EFFECT OF POLYOLS ON FLAVOR RELEASE DURING MASTICATION OF SUGAR-FREE CONFECTIONS

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ABSTRACT

Flavor is one of the most important attributes influencing food choice. In confectionery products, a major focus on product innovation involves developing more desirable flavor attributes for maximum impact and duration as well as providing healthier products.

In addition to advances in flavor technology, the confectionery industry has moved towards products that are sugar-free by utilizing polyols (sugar alcohols) instead of traditional sugar ingredients, such as sucrose or glucose. Polyols, like sugar, provide sweetness to a confectionery product and can also impact the product performance in multiple ways such as the texture and the flavor release properties. Currently there is limited knowledge on how polyols alter flavor delivery and ultimately impedes product innovation. The overall objective of this thesis was to investigate the influence of polyols on delivery of flavor components, volatiles (aroma) and non-volatiles (taste), in confectionery products.

As an initial study (presented in Chapter 3) a model confectionery product was formulated with different polyols: xylitol, mannitol or sorbitol that ranged in physical-chemical properties. Release of volatile and non-volatile flavor components was monitored close to the respective biological receptors of three trained panelists over a 12 min mastication period. Real-time release of volatile flavor compounds (aroma) were measured in exhaled breath by APCI-MS analysis, and non-volatile flavor compounds (taste, polyols and high intensity sweeteners, HIS: acesulfame K and aspartame) were monitored in expectorated saliva by LC/MS/MS analysis. In general, the rate of delivery of both aroma and taste components were inversely related to the water solubility of the

polyol utilized. The flavor release profiles for both the aroma and HIS compounds were highest for the sample formulated with the least water-soluble polyol (mannitol) and lowest for sample with the most water-soluble polyol (sorbitol). Time-intensity sensory evaluation of these samples was also in agreement with the instrumental data supporting the observed chemical changes in flavor delivery resulted in changes in flavor perception.

Based on the observation that water solubility of polyols influenced flavor delivery, we further hypothesized that surface area of the polyol material would also influence the flavor release profile. In chapter 4, the influence of polyol-type and particle size on the flavor release profile in a sugar-free confection was investigated *in vivo*. Four samples with an average particle size of 62 or 246μm (sorbitol) and 57 or 184μm (mannitol) were analyzed. Two trained panelists masticated the samples at a controlled chewing rate for 12 minutes period. APCI-MS analysis of the expired breath reported the samples formulated with mannitol, in general, had a higher aroma release profile than when formulated with sorbitol. LC/MS analysis of the expectorated saliva reported the HIS had a significantly higher release profile for the smaller polyol particle size samples; the release rate of polyol was not significantly changed by the particle size. Sensory time-intensity analysis of the sorbitol samples was also in agreement with the HIS delivery, the smaller particle size sorbitol gum was significantly higher in perceived sweetness intensity (compared to the larger particle sized sample). In summary, unique polyol-flavor interactions were reported to alter flavor delivery; the aroma compounds were mainly influenced by the polyol-type whereas the HIS by particle size (surface area).

In the final research phase the mechanisms of flavor delivery in confections were further investigated by conducting a mass balance analysis of the volatile and non-volatile flavor compounds during a 12 min mastication of samples formulated with sorbitol of two particle sizes (Chapter 5). The mass and release rate of the volatile compounds (ethyl butyrate, benzaldehyde, menthol, menthone and limonene) were monitored in the exhaled breath, in the saliva and the sample bolus; whereas the non-volatiles compounds (sorbitol or mannitol, aspartame and acesulfame K) were monitored in the expectorated saliva and the sample bolus. In general, the percent recovery of the volatile compounds released during mastication was lower or was less quantifiable in comparison to the more polar non-volatile compounds. For the volatile compounds analyzed, the medium hydrophobicity region showed the lowest % recovery (< 10%) and suggested biological absorption was the main quantitative route of delivery in the oral cavity. The aroma release profile in the exhaled breath was reported not to be influenced by the compound concentration in the saliva or sample bolus during mastication but was suggested to be mainly controlled by residual levels of these compounds in the oral cavity or the lungs. Conversely, the percent recovery of the non-volatile compounds released from the samples during mastication was relatively high (> 90%). The release profile of both the polyol (sorbitol) and HIS compounds were reported not to be concentration dependent during the first 4 minutes of mastication. This suggested the polyol and HIS were physically entrapped in the sample matrix and the release mechanism was related to the surface exposure rate these compounds to the oral cavity during chewing (mechanical stress) for release to occur during mastication.

In summary, we have reported novel mechanisms by which polyols influence the delivery of the aroma and taste compounds in sugar-free confectionery products as well as oral processing mechanisms that govern flavor delivery (absorption in the oral cavity). This information provides new knowledge for the food (and pharmaceutical) industry to tailor product quality.

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Chapter 1 : Literature Review

1.1 HISTORY OF CHEWING GUM

The need for humans to chew on something either for enjoyment or other purposes is not new. According to anthropologists, the ancient Greeks used to chew the bark of the mastic tree, which could be found in Greece and Turkey. Similarly, ancient Mayans from southern Mexico used to chew on the sap from the sapodilla tree, known as chicle. This material was widely used in production of gums in the early 1860s (Huzinec, 2009). Colonists coming to the state of Maine noticed that the native Indians chewed a gummy substance from the local spruce trees but did not swallow. In 1848, an entrepreneur John Curtis used this ingredient to make chewing gum and became the first official chewing gum manufacturer in the world. His gums were called the "State of Maine Spruce Gum" (Fenimore, 2008). Ever since, the demand for chewing gum has been increasing all over the world as well as the ever-increasing research and development. As the product witnessed widespread popularity, people were looking for new uses. In 1881, a druggist named Dr. Edward E. Beeman incorporated his pepsin compound to chewing gum (Fenimore, 2008). Although not a commercial success in the beginning, this set a milestone as the first-known medicated gum, which is an appealing idea to this day.

1.2 COMPOSITION OF CHEWING GUM

Chewing gum is primarily a two phase system: a water soluble phase (sugar, sugar alcohol, high intensity sweeteners) and a water insoluble phase (gum base) composed roughly in the ratio of 3 to 1. In addition, many other ingredients shown in **Table 1-1** complete the formation of a chewing gum. The ratios of all these ingredients

play a critical role in obtaining both desirable sensory properties as well as production efficiency (Mestres, 2008).

Table 1-1. General formulations for sugar and sugar-free chewing gums. (Ponkala et al., 2008)

Ingredient	Sugar Gum (% by wt.)	Sugar-Free Gum (% by wt.)
Gum base	20	28
Sugar	60	--
Corn/Glucose syrup	18-20	--
Polyol	--	40-65
Glycerin	0.5	6
Flavor	0.5-1	0.6-1.5
High intensity sweetener	--	0.05-0.1

1.2.1 Gum Base

Unlike other food products, chewing gum is a unique food that is not swallowed but rather can be enjoyed for several minutes. The ingredient that renders chewing gum its unique quality is the gum base which is responsible for its water insolubility as well as elasticity. Gum base itself is composed of many ingredients such as elastomer, elastomer solvent, polyvinyl acetate, emulsifier, low molecular weight polyethylene, waxes, plasticizer and fillers (Cherukuri et al., 1985). These ingredients are very compatible among themselves and do not undergo any chemical reaction (Estrunch, 2008). Changing the ratio of any of these ingredients even slightly affects the quality of the resulting gum base, which in turn, affects the quality of the resulting chewing gum. It is

perhaps due to this reason that the composition of gum base is considered a valued trade secret and findings are often published as patents rather than peer-reviewed journal articles. Major ingredients and composition of a typical non-adhesive gum base are explained below and tabulated in Table 1-2.

