucose polymer stimuli saccharide profile. Another study in our lab found that some subjects, regardless of salivary α-amylase activity, were sensitive to the taste of cooked starch after 2-30 seconds (Lapis et al., submitted). The subjects sensitive to the taste of cooked starch with high α-amylase activity had a significantly higher taste response to the cooked starch likely due to higher production of glucose polymers from starch hydrolysis. Interestingly, subjects sensitive to the taste of cooked starch did not necessarily have a higher α-amylase activity. Therefore, it was thought that the relative salivary α-amylase activity range of the subjects would not influence the overall taste detection of the glucose polymer stimuli, which was shown here to be an accurate assessment.

5. Summary

This research evaluated the human detection of three glucose polymer stimuli (i.e., Samples 1-3) and found that Sample 1 and Sample 2 were significantly discriminated from water, which sustains the findings of Lapis et al. (2014) that humans can taste glucose polymers of various polymeric chain lengths and that the taste of glucose polymers is independent of that of simple sugars. Overall, the human taste detection of the glucose polymer stimuli devoid of simple sugars demonstrated in this study supports the existence of a postulated complex carbohydrate taste receptor.

Chapter 4: General Conclusion

The objectives of this thesis were to develop a methodology to produce three glucose polymer stimuli devoid of glucose and maltose with distinct chain length ranges and to evaluate the taste perception of the three stimuli. First, an economical highly-adaptable methodology to produce large quantities of three compositionally distinct food-grade glucose polymer preparations (i.e., Samples 1-3) was developed using corn syrup solids STAR-DRI DE20 as a starting material. Aqueous ethanol differential solubility was used to 1) remove glucose and maltose and 2) to fractionate samples into MOS/MPS before processing for consumption (i.e., rotary evaporation and freeze-drying). The average percent composition of Samples 1-3 were DP3-DP8, DP9+: 26.4 ± 1.5%, 73.6 ± 1.5%; 75.5 ± 1.8%, 24.5 ± 1.8%; and 0%, 100%, respectively.

The human taste detection of Samples 1-3 was evaluated using triangle tests at two concentrations with and without acarbose, an α-amylase inhibitor, to prevent the salivary α-amylase hydrolysis of the stimuli. It was found that the glucose polymer stimuli detection was related to the proportion of short chain glucose polymers in each stimuli. The degree of differentiation of the glucose polymer stimuli from water increased with an increase in short chain glucose polymer concentration. Specifically, Sample 2, with the highest concentration of short chain glucose polymers, was greater differentiated from water than Sample 1. Sample 3 was not significantly differentiated from water and has the lowest concentration of short chain glucose polymers. Except for the glucose polymer stimulus with the highest proportion of short chain glucose polymers (i.e., Sample 2), there was no difference between the taste detection of the

test stimuli across concentration likely due to an insignificant increase in the concentration of detectable short chain glucose polymers for Samples 1 and 3. Acarbose did not significantly affect the degree of taste detection of the glucose polymer stimuli, which suggests that salivary α-amylase did not significantly alter the test stimuli composition.

This was the first human study that investigated the taste detection of glucose polymer stimuli devoid of glucose and maltose and the results support the existence of a taste receptor that can detect short chain glucose polymers. Although short chain glucose polymers are not highly abundant in foods, starch is highly prevalent in the human diet. Soluble starch is broken down by salivary α-amylase into short chain glucose polymers during mastication. Therefore, it is possible that short chain glucose polymers could be orally detected through taste, which could signal for a large source potential energy.

Further research is necessary to define and evaluate the taste detection of equimolar glucose polymer stimuli solutions as the glucose polymer stimuli were not equally represented on a molar basis in this present study like most maltodextrin-based psychophysical studies. Future work will focus on the production and the taste detection evaluation of short chain glucose polymers with singular chain lengths (i.e., maltotriose, maltotetraose) to define the specific glucose polymer range that humans can detect through taste.

Bibliography

Alder, E., Hoon, M.A., Mueller, K.L., Chandrashekar, J., Ryba, N.J.P., Zucker, C.S. (2000) "A Novel Family of Mammalian Taste Receptors" *Cell*. 100, 693-702.

Alves, L.A., Almeida e Silva, J.B., and Giulietti, M. (2007) "Solubility of D-Glucose in Water and Ethanol Water/Mixtures" *J. Chem. Eng. Data*. 52, 2166-2176

Autio, K., Niskanen, L., Putanen, K. (2004). "Chapter 18: Starch in food diabetes and coronary heart disease." In "Functional Foods, Cardiovascular Disease, and Diabetes" Edt. Arnoldi, A. CRC Press: FL. 377-401.

Avaltroni, F., Bouquerand, P.E., and Normand, V. (2004) "Maltodextrin molecular weight distribution influence of the glass transition temperature and viscosity in aqueous solutions" *Carb. Polymers* 58, 323-334

Balfour, J.A. and McTavish, D. (1993) "Acarbose: An Update of its Pharmacology and Therapeutic Use in Diabetes Mellitus" *Drugs*. 46, 1025-1054

BeMiller, J. (2007) Carbohydrate Chemistry for Food Scientists. Second Edition. AACC International: Minnesota

BeMiller, J. and Whistler, R. (2009) Starch: Chemistry and Technology. Third Edition. Elsevier Inc.: USA

Blanchard, P.H. and Katz, F.R. (2006) "Starch hydrolysates" in Food Polysaccharides and Their Applications Second Edition (A.M. Stephen, G.O. Phillips and P.A. Williams, Edts.), CRC Press, 119-145

Bouchard, A., Hofland, G.W., and Witkamp, G.-J. (2007) "Properties of sugar, polyol, and polysaccharide water-ethanol solutions" *J. Chem. Eng. Data* 52, 1838-1842

Braquehais, F.R. and Cava, M.J.B (2011) "Functionality of α-glucans in special formulas for infant and clinical nutrition" *Starch/Stärke* 63, 432-442

Bertolini, A. (2010) Starches: Characterization, Properties, and Applications. CRC Press: FL

Breslin, P.A.S. and Spector, A.C. (2008) "Mammalian taste perception" *Current Biology*. 18, R148-R155

Brooks, J. R., Griffin, V. K., and Kattan, M. W. (1986) "A Modified Method for Total Carbohydrate Analysis of Glucose Syrups, Maltodextrins, and Other Starch Hydrolysis Products" *Cereal Chem*. 63, 465–466

Carter, J.M., Jeukendrup, A.E., and Jones, D.A. (2004) "The effect of carbohydrate mouth rinse on 1-h cycle time trial performance" *Med. Sci. Sports Exerc*. 36, 2107-2111

Chambers, E.S., Bridge, M.W., and Jones, D.A. (2009) "Carbohydrate sensing in the human mouth: Effects on exercise performance and brain activity" *J. Physiol*. 587, 1779–1794

Chronakis, I.S. (1998) "On the molecular characteristics, compositional properties, and structural-functional mechanisms of maltodextrins: a review" *Crit. Rev. Food Sci*. 38, 599-637

Damodaran, S., Parkin, K.L., and Fennema, O.W. (2008) Fennema's Food Chemistry. Fourth Edition. CRC Press: USA, 130, 368-369

Davis, J.D. and Breslin, P.A.S. (2000) "A behavioral analysis of the ingestion of glucose, maltose, and maltooligosaccharide by rats" *Physiology & Behavior*. 69, 477-485

Davis, J.D., Perez, M.C., and Kung, T.M. (1994). "Conditioned Control of Ingestive Behavior by Acarbose-Induced Inhibition of Sucrose Ingestion in the Rat" *Physiology & Behavior*. 55, 511-518

de Ataide e Silva, T., de Souza, M.E.D.C.A., de Amorim, J.F., Stathis, C.G., Leandro, C.G., and Lima-Silva, A.E. (2014) "Can Carbohydrate Mouth Rinse Improve Performance during Exercise? A Systematic Review" *Nutrients*. 6, 1-10

de Sales, M., de Souza, P.M., Simeoni, L.A., de Oliveira Magalhaes, P., and Silveira, D. (2012) "α-amylase Inhibitors: A Review of Raw Material and Isolated Compounds from Plant Source" *J. Pharm. Pharmaceut. Sci*. 1, 141-183

Defloor, I., Vandenreyken, V., Grobet, P.J., and Delcour, J.A. (1998) "Fractionation of maltodextrins by ethanol" *J. Chrom. A* 803, 103-109