Table 1-2. Typical formulation of chewing gum base.

Ingredients	**Examples** [1,2,3]	**Weight (%)** [1,2]
Elastomer	Synthetic elastomers like Butadiene-styrene copolymers, polyisobutylene and isobutylene-isoprene copolymers. Natural elastomers like chicle, rubber, jetulong, balata, guttapercha, sorva	8-30
Elastomer solvent	Methyl, glycerol or pentaerythritol esters of rosins or modified rosins	2.5-13
Emulsifier	Glycerol monostearate, lecithin fatty acid monoglycerides, diglycerides, triglycerides, propylene glycol monostearate	0.5-10
Filler/Texturizer	Magnesium and calcium carbonate, magnesium and aluminum silicate, talc, mono, di and tri calcium phosphates	0-5
Low molecular weight polyethylene		0.5-15
Polyvinyl Acetate		15-45
Softener/Plasticizer	Hydrogenated vegetable oils, lanolin, stearic acid, sodium stearate, potassium stearate	9-40
Wax	Microcrystalline wax, natural wax, petroleum wax	0.5-10

[1] Estrunch, 2008; [2] Cheruki et al., 1985; [3] Koch et al., 1983.

Elastomer is the ingredient that imparts the rubbery, elastic and cohesive nature to the gum. Pioneers of gum manufacturing relied on natural elastomers extensively. However due to the need for constant supply, quality and economic considerations, synthetic polymers are the norm today. Selection of polymers determines the chewy, elastic quality of the final chewing gum. For example, the polymer butadiene-styrene rubber (SBR) gives a very good elastic and film forming capacities to chewing gum, making it a good elastomer for bubble gums. Isobutylene-isoprene copolymer (PIIB or butyl), on the other hand, gives a very balanced elastic-plastic properties to chewing gum, making it appropriate for a wide range of products, from bubble gum to chewing gum. (Estruch, 2008).

Elastomer solvents are used in gum base formula in order to plasticize the elastomers in the beginning of the process. Some common elastomer solvents are ester resin gums which can be modified to various forms that differ in the firmness they provide to the gum base. Others like terpene resins are attributed to reducing stickiness and may also improve flavor perception in chewing gum (Estruch, 2008).

Texturizers or fillers are the low cost ingredients that modify the texture of gum base as well as help in processing. Some examples of texturizers are magnesium and calcium carbonate, magnesium and aluminum silicate, etc. Use of certain texturizes such as calcium carbonate poses a problem in formulations that require addition of acidic flavors since acid readily reacts with calcium carbonate. This not only results in loss of acids but also can damage chewing gum packages and candy coated gums due to

production of carbon dioxide gas. In such cases, talc is commonly used since it does not react when in contact with acids, however the cost is high (Fritz, 1995).

Other ingredients such as waxes and plasticizers are used to improve the texture of gum bases so as to give a better chew quality to the gum. By softening the gum base, they also help in the blending procedure. Emulsifiers bring the normally incompatible constituents of the gum base together so that a single continuous and finely dispersed stable system can be formed. They may also help in incorporation of flavors into the gum base giving a uniform flavor distribution, and also reduce stickiness of gum to teeth and lips (Estruch, 2008).

Various patents have described the process of making gum base. (Ehrgott et al., 1976; Koch et al., 1980; DeTora et al., 1984). Generally, the process starts with softening the elastomer by mixing with the elastomer solvent and/or plasticizers, and/or emulsifiers for about 30 to 120 minutes. After this initial mixing is complete, the remaining base constituents are added in bulk, incrementally or stepwise and the resulting mixture is blended for an equivalent period of time (Cherukuri et al., 1985). The resulting gum base can be in various shapes and forms such as slabs, sheets, pellets, pearls and drops. While the primary focus of the various patents describing the process of gum base production has been on texture aspects, interest in creating a gum base material that is bio degradable and therefore environment-friendly has been gaining momentum, and has been the subject of many patents (Cook, 1996; Li et al., 2000; Neergaard et al., 2011).

1.2.2 Sweeteners

Sweetness is the foremost taste quality associated with chewing gum regardless of the type of flavoring. Often times, people consider a chewing gum to be "flavorful" as long as the sweetness persists. Davidson et al. (1999) found that the perception of chewing gum mint flavor followed the release of sucrose rather than the "mint flavor" stimuli – menthone. Such interaction between taste and aroma will be discussed in depth in subsequent parts. For the purpose of this section, it can be concluded that sweeteners are an important part of a chewing gum formulation. In addition to this obvious role in chewing gum, sweeteners are also important for providing bulk to the product. Depending on the type of sweeteners used, gums can be grouped as sugar gums and non-sugar gums, as described below.

1.2.2.1 Sugar Gums

Sucrose is the most common sweetener used in sugar gums due to its universal availability and versatility (Nicol, 1991). Despite a lack of systematic study, manufacturers of chewing gum have found that the particle size of sucrose does have impact on not only the manufacturing process but also on the final texture of the gum. Sizes below 40 μm are found to make the product firmer whereas those over 150 μm give a sandy texture. (Carcasona, 2008). Dextrose is another sugar used mainly for lowering costs compared to sucrose and the cooling effect it imparts. However, it cannot be used to completely replace sucrose since it has a negative effect on both chewing character as well as processing of the gum. Often times, glucose/corn syrups are also added as plasticizers.

1.2.2.2 Non-Sugar Gums

In non-sugar gums, sucrose is replaced by polyols or sugar alcohols. They provide fewer calories per gram than sugar, do not cause sudden increases in blood glucose levels and do not promote growth of dental caries (Calorie Control Council, 2012). The first sugar free chewing gum was commercially made and distributed in Denmark in the mid 1960s (Piotrowski, 1994). Although polyols have inherent sweet quality, their sweetness is lower than that of sucrose. So, to compensate for the reduced sweetness, products made with polyols are complemented with non-nutritive high intensity sweeteners (HIS), which will be explained in subsequent sections.

Polyols or sugar alcohols are carbohydrate derivatives that contain only hydroxyl groups as functional groups, hence the name sugar alcohol; however they are neither sugars nor alcohols. Many polyols are naturally found in plants; however for efficiency and cost effectiveness, they are manufactured industrially by catalytic hydrogenation of the corresponding saccharides (Billaux et al., 1991) as shown in Figure 1-1. Polyols are incompletely absorbed in the digestive tract which is responsible for fewer calories per gram consumed. However, if consumed at elevated levels, they can cause diarrhea (American Dietic Association, 2004). Some of the commonly used polyols in many food applications today are sorbitol, xylitol, erythritol, mannitol, maltitol, lactitol, and trehalose. Some of these polyols will be discussed in detail below.

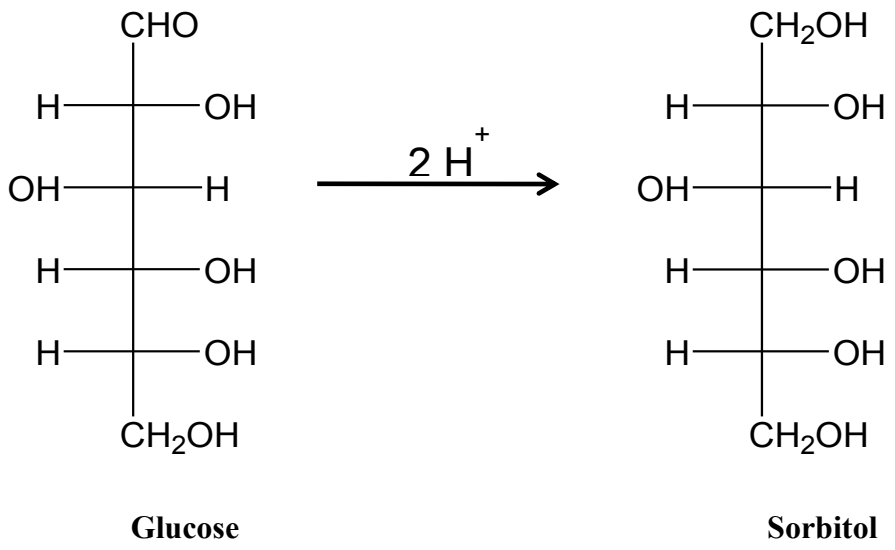


Figure 1-1. Conversion of sorbitol from glucose

1.2.2.2.1 Polyols

Sorbitol is one of the most commonly used polyol in sugar-free chewing gum. It is a hexitol that is found naturally in animals and plants as well as easily synthesized by hydrogenation of glucose as shown in Figure 1-1. It is relatively cheaper than other polyols and is widely available. Among other properties, its high water solubility (235g in 100g water at 20˚C), high melting point (93-97˚C), high heat of solution (-111kJ/kg), relative sweetness (0.6 compared to 10% sucrose solution rated as 1) and GRAS status makes sorbitol a choice for many applications (Lawson, 2007b). Some common uses of sorbitol are in hard-boiled candies, chewing gum, chocolate, etc. However, if consumed in excess, flatulence or a laxative effect can be induced.

Mannitol is another hexitol found naturally in plants but mostly synthesized by catalytic hydrogenation of D-fructose. Being an isomer of sorbitol, chemical-physical properties of mannitol are markedly different. Mannitol is sparingly soluble in water (18g

in 100g water at 20˚C), the least sweet of all the polyols, and does not give a strong cooling effect in the mouth despite having a higher negative heat of solution than sorbitol (-121kJ/kg) due to low water solubility (Lawson, 2007c). Use of mannitol is not cost-effective but is used in small quantities as a dusting material to prevent chewing gum from sticking to machinery during manufacture.

Xylitol is a pentitol widely found in nature. Compared to other polyols, xylitol is expensive; however it seems to be garnering special interest for prevention of dental caries. Unlike most sugar alcohols, xylitol is almost as sweet as sucrose. Also due to its high negative heat of solution and high water solubility, xylitol has a pleasant cooling effect suitable mostly for mint flavored products (Fritz, 1995).

1.2.2.2.2 High Intensity Sweeteners (HIS)

Acesulfame K is a white crystalline powder sweetener widely used in chewing gums. It is approximately 200 times sweeter than 3% (w/w) sucrose solution and is highly water soluble (Rathjen and Lipinski, 2007). It has a pleasant clean sweet taste like sucrose but fades quickly. It is a very stable sweetener and does not decompose at temperate and pH conditions encountered in many cooking/baking processes. It is also stable in aqueous form and pH range common for beverages, making it a good candidate for use in low-calorie beverages.

Aspartame is another sweetener commonly used in chewing gums. It is a synthesized sweetener: a dipeptide of aspartic acid and the methyl ester of phenylalanine. It has a clean sweet taste like sucrose (Meyer, 2007) but is about 160-220 times sweeter. The sweetness potency of aspartame can vary as a function of concentration, temperature

and pH. In dry powder form, aspartame is very stable however solution stability is affected by pH and temperature. A cool temperature and pH 4.2 is considered to be the optimal condition for stability of aspartame solutions.

Acesulfame K and aspartame are known to be synergistic to each other. They balance and enhance the final sweet perception. Due to its high water solubility, acesulfame K is perceived quickly and fades quickly as well. So, it is used to mask initial off flavors introduced by sweeteners like aspartame which has slower onset of sweetness but lasts longer. This results in a more rounded sweetness quality. Different blends of these two sweeteners show high synergy with 40 to 50% increase in sweetness intensity with the 1:1 ratio of acesulfame K with aspartame showing the highest synergism (Lipinski, 1996).

1.2.3 Flavor

A number of flavoring options are available for chewing gum. Depending on the flavor attribute required such as fruity or minty, flavorings are added to chewing gum in liquid form. Compared to other constituents of chewing gum such as sugars and gum base, the percentage of flavor is low (around 1%). However, this flavor load is relatively higher than other foods because only a very small portion of the added flavor is released (Kendall 1974; De Roos, 1990). Flavors are important constituents of chewing gum not only for the obvious sensory reasons but they also act as plasticizers for the gum base (Fritz, 1995). By changing the proportion of flavors, the texture of the resulting gum product can be modified.

Table 1-3. Properties of some common sugar alcohols

	kcal/g [1]	Regulatory Status (US)[1]	Relative Sweetness (10% Sucrose = 1)[4,7]	#hydroxyl groups	Solubility (g/100g water at 25°C, unless specified) [2,3,4,5,6,7,8]	Heat of Solution KJ/kg[4]
Monosaccharide polyols						
Erythritol	0.2	GRAS	0.6-0.7	4	37	-184
Xylitol	2.4	Food additive for use in special dietary uses	1	5	65	-153
Sorbitol	2.6	GRAS with laxative warning	0.6	6	235	-111
Mannitol	1.6	Food additive with laxative warning	0.5	6	22	-121
Disaccharide polyols						
Maltitol	2.1	GRAS	0.8	9	165	-69
Isomalt	2	GRAS	0.5-0.6	9	24 @20°C	-39.4
Lactitol	2	GRAS	0.3-0.4		60	-74
Trehalose	4	GRAS	0.45	9	68.9 @20°C	

[1]American Dietic Association (2004).; [2]Dwivedi, 1991; [3]Lawson, 2007; [4]Bond, 1991;[5]Billaux et al,1991; [6]Ferguson et al., 2007; [7]Higashiyama,2002; [8]Kappas, 2007;

Table 1-4. Properties of some common high intensity sweeteners.