Dokic-Baucal, L., Dokic, P., and Jakovljevic, J. (2004) "Influence of different maltodextrins on properties of O/W emulsions" *Food Hydrocolloids* 18, 233-239

Feigin, M.B., Sclafani, A., and Sunday, S.R. (1987) "Species Differences in Polysaccharide and Sugar Taste Preferences" *Neurosci. Biobehav. Rev*. 11, 231-240

Fernstrom, J.D., Munger, S.D., Sclafani, A., de Araujo, I.E., Roberts, A., and Molinary, S. (2012) "Mechanism for Sweetness" *Journal of Nutrition*. 142, 1134S-1141S

Forker, A., Zahn, S., and Rohm, H. (2012) "A combination of fat replacers enables the production of fat-reduced shortdough biscuits with high-sensory quality" *Food Bioprocess Technol.* 5, 2497-2505

French, D., Robyt, J.F., Weintraub, M., and Knock, P. (1966) "Separation of maltodextrins by charcoal chromatography." *J. Chromatog*. 24, 68-75

Frigård, T., Andersson, R., and Åman, P. (2002) "Gradual enzymatic modification of barley and potato amylopectin" *Carb. Polym.* 47, 169-179

Galindo, M.M., Voigt, N., Stein, J., van Lengerich, J., Raguse, J.D., Hofmann, T., Meyerhof, W., Behrens, M. (2012) "G Protein-Coupled Receptors in Human Fat Taste Perception" *Chem. Senses.* 37, 123-139.

Garcia, E., Johnston, D., Whitaker, J., and Shoemaker, S. (1993) "Assessment of Endo-1, 4-beta-D-glucanase activity by a rapid colorimetric assay using disodium 2, 2'-bicinchoninate" *Journal of Food Biochemistry*. 17, 135-145

Gelders, G.G., Bijnens, L., Loosveld, A., Vidts, A., and Delcour, J.A. (2003) "Fractionation of starch hydrolysates into dextrins with narrow molecular mass distribution and their detection by high-performance anion-exchange chromatography with pulsed amperometric detection" *Journal of Chromatography*. 992, 75-83

Giddings, J.C. (1991) Introduction. In *Unified Separation Science*. John Wiley & Son, Inc.: New York, 1-15

Gosling, A., Stevens, G.W., Barber, A.R., Kentish, S.E., and Gras, S.L. (2010) "Recent advances in refining galactooligosaccharide production from lactose" *Food Chem.* 121, 307-318

Gottlieb, H.E., Kotlyar, V., and Nudelman, A. (1997) "NMR Chemical Shifts of Common Laboratory Solvents as Trace Impurities" *J. Org. Chem*. 62, 7512-7515

Hadnađev, M., Hadnađev, T.D., Dokić, L., Pajin, B., Torbica, A., Šarić, L., and Ikonić, P. (2014) "Physical and sensory aspects of maltodextrin gel addition used as fat replacers in confectionery filling systems" *LWT – Food Sci. Technol.* 59, 495-503

Harmon, A.G, Towe-Goodman, N.R., Fortunato, C.K., and Granger, D.A. (2008) "Differences in saliva collection location and disparities in baseline and diurnal rhythms of alpha-amylase: A preliminary note of caution" *Hormones and Behavior*. 54, 592-596.

Hettinger, T.P., Frank, M.E. and Myers, W.E. (1996) "Are the tastes of polycose and monosodium glutamate unique?" *Chem. Senses* 21, 341-347

Hofman, D.L., Van Buul, V.J., and Brouns, F.J.P.H. (2015) "Nutrition, Health, and Regulatory Aspects of Digestible Maltodextrins" *Crit. Rev. Food Sci. Nutr.*

Kamada, T., Nakajima, M., Nabetani, H., Saglam, N., and Iwamoto, S. (2002) "Availability of membrane technology for purifying and concentrating oligosaccharides" *Eur. Food Res. Technol.* 214, 435–440

Kalathenos, P. and Russell, N.J. (2003) "Ethanol as a food preservative" in *Food Preservatives Second Edition* (N.J. Russell and G.W. Gould, Edts.) Kluwer Academic/Plenum Publishers, New York, 196-217

Koch, K., Andelrsson, R., and Aman, P. (1998) "Quantitative analysis of amylopectin unit chains by means of high-performance anion-exchange chromatography with pulsed amperometric detection" *Journal of Chromatography*. 800, 199-206

Kongruang, S., Joo Han, M., Breton, C., and Penner, M. (2004) "Quantitative analysis of cellulose-reducing ends" *Applied Biochemistry and Biotechnology*, 113, 213-231.