High Intensity Sweetener	**kcal/g** [1]	**US Regulatory Status** [1,4,6,8]	**Carcinogenic**[4]	**Relative sweetness (Sucrose = 1)** [1,2,7]	**Sweetness Profile** [2,3]	**Solubility (g/100g water at 25°C, unless specified)** [2,3]
Aspartame	4	GRAS as general-purpose sweetener	No	160-220	Clean sweet, very similar to sucrose	1.1
Acesulfame K	0	GRAS as general-purpose sweetener	No	200	Clean sweet taste, fades quickly, no aftertaste	27 @20°C
Alitame	1.4	Petition pending		4500	Intensely sweet, rapid onset and disappearance, no aftertaste	13.1
Cyclamate	0	Petition postponed	No	40	Long-lasting pleasant sweet taste, bitter and salty aftertastes at high dose	21
Neotame	0	GRAS as general-purpose sweetener	No	8000	Clean sweet, no aftertaste even at high concentration	1.26

Saccharin	0	Approved as a sweetener for beverages and as tabletop sweetener in foods with specific maximum amounts allowed	No	200-700	Sweet, metallic aftertaste in high concentrations	80
Stevioside	0	Not approved as sweetener but can be used in dietary supplement	No	300	Bitter aftertaste, astringent	0.125
Sucralose	0	GRAS as general-purpose sweetener	No	600	Sweet, close to sucrose, slightly longer sweet aftertaste	26
Thaumatin	4	GRAS as flavor enhancer in some foods	No	2000	Delayed onset but lingering sweetness, liquorice-like aftertaste at high dose	60
Aspartame-Acesulfame K (60:40) (Twinsweet)[5]	--	GRAS as general-purpose sweetener	No	350	Clean sweet, rapid onset, no lingering sweetness or aftertaste	2.75 @°21C

[1] American Dietic Association (2004).; [2]Wiet et al.,1992; [3]Grency, 1991; [4]Ponakala et al., 2008; [5]Hoek, 2008; [6]Kroger et al., 2006; [7] Kemp, 2006; [8]www.fda.gov

1.3 FLAVOR RELEASE AND PERCEPTION

Flavor is the one of the important parameter for quality and acceptance of foodstuff (Buttery, 1989). The flavor of foods that we experience is the result of various stimuli such as taste, aroma (odor), mouth feel (texture) and any pain/irritation, making it a multimodal process. Visual appearance, temperature and even mood also may have effect on flavor perception. When we eat a food, the flavor that is perceived is the final sensation produced by interaction of all these different inputs. In order for flavor to be perceived, the stimuli responsible for each of the modalities of flavor has to be released from food and transported to respective receptors (Overbosch et al., 1991).

Aroma or smell is generated due to interaction of volatile compounds with olfactory receptors of the nasal cavity. Taste, on the other hand, is the sensation generated by the interaction of non-volatile chemicals with the gustatory cells in the mouth. Flavors are initially released into the saliva phase in the mouth. From saliva, volatiles are then transferred to the mouth headspace before being finally transported to the olfactory epithelium in the nose. Non-volatiles are solubilized in the saliva and sensed by taste buds on the tongue. The trigeminal sensations such as astringency, pungency and cooling or burning sensations have similar mode of action as the non-volatiles in the sense that they need to be soluble in saliva first. The physical and chemical properties of the flavor components determine how they are released from a food matrix (Linforth and Taylor, 2006). Mouthfeel is the sensation produced by physical stimulation of receptors in the mouth such as by texture or temperature. It has been maintained that to ideally characterize flavor completely, it is necessary to measure all these stimuli close to the site

where stimulation occurs (Taylor, 2000). This can however present a big challenge to researchers since there are a number of physiological and physic-chemical factors that can affect flavor release and perception.

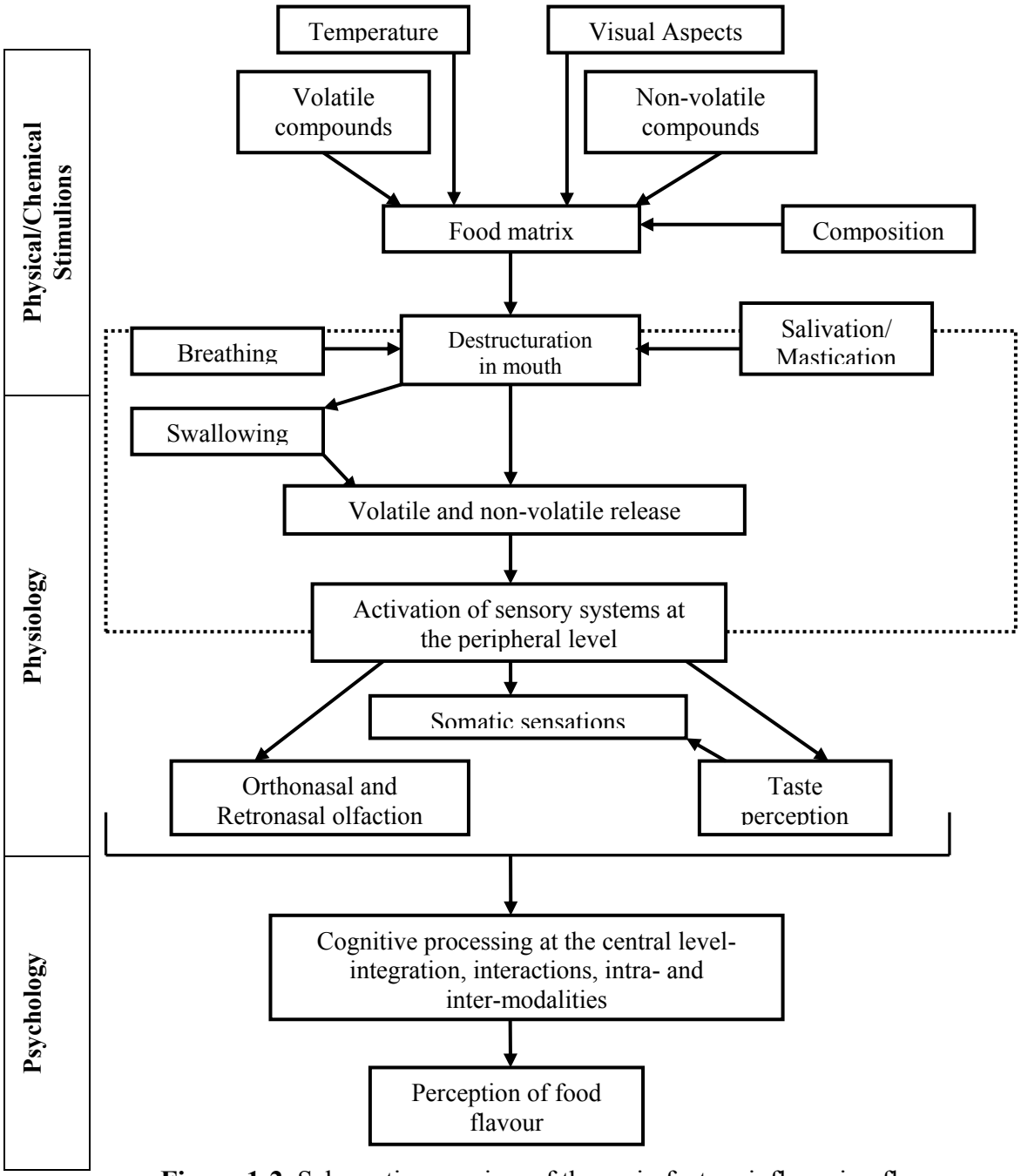


Figure 1-2. Schematic overview of the main factors influencing flavor perception (Adapted from Salles, 2006).