Lakshminarayan, S.M., Rathinam, V., and KrishnaRau, L. (2006) "Effect of maltodextrin and emulsifiers on the viscosity of cake batter and on the quality of cakes" *J. Sci. Food Agric.* 86, 706-712

Lapis, T., Penner, M.H., and Lim, J. (2014) "Evidence that Humans Can Taste Glucose Polymers" *Chem. Senses*. 39, 737-747

Lapis, T., Penner, M.H., Balto, A.S., and Lim, J. "Oral Digestion and Taste Perception of Starch" Manuscript submitted for publication

Leemhuis, H., Dobruchowska, J.M., Ebbelaar, M., Faber, F., Buwalda, P.L., van der Maarel, M.J.E.C., Kamerling, J.P., and Dijkhuizen, L. (2014) "Isomalto/malto polysaccharide, a novel soluble dietary fiber made via enzymatic conversions of starch" *J. Agric. Food Chem.* 62, 12034-12044

Li, C.; Begum, A., Numao, S., Park, K.H., Withers, S.G., and Brayer, G.D. (2005) "Acarbose Rearrangement Mechanism Implied by the Kinetic and Structural Analysis of Human Pancreatic R-Amylase in Complex with Analogues and Their Elongated Counterparts" *Biochemistry*. 44, 3347-3357

Li, X., Miao, M., Jiang, H., Xue, J., Jiang, B., Zhang, T., Gao, Y., and Jia, Y. (2014) "Partial branching enzyme treatment increase the low glycaemic property and α-1, 6 branching ratio of maize starch" *Food Chemistry*. 164, 502-509

Loret, C., Meunier, V., Frith, W.J., and Fryer, P.J. (2004) "Rheological

characterization of the gelation behavior of maltodextrin aqueous solutions" *Carb. Polymers* 57, 153-163

Madene, A., Jacquot, M., Scher, J., and Desobry, S. (2006) "Flavour encapsulation and controlled release – a review" *Int. J. Food Sci. Technol.* 41, 1-21

Marchal, L.M., Beeftink, H.H., and Tramper, J. (1999) "Toward a rational design of commercial maltodextrins" *Trends Food Sci. Technol.* 10, 345-355

Mattes, R.D. and DiMeglio, D. (2001) "Ethanol perception and ingestion" *Physiology & Behavior*. 72, 217-229

McPherson, A.E. and Seib, P.A. (1997) "Preparation and properties of wheat and corn starch maltodextrins with low dextrose equivalent" *Cereal Chem.* 74, 424-430

Mishra, P.J., Ragunath, C., and Ramasubbu, N. (2002) "The mechanism of salivary alpha-amylase hydrolysis: Role of residues at subsite S2. *Biochem. Bioph. Res. Co.* 292, 468-473.

Mueller, K.L., Hoon, M.A., Erlenchbach, I., Chandrashekar, J., Zuker, C.S., and Ryba, N.J.P. (2005) "The Receptors and coding logic for bitter tastes" *Nature*. 434: 225-229

Nater, U.M., Rohleder N., Schlotz, W., Ehlert, and U. Kirscbaum, C. (2007) "Determinants of the diurnal course of salivary alpha-amylase" *Psychoneuroendocrinology*. 32, 392-401

Neelam, K., Vijay, S., and Lalit, S. (2012) "Various techniques for the modification of starch and the applications of its derivatives" *International Research J. of Pharm*. 3, 25-31

Nelson, G., Hoon, M.A., Chandrashekar, J., Zhang, Y., Ryba, N.J., Zucker, C.S. (2001) "Mammalian sweet taste receptors" *Cell*. 416, 199-202.