1.3.1 Effect of oral parameters on flavor release and perception

When a food is placed in the mouth, a number of events take place before the food is swallowed. First, the temperature of the food equilibrates to mouth temperature; it then gets mixed with saliva so that various salivary enzymes can act, and mastication of the food particles results in fracture of solid foods and shear thinning of liquids and semi-solids before being finally swallowed (Prinz et al., 2004). Mastication has been found to produce pulses of aroma to the nose (Overbosch et al, 1991; Hodgson et al., 2003). The flavor also undergoes dilution due to the expired air in case of the volatiles and saliva in case of the non-volatile taste compounds. All these processes change the physical properties of food, affecting the release and perception of flavor.

1.3.1.1 Mastication and Swallowing

Mastication is a complex process brought about by rhythmic jaw movements resulting in fracture and deformation of the product to form bolus (Foster, et al., 2011). This increases the surface of the food exposed to saliva and air, thus facilitating the dissolution of taste compounds in saliva and the release of volatiles in the mouth headspace, leading to flavor sensations. The taste compounds are perceived by the taste buds located at the tongue. Various researchers have investigated the role of mastication parameters on the release of non volatile taste compounds. Pioneer et al. (2004b) showed that the release of non-volatiles compounds from model cheese was related to masticatory rate, duration, efficiency and salivary flow rate. They found that a low chewing rate was associated with a long chewing time and a low salivary flow rate, which delayed the time

to reach the maximum concentration of tastants in saliva. Neyraud et al. (2005) demonstrated that the subjects who chewed for a longer time rated bitterness to be more intense. These findings indicate that different masticatory actions affect release and perception of tastants, suggesting an influence of mouth geometry which varies widely among individuals. The density of taste papillae responsible for taste recognition is said to vary across individuals by as much as 16-fold and may also modify taste sensitivity (Salles et al.,2011).

In case of volatile aroma compounds, after being released in the mouth headspace, they are transferred to the olfactory epithelium located in the nasal cavity via the respiratory tract. However, not all the volatiles are transported to the olfactory epithelium but are lost in the way due to absorption in the upper respiratory system (Overbosch et al., 1991) and in the oral mucosa (Hussein et al., 1983; Buettner et al., 2002). Such adsorption of volatiles to the internal membranes has also been considered to be the cause for persistence of flavor, generally referred to as aftertaste (Linforth et al., 2002). Since aroma is transported along with expired air, a higher respiratory rate results in more transport of volatiles to the nose (Hanoka et al., 2001; Pioneer et al., 2004a). Effect of mastication on aroma release and perception has been a subject of study of many researchers as well. Van Ruth et al. (2002) showed that increasing the rate of mastication from 26 to 52 or 78 chews per second increased not only the maximum concentration in air (I_{max}) but also the time to reach I_{max} (t_{max}). However, the extent of the effect of mastication rate depends on the mass transfer coefficients of different compounds. Similarly, the number of chews prior to swallowing, masticatory muscle activity and

chewing frequency were also found to positively correlate with high concentration of volatiles in the nose (Pioneer et al, 2004a; Haahr et al., 2004). Therefore, it can be said that the masticatory activities have profound effects in release and perception of aroma. Several authors have suggested that the swallowing process plays an important role in perception of retronasal aroma (Land, 1994; Buettner et al., 2001; Buettner et al, 2002) as an effect of periodical jaw closure and opening, allowing bursts of air to be transported to nose. The maximum perception of aroma was actually found to occur close to the moment of swallowing (Linforth and Taylor, 2000).

1.3.1.2 Saliva

Saliva is a complex dilute aqueous solution composed of both inorganic and organic components secreted by various glands into the oral cavity. Most of the saliva is produced by three paired salivary glands: the parotid, submandibular (or submaxillary), and sublingual, accounting to about 90% of the total saliva volume (Bradley, 1991). A number of smaller salivary glands in the mucous membrane of the lips (labial), palate (palatine), and cheek (buccal) also contribute to the overall saliva production (Bradley and Biedler, 2003). The labial glands in the tongue are considered to be important in taste function since they drain into the clefts of the circumvillate and foliate papillae consisting of more than 70% of the taste buds (Reineccius, 2006). Salivary glands are estimated to produce 0.5-0.75 L of fluid in one day (Bradley, 1991). However, this may be changed due to stimulation as salivary secretion is controlled by the autonomic nervous system (Garett and Proctor, 1998). Other factors such as aging, disease, gender and drugs are

also considered to affect saliva flow (Dodds et al., 2005). Saliva is composed of 99% water and the rest is made up of ions and organic constituents (Schneyer, et al., 1972). Sodium and potassium are the most important cations in saliva whereas the major active anions are chloride and bicarbonate. Other electrolytes such as calcium, phosphate, fluoride, thiocyanate, magnesium, sulfate, and iodide are also present in saliva. (Schneyer, et al., 1972). Organic constituents of saliva include the enzymes amylase and lipase, as well as salivary proteins: mucin, histatin, statherin, proline-rich proteins (PRPs), lysozyme, etc. It is because of these various components that saliva serves multiple functions such as lubrication, buffering, maintenance of tooth integrity, antibacterial activity, and taste and digestion (Humphrey and Williamson, 2001).

Some studies have investigated the correlation between salivary flow rate and the release and perception of flavor compounds. The role of saliva in taste perception stems from the fact that the process requires dissolution of tastants in the saliva before being detected by the taste buds. However, there is no clear consensus on the effect of salivary flow rate on the release and perception of flavor. Fischer et al. (1994) found that during wine consumption, panelists with low salivary flow perceived bitterness and astringency for longer durations than those with high salivary flow. Noble (1995) also found that the low-flow subjects perceived bitterness and astringency more intensely, and lasted longer. However, the same study showed no difference in perception of sweetness or sourness. Similar results for sweetness, sourness and fruitiness have been reported by others (Ishikawa and Noble, 1995; Bonnana and Noble, 1995). Guinard et al., (1997), on the other hand, found a significant positive correlation between saliva flow and time to reach

maximum intensity of sweetness in chewing gums for panliests with high-flow but there was no effect on the intensity of sweetness.