Nelson, G., Chandrashekar, J. Hoon, M.A., Feng, L., Zhao, G., Ryba, N.J.P., Zucker, C.S. (2002) "An amino-acid taste receptor" *Nature*. 28, 58-63.

Nilsson, G.S., Bergquist, K.E., Nilsson, U., and Gorton, L. (1996) "Determination of the Degree of Branching in Normal and Amylopectin Type Potato Starch with H-NMR Spectroscopy Improved resolution and two-dimensional spectroscopy *Starch/ Stärke* 48, 352-357

Nissenbaum, J.W. and Sclafani, A. (1985) "Qualitative Differences in Polysaccharide and Sugar Tastes in the Rat: A Two-Carbohydrate Taste Model" *Neurosci. Biobehav. Rev.* 11, 187-196

Patil, N.B., Somvanshi, B.S., Gupte, S.P., and Kale, N.R. (1974) "Isolation and characterization of high-molecular–weight amylose by dispersion of starch granules with urea" *Makromol. Chemie.* 175, 1979-1994

Perry, G.H., Dominy, N.J., Claw, K.G., Lee, A.S., Fiegler, H., Redon, R., Werner, J., Villanea, F.A., Mounain, J.L., Misra, R., Carter, N.P., Lee, C., and Stone, A.C. (2007) "Diet and the evolution of the human amylase gene copy number variation. *Nat. Genet*. 39, 1256-1260

Psimouli, V. and Oreopoulou, V. (2013) "The effect of fat replacers on batter and cake properties" *J. Food Sci*. 78, C1495-C1502

Ramirez, I. (1991) "Does Starch Taste Like Polycose?" *Physiology & Behavior*. 50, 389-392

Ramirez, I. (1994) "Glucose Polymer Taste is Not Unitary for Rats. *Physiology & Behavior*. 55, 355-360

Rohleder, N. and Nater, U.M. (2009) "Determinants of salivary a-amylase in humans and methodological considerations" *Psychoneuroendocrinology*. 34, 469-485

Robyt, J.F. (2008) Starch: Structure, Properties, Chemistry, and Enzymology, in: B.O.F.-R.P. Dr., K.T.P. Dr., (Eds.), Glycoscience, Springer Berlin Heidelberg: pp. 1437-1472

Robyt, J.F. (2005) "Inhibition, activation, and stabilization of α-amylase family enzymes" *Biologia*. 60, 17-26.

Robyt, J.F. and French, D. (1967) "Multiple attack hypothesis of α-amylase action: action of porcine pancreatic, human salivary, and *Aspergilllus oryzae* α amylases" *Arch. Biochem. Biophys.* 122, 8-16

Robyt, J.F and French, D. (1970) "The action pattern of porcine pancreatic α-amylase in relationship to the substrate binding site of the enzyme. *J. Biol. Chem*. 245, 3917-3927

Sako, N., Shimura, T., Komure, M., Mochizuki, R., Matsuo, R., and Yamamato, T. (1994) "Differences in Taste Responses to Polycose and Common Sugars in the Rat as Revealed by Behavioral and Electrophysiological Studies" *Physiology & Behavior*. 4, 741-745

Sanz, M. and Martínez-Castro, I. (2007) "Recent Developments in Sample Preparation for Chromatographic Analysis of Carbohydrates" *Journal of Chromatography A*. 1153, 74–89

Sclafani, A. (1986) "Influence of Taste and Nutrition on the Sugar Appetite of Rats" *Nutrition and Behavior*. 3, 57-74.

Sclafani, A. (1987) "Carbohydrate Taste, Appetite, and Obesity: An Overview" *Neurosci. Biobehav. Rev*. 11, 131-153

Sclafani, A., Nissenbaum, J.W., and Vigorito, M. (1987) "Starch Preference in Rats" *Neuroscience & Biobehavioral Reviews*. 11, 253-262

Sclafani, A. and Clyne, A.E. (1987) "Hedonic Response of Rats to Polysaccharide and Sugar Solutions" *Neuroscience & Biobehavioral Reviews*. 11, 173-180

Sclafani, A., Hertwig, H., and Vigorito, M. (1987) "Influence of Saccharide Length on Polysaccharide Appetite in the Rat" *Neuroscience & Biobehavioral Reviews*. 11, 197-200

Sclafani, A., Thompson, B., Smith, J.C. (1998) "The Rat's Acceptance and Preference for Sucrose, Maltodextrin, and Saccharin Solutions and Mixtures" *Physiology & Behavior*. 63, 499-503

Scott, T.R. and Verhagen, J.V. (2000) "Taste as a factor in the management of Nutrition" *Nutrition*. 16, 874–885

Sen, D., Gosling, A., and Stevens, G.W. (2011) "Galactosyl Oligosaccharide Purification by Ethanol Precipitation" *Food Chemistry*. 128, 773–77

Shah, A.B., Jones, G.P., and Vasiljevic, T. (2010) "Sucrose-free chocolate sweetened with Stevia rebaudiana extract and containing different bulking agents – effects on physicochemical and sensory properties" *Int. J. Food Sci. Technol*. 45, 1426-1435

Sheldrake, P. (2010) "Chapter 16: Starch." In "Food Stabilisers, Thickeners, and Gelling Agents." Edt. Imeson, A. Wiley Blackwell: UK. 293-324.

Tordoff M.G., Alarcón, L.K., Valmeli, S., and Jiang, P. (2012) "T1R3: a human calcium taste receptor" *Sci Rep*. 2, 496

Treesuukol, Y., Smith, K.R., and Spector, A.C. (2011) "Behavioral Evidence for a Glucose Polymer Taste Receptor That Is Independent of the T1R2+3 Heterodimer in a Mouse Model" *Journal of Neuroscience*. 31, 13527-13534

Turner, C.E., Byblow, W.D., Stinear, C.M., and Gant, N. (2014) "Carbohydrate in the mouth enhances activation of brain circuitry involved in motor performance and sensory perception" *Appetite* 80, 212-219

Vasselli, J.R., Haraczkiewicz, E., Maggio, C.A., and Greenwood, M.R.C "Effects of a

glucosidase inhibitor (Acarbose, BAY g 5421) on the development of obesity and food motivated behavior in Zucker (fafa) rats" *Pharmacology Biochemistry & Behavior*. 19, 85-95

Vigorito, M. and Sclafani, A. (1987) "Effects of anosmia on Polycose appetite in the rat. *Neurosci. Biobehav Rev*. 11, 211-213.

Wang, Y.-J. and Wang, L. (2000) "Structures and properties of commercial maltodextrins from corn, potato, and rice starches" *Starch/Stärke* 52, 296-304

Werner, S.R.L., Fanshawe, R.L., Paterson, A.H.J., Jones, J.R., and Pearce, D.L. (2006) "Stickiness of corn syrup powders by fluidized bed test" *Int. J. Food Eng*. 2, Article 7

Wheelan, W.J., Bailey, J.M., and Roberts, P.JP. (1953) "The mechanism of carbohydrase action. Part I. The preparation and properties of maltodextrin substrate" *J. Chem. Soc. (Resumed)*. 260, 1293-1298

Whistler R.L. and BeMiller, J.N. (1997) "Carbohydrate Chemistry for Food Scientists." Eagan Press: St. Paul, MN

White Jr., D.R., Hudson, P., and Adamson, J.T. (2003) "Dextrin characterization by high-performance anion-exchange chromatography-pulsed amperometric detection and size-exclusion chromatography-multi-angle light scattering-refractive index detection" *J. Chromatog. A* 997, 79-85

Wijk, R.A., Prinz, J.F., Engelen, L., and Weenen, H. (2004) "The role of a-amylase in the perception of oral texture and flavour in custards" *Psychology & Behavior*. 83, 81-91

Yoon, S.H. and Roybt, J.F. (2003) "Study of the inhibition of four alpha amylases by acarbose and its 4IV-a-maltohexaosyl and 4IV-a-maltododecaosyl analogues" *Carbohydrate Research*. 338, 1969-1980

Zuckerman, S, Glendinning, J.I., Margolskee, R.F., and Sclafani, A. (2009) "T1R3 taste receptor is critical for sucrose but not Polycose taste" *Am J Physiol Regul Integr Comp Physiol*. 296, R866–R876

IUPAC. (2006) Compendium of Chemical Terminology, 2nd ed. (the "Gold Book"). Compiled by A. D. McNaught and A. Wilkinson. Blackwell Scientific Publications, Oxford (1997). XML on-line corrected version: http://goldbook.iupac.org