Saliva may also have an effect on aroma release. Taylor (1996) concluded that dilution of foods by saliva affects the partitioning of the volatile compounds over the food, saliva and vapor phase. van Ruth and Roozen (2000) found that an increase in saliva volume resulted in a significant decrease in release of aroma compounds from rehydrated French beans, similar to findings of Odake et al. (1998) and Harrison (1998). In contrast, Diebler et al. (2001) found that increasing saliva ratio increased volatility of esters but not aldehydes and ketones. Such contrasting results indicate that dilution is not the simple way in which saliva affects aroma. Friel and Taylor (2001) conducted a study to examine interaction of mucin with various aroma compounds. They found that mucin affected volatility of certain compounds whereas some were not affected, which can explain increased volatility of some compounds due to increase in saliva. In another study conducted in water and oil model systems, a salting-out effect was found for hydrophilic compounds, resulting in increased volatility whereas the volatility of hydrophobic compounds was reduced due to binding with salivary proteins (van Ruth et al., 2001). However, another study found no significant effect of saliva on in-mouth release of various aroma compounds from liquids in the initial 30 sec of ingestion, and concluded that enzymatic reactions were irrelevant for this duration (Rabe et al., 2004).

1.3.1.3 Absorption

Flavor release and perception can be affected by absorption of the released flavor components in the tissues of the mouth or throat. So, the proportion of the flavor that reaches the receptors may be different from what was originally released. Reabsorption may occur for many volatiles in the course of transport to the olfactory epithelium (Speizer and Frank, 1966). Either of these events may result in reduction or increment of the final sensory perception (Overbosch et al. 1991).

Such instances of absorption and resorption of chemical compounds in oral tissues have huge implication for the drug industry, and therefore have been studied (Beckett and Hossie 1971; Siegel, 1984) extensively. Chemical compounds have been found to permeate the oral epithelium based on their oil-to-water partition coefficient, molecular volume and presence of surfactants. When cigarette smoke was allowed to stay in mouth for two seconds, different components in the smoke were found to be absorbed by mouth tissue to different extents (Dalhamn and Rylander, 1968). Absorption of water soluble components was found to be around 60% whereas only 20% absorption was reported for non-water-soluble compounds. Similar finding was observed in a study of uptake and release of a highly soluble gas SO_2. During inspiration, almost the entire compound was absorbed by the nasal mucosa, of which 15% was desorbed during expiration. Like volatiles, taste compounds are also absorbed in the oral mucosa and has been found to be stereospecific (Manning and Evered 1976). The buccal absorption was favorable for D-glucose and L-arabinose, and was found to be partly dependent on the presence of sodium ions.

1.3.2 Physical-Chemical Parameters

1.3.2.1 Taste-Aroma Interaction

Taste and aroma are different chemical stimuli and produce different sensory sensations on their own. However, often times while consuming a food, it is difficult to characterize the taste and aroma aspects of the flavor as such. Consequently, people tend to confuse taste and aroma, suggesting interaction of some sort happening between taste and aroma (Rozin, 1982). Such interactions result from physicochemical, physiological and psychological effects, making flavor perception a complex process (Salles, 2006).

It is an everyday experience that certain tastes enhance aroma and vice versa. Murphy and Cain (1980) observed an increase in sweetness intensity of a sucrose solution when a citrus solution was sniffed simultaneously. Similar observations were also reported for sweet solutions and aroma of strawberry (Frank et al., 1989), peach (Cliff and Noble 1990), vanilla (Kuo et al. 1993), fruity (Stampanoni, 1993) and caramel (Stevenson et al. 1999). Further evidence for taste-aroma interaction can be obtained by interrupting either of the two stimuli. It is comparatively easier to block aroma stimulation by simply pinching the nose than to block taste. Frank and Byram (1988) found an 85% reduction in the enhancement of sweetness by strawberry aroma. Similarly, Murphy and Cain (1980) also reported a drop in sweetness intensity of a solution containing sucrose and citrus aroma when the nose was pinched than when it was not.

The phenomenon of taste-aroma interaction is not universal for all taste-aroma combinations but usually limited within congruent mixtures that are usually encountered

in foods. Examples of such congruent mixtures are sweetness and the so called "sweet-flavors" like strawberry, vanilla; saltiness with cheese; sourness with lemon, and so on. Baldwin et al. (1998) showed the association of fruity notes with sweetness and green note with sourness in tomato. Frank and Byram (1988) also concluded that interaction between taste and aroma are dependent on both taste and aroma. They reported an increase in sweetness of a beaten cream when strawberry aroma was added however no such effect was observed after addition of peanut butter aroma. They also found that strawberry aroma was unable to enhance saltiness of sodium chloride. Since strawberry aroma is not connected with being salty, this result further suggests the importance of congruency.

An increased concentration of aroma has been generally associated with enhancement of taste (Cliff and Noble 1990; Schifferstein and Verlegh, 1996), especially in the case of congruent mixtures. In case of incongruent mixtures, taste intensity is either reduced or unaffected (Stevenson et al., 1999). The same study reported that caramel aroma masked the sourness of a citric acid solution while enhancing sweetness (Stevenson et al. 1999).

Such taste-aroma interaction has also been explained by associative learning (Stevenson, 1995, 1998). People very often associate sweetness with fruity aromas (Dravneiks, 1985). Stevenson (1995, 1998) paired novel aroma such as lychee and water chestnut with either sucrose or citric acid. When panelists rated the sweetness and sourness of the respective aromas associated with the two tastes, the ratings were found to be significantly increased for the particular taste and not the other. Such associative

learning might arise from a cultural standpoint. Nguyen et al. (2002) reported that compared to French panelists, the American panelists observed a stronger strawberry-sweetness enhancement. Similarly, Viatnamese panelists showed a stronger effect of vanilla on sourness than the French. All these examples point to the common fact that understanding flavor perception is multi-faceted and hence very challenging.

As discussed above, an aroma with certain taste qualities such as sweet can enhance that taste in solution. This effect is however found to be dependent on what the subject is asked to judge. While Frank et al. (1993) had found that strawberry aroma enhanced sweetness of sucrose in solution when panelists were asked to rate sweetness only; the effect was lost once sourness and fruitiness were also added to the attributes to be tested. Interestingly, including too many responses such as sweet, salty, bitter, sour for one testing resulted in the suppression of the sweetness of the strawberry-sucrose mixtures due to inappropriate partitioning of responses (Frank et al., 1991, 1993). Such apparent enhancement in sensory perception due to the lack of appropriate attribute is termed as "halo-dumping" (Lawless and Clark, 1992). In absence of relevant attributes, panelists tend to "dump" sensory ratings into the attribute that is provided. Such cases are usually more prevalent while evaluating similar stimuli (Frank et al., 1993). Clark and Lawless (1994) reported that psychological bias can be introduced to time-intensity scaling results due to halo-dumping when sensory ratings for an aroma-sucrose solution was limited to sweetness. However, when the attribute flavor was added to the sensory ballot, enhancement of sweetness due to aroma was reduced. Therefore, it can be

concluded that one way to reduce "halo-dumping" effect is to establish relevant flavor attributes to characterize a sample, especially when similar attributes are present.

It is of utmost interest to flavor scientists to understand where and how the sensory information is processed in the brain. De Arauja et al. (2003) used magnetic resonance imaging (fMRI) technology to reveal brain areas that responded to either only taste (sucrose) or aroma (strawberry), and also areas that were activated by both taste and aroma. They also found more activation by the sucrose-strawberry mixture than by the sucrose or strawberry aroma solutions alone, suggesting sweetness enhancing effect due to taste-aroma interaction.