National Research Council. (2003) Food Chemicals Codex: Fifth Edition. Washington, DC: The National Academies Press

Appendix: Supplemental Data

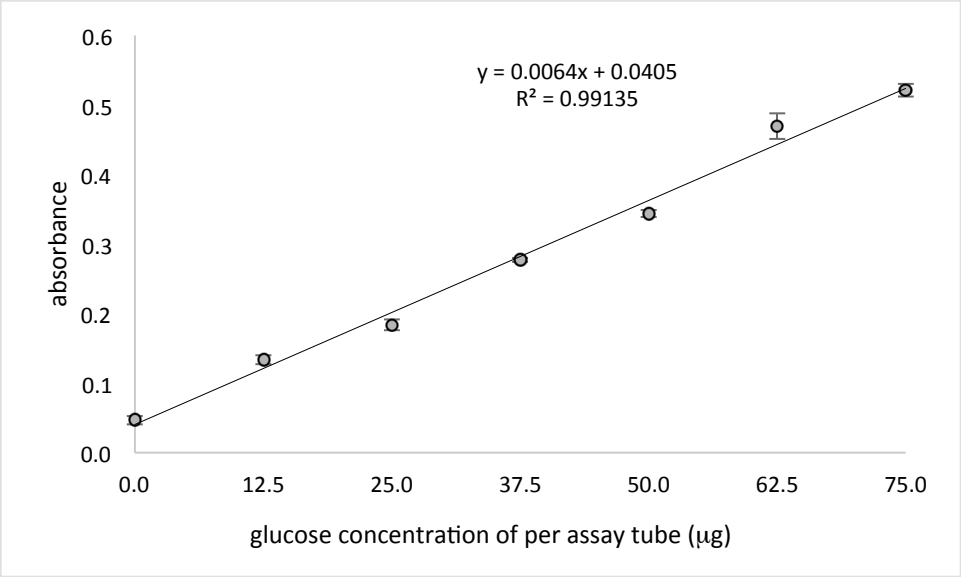

Figure 1. Glucose calibration curve of glucose concentration (0-75 μg) per assay tube against absorbance.

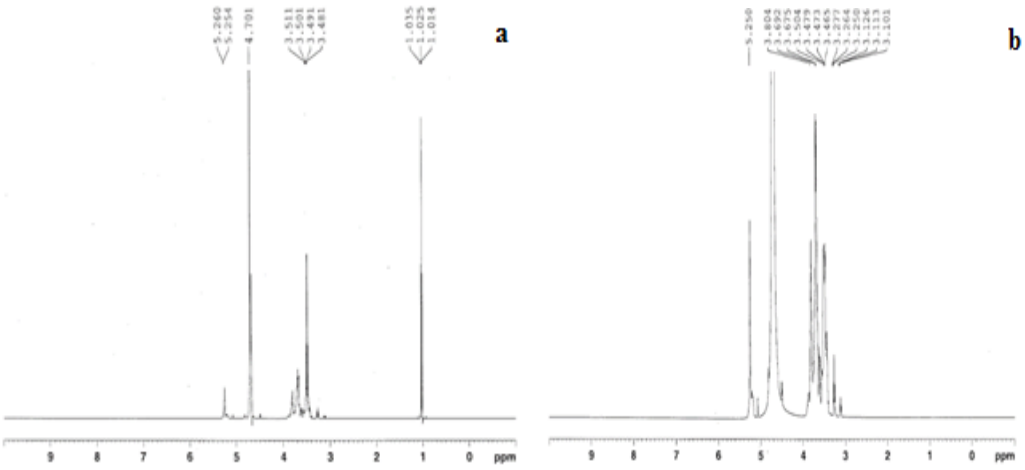

Figure 2. Representative ^{1}H NMR spectra of 90EI-CSS (Acronym defined as in Figure 1) dissolved in D_2O (**a**) prior to complete ethanol removal and (**b)** following multiple solvent-exchanges for complete ethanol removal. Ethanol in D_2O produces a triplet at 1.17 and a quadruplet at 3.60 ppm (Gottlieb, 1997).

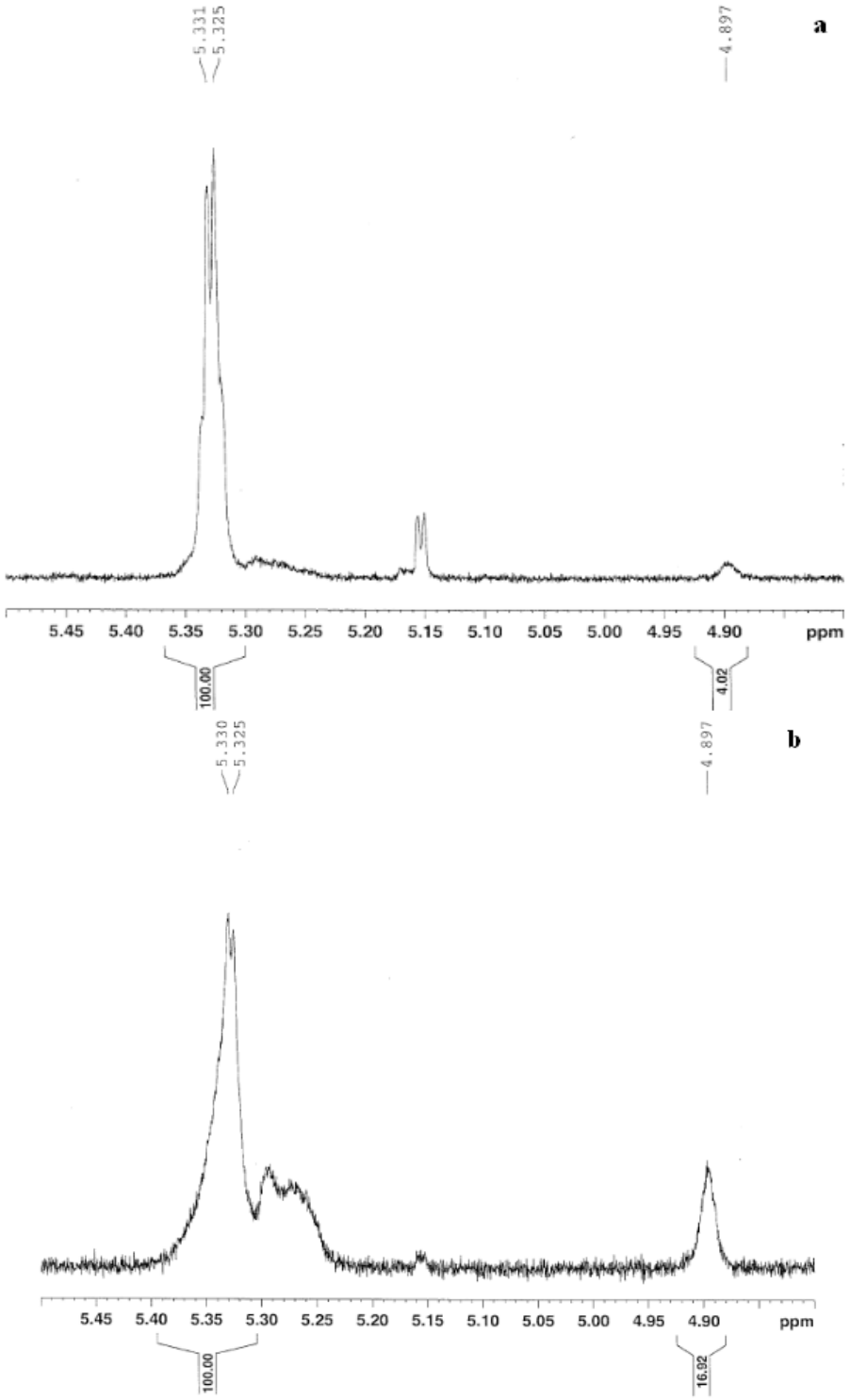

Figure 3. Representative ^{1}H NMR spectra of 90EI/70ES-CSS dissolved in D_2O (**a**) showing full spectrum and (**b**) magnified spectrum of ppm range containing peaks corresponding to α-(1→4) (5.305-5.395 ppm) and α-(1→6) (4.881-4.924 ppm) linkages (Nilsson et al., 1996).