1.3.2.2 Texture-Aroma Interaction

Along with aroma and taste, the importance of texture on the overall flavor quality and food acceptance is undeniable. Studies have shown the effect of texture on the release and perception of aroma in foods and model systems. An early work by Pangborn et al. (1978) established that the perceived intensity of the aromas of coffee, orange and tomato decreased with an increase in the perception of the viscous character (Pangborn et al., 1978). They also indicated that the type of thickening agent used might affect the degree of such decrease. Roberts et al. (1996) reported in solutions of equal viscosity, using sucrose as the texturizer resulted in the greatest reduction in release of aroma than using guar gum or carboxymethylcellulose. They also found that the extent of this reduction was more pronounced for the highly volatile compounds than the less volatile. They suggested some sort of binding mechanism occurs between the aroma and

the thickening agent that prevents transfer of the volatiles from the food to the nasal cavity during mastication.

Since eating is a dynamic process, in-vivo studies have been conducted to investigate the effect of texture on flavor release and perception. Weel et al. (2002) examined the relation between gel strength, sensory perception and in-nose aroma concentration. The time-intensity sensory measurement showed a reduction in the perceived intensity of the flavor with an increase in the firmness of the gel although there was no difference in the nosespace concentration between the samples. From this, they concluded that flavor perception is determined by psychophysical interactions between texture of gels and aroma rather than the in-nose flavor concentration. Similar result was obtained by Baek et al. (1999) in gelatin gels. They further attributed the disparity between in-nose concentration and sensory perception to the different rates of initial release of the volatiles, with softer gels having higher rate than the harder ones.

1.3.2.3 Matrix Interaction

1.3.2.3.1 Carbohydrate

Carbohydrates are one of the most common constituent of many foods. Interactions between flavor and carbohydrates have been reported. Mono and disaccharides are known to affect flavor release by affecting the partition behavior of some aroma compounds due to a "salting out" effect (Hansson et al. 2001). Hansson et al. (2001) consequently showed increased release of five out of six flavor compounds tested due to sucrose and inverted sugar when the sugar concentrations were 60% (w/w) in

water. However the amount of sugar in the system is very high and may not be the case in real foods. When using low levels of fructose (1.4 to 4% w/w) in yoghurt, no change was found in the headspace concentration of the 15 compounds of a strawberry aroma (Decourcelle et al. 2004). A similar result was found from an in vivo release study of different volatiles in model custards with lower sucrose concentration (2.5-10%). The differences in flavor perception due to addition of sugar (as explained in the taste-aroma interaction section) are therefore more likely to be due to perceptual interactions rather than aroma release as suggested by Davidson et al. (1999). They reported that the perception of mint flavor during mastication of chewing gum was dependent on the release of sucrose rather than on that of menthone.

Polysaccharides have the potential to interact with flavor in many ways due to their diverse structures. As discussed in the texture section above, hydrocolloids such as starch, carboxymethylcellullose and pectin are known to change viscosity of food systems by gel formation at a critical concentration (c*), leading to reduced flavor perception (Moris, 1987; Roberts et al. 1996). Similarly, softer gels have been shown to release volatiles faster than harder gels, consequently resulting in increased intensity for flavor perception (Baek et al. 1999). Another way of interaction of carbohydrate with flavor is by the formation of inclusion complexes, esp. with starches and cyclodextrins. Flavor compounds are trapped inside these complexes due to hydrophobic interactions and are therefore unavailable for release. Analysis of headspace of volatiles above an ethanol:water containing cyclodextrin showed a reduction in volatile concentration, leading to a decrease in sensory character (Reineccius et al., 2003).

1.3.2.3.2 Lipid

Interaction between lipid and aroma is an expected effect because of the hydrophobic nature of most aroma compounds. Roles of lipids in the storage and release of aroma compounds are known (de Roos, 1997). Lipids act as a flavor solvent, hence resulting in delayed release and perception. An increase in lipid level has therefore been shown to reduce flavor release and perception, with maximum effects for lipophilic compounds and less to no effect for hydrophilic compounds with log P (ratio of concentrations of a compound in a mixture of two immiscible phases at equilibrium) values near or below zero (Brauss et al., 1999; Piparez et al., 1998; Guichard, 2002). Piparez et al. (1998) reported an increased retention of volatile compounds in a cheese matrix with an increase in the lipid triolein content, resulting in reduced release. They further concluded that this phenomenon is compound dependent.

Studies have also been conducted to understand the effect of lipid on flavor perception during dynamic conditions of eating. An in-vivo measurement of flavor release and perception from yogurts showed that decreasing the fat content resulted in a faster release rate initially and high intensity (Brauss et al., 1999). The instrumental data was found to correlate with sensory panelists who noted the difference in intensity as well as the initial release rate. Contrary to this result, Trorila et al. (1994) had reported no effect on timing of flavor perception during eating yogurt. Similarly, a time-intensity sensory panel conducted in ice cream with varying fat content found no significant difference in sweetness perception and vanilla flavor perception. They however reported

a difference in the time required to reach maximum vanilla intensity (Li et al., 1997). Another study conducted in low and high fat ice cream found faster release and higher intensity of stale and cherry flavors as fat content decreased but had the opposite effect on vanilla flavor release (Chung et al., 2003). Such contrasting data may be attributed to the instrumental or sensory methods and experimental designs chosen and points towards yet another difficulty in conducting flavor release studies.

1.3.2.3.3 Protein

The structure and conformation of proteins are the factors that make interaction with flavor possible. By virtue of these two qualities, proteins can interact with flavor either chemically or physically by reversible weak hydrophobic interactions or by stronger ionic and covalent bonds. In addition to the type of protein and flavor, factors that influence the characteristics of protein structure and shape also have an impact on the protein-flavor interaction. Among other factors, ionic strength, pH, temperature and time are important since they can have denaturing effects (Guichard, 2002). Denaturation causes exposure of the hydrophobic pockets of the protein, which are now more accessible for binding flavors (O'Neill and Kinsella, 1988).

1.3.2.4 Chemesthesis

In addition to taste and smell, chemesthesis is another integral component of flavor. It is the unique sensation in mouth produced by certain chemicals such as the cooling sensation produced by menthol, the burning of hot peppers and cinnamon, the

tingle and prickling of carbonated beverages, the sting of radish, the tartness and ting of lemon, and so on. Such chemical sensitivity in the mucous membrane is produced due to the stimulation of the "somesthetic senses", i.e. temperature, pain and touch (Treede, 1995). The burning sensation of capsaicin is enhanced by increasing temperature of the heat receptor VR1 while it is being stimulated (Ringkamp et al. 2001). Similarly, the cooling of menthol was found to be stronger at lower temperatures (Green, 1985). In addition to temperature, simple mechanical stimulations such as sipping and expectorating a liquid or moving the tongue and lips during tasting may have the potential to reduce capsaicin burn or pungency of menthol (Green, 1986; Green and Pope 2003).

Continuous chemesthetic stimulation is found to cause either sensitization or desensitization in perception depending on the interval between successive stimulations. Shorter intervals are conducive to sensitization whereby the chemesthetic perception is enhanced (Green 1998) and longer intervals are conducive to desensitization and therefore decrease in sensitivity (Green and Rentmeister-Bryant 1998). However, continuous re-stimulation is found to re-establish the sensation (Green, 1998). This is in contrast to other sensory stimuli, where continuous and repeated stimuli cause continuous decline in sensitivity due to sensory adaptation.

There are some evidences of interaction of chemesthetic stimuli with taste and smell. Primarily, masking effects have been observed for sweet, bitter and umami tastes (Simons et al., 2003) as well as retronasal aroma perception (Green, 1996). However, these effects are small and inconsistent. Carbonation, on the other hand, is found to

enhance the overall taste intensity, especially sweetness, and sourness and saltiness to some extent (Cowart, 1998).

1.4 TECHNIQUES FOR MEASURING FLAVOR

1.4.1 Aroma

1.4.1.1 Sample preparation

Sample preparation is the first and a vital step while measuring flavor (Schreier, 1984). More often than not, samples have to be modified in the way that makes it possible for them to be analyzed by an instrument. However, in the process, the sample can be deteriorated if an appropriate method is not utilized. Furthermore, sample preparation is complicated by many factors: matrix, instability, low concentration levels of aroma, complexities of aroma due to the sheer number of compounds in the aromatic composition of a food, variation of volatility (Parliment 1986). Consequently, there is no universal method that is optimal for every sample but whichever method is used, evaluations should be made to ensure that decomposition of heat labile compounds, loss of volatile compounds by distillation, or loss of low solubility compounds in extractions do not occur (Parliment, 1997; Teranishi, 1998).

The simplest method of sample preparation is direct injection of the sample into the instrument. This method is most suitable for analysis of simple samples that are not complicated by matrix effects (Parliment, 1997). Most sample preparation techniques usually involve extraction or distillation or a combination of both. While distillation is dictated by differences in vapor pressures, extraction is based on differences in solubility

in different solvents (van Ruth, 2001). Steam distillation followed by solvent extraction is one of the most common and easy to use sample preparation techniques for slightly volatile and water insoluble compounds. For samples that decompose under steam conditions, distillation may be done under vacuum. Simultaneous steam distillation/extraction (SDE) is another popular technique in flavor analysis. The volatiles are extracted from sample mixture and concentrated in a single operation, and recoveries of aroma compounds are generally high. Solid samples can also be extracted directly with a solvent. Solvents such as diethyl ether or methylene chloride are used to extract finely ground and dried samples such as a spice, coffee or a grain multiple times. The different solvent fractions are then combined and concentrated. High vacuum distillation is another effective method, especially when large amounts of lipids are present in sample. Another technique is the co-distillation in which a solvent is dispersed in the sample and is distilled rapidly (15-20 minutes) which generates samples without a boiled note. Other sampling methods such as headspace and solid phase microextraction (SPME) are also useful when the purpose to monitor aroma profile during certain processes such as consumption (Vidrih et al., 2009), cooking (Zeng et al., 2008), roasting (Mondello et al., 2005), storage (Paik and Venables, 1991) and so on versus characterization in the original food product. While such methods can be used for qualitative information, reliable quantification cannot be done (Lambropoulou and Albanis, 2007).

1.4.1.2 Gas Chromatography

Gas chromatography is a very widely used method for analysis of volatile aroma compounds. Aroma compounds, by nature, are required to volatilize into the mouth headspace and travel via air to be perceived. This inherent property of aroma compounds makes gas chromatography an excellent method of analysis for the volatiles such as food aroma (Wampler, 1997). With the availability of a variety of more stable columns with greater resolution, use of gas chromatography in aroma analysis has become even more powerful. A typical gas chromatograph is usually complemented by some chemical/physical detectors like flame ionization (FID), electron capture, thermal conductivity, mass spectrometric and many other detectors (Acree and Barnard, 1994). FID is one of the most popular detectors due to its high sensitivity towards a wide range of compounds as well as reliability. Components eluting from the column are directed to a hydrogen-air diffusion flame and and electrically charged species are formed. The current resulting from movement of these species is transmitted as signal (Colon and Baird, 2004). However, like many other detectors, FID is also unable to resolve two species with the same m/z value and lumps them as a single peak.

Coupling mass spectrometry (MS) with GC analysis has opened up new possibilities for flavor analysis since it provides both qualitative and quantitative information (Lehatay and Hajslova`, 2002) in a reduced time. Various kinds of MS can be coupled to GC. Quadrupole mass spectrometric systems provide the advantage of fast scan rates for fast GCMS (Rubiolo et al., 2008) and TOF analyzers are capable of giving wide spectral mass range and mass resolution (Hubschmann, 2009). Analysis of food

samples present a daunting challenge due to their compositional complexity and such analysis often requires combination of various techniques. So, the use of multi-dimensional analysis for aroma analysis has been increasing. Marriott et al. (2000) used GCxGC-TOF-MS analysis to identify components of lavender oil. They successfully resolved the otherwise overlapping components: monoterpene and sesquiterpene hydrocarbons, and their oxygenated analogues. Another study combined SPME with GCxGC-FID for comprehensive analysis of garlic powder (Adahchour et al., 2002). They reported an improved resolution as well as analyte detectability and concluded that this technique has potential to become a powerful tool for solving complex aroma identification problems of a wide range of foods. Similarly, tandem mass spectrometry provides another technique for analysis of complex mixtures (Fay et al., 1996). Given the complexity of food materials and availability of a wide array of techniques, the future of multidimensional and tandem mass spectrometry looks bright and will continuously evolve.

1.4.1.3 Model Mouth Systems

In an attempt to understand flavor release during eating, various authors have developed model mouth systems simulating human mouth conditions (Lee, 1986; vanRuth et al., 1994; Roberts and Acree, 1995; Weel et al., 2004; Arvisenet et al., 2008). However due to the complexity introduced by the mouth and the eating process, none of the attempts to use model mouth systems completely mimic the actual events. Effectiveness of the models can be gauged by the number of processes that imitate

changes during eating. The easiest factor to control is temperature and is generally set to the temperature of the human mouth (Arvisenet et al., 2008). In order to mimic saliva, the sample is humidified with water or artificial saliva (van Ruth and Roozen, 2000; Roberts and Acree, 1995). Although there are various formulations of artificial saliva published in literature (Muhler and Swenson, 1947; van Ruth et al., 1995; Gal et al., 2001; Preetha and Banerjee 2005), there is no clear answer if they have the exact same impact on flavor release as natural human saliva. Mastication is simulated by either stirring (Nassl et al., 1995) or crushing the samples with a plunger (van Ruth et al., 1994). Arvisenet et al. (2008) developed a more advanced mouth model system. The device included a notched plunger mimicking teeth and variable speed motors controlling the speed of compression (to simulate different mastication rates) and rotation (to assume the role of the tongue and jaw in the human mouth) to give different crushed apple samples. These samples were compared with apples crushed in the human mouth to measure the similarity between apple particles. Although such attention to details in constructing a model mouth increases the resemblance to real human mouth, the real test lies with direct comparison with in-vivo flavor release. This can be possible if the model mouth is coupled with more sensitive analysis or with online analysis such as APCI-MS or PTR-MS (to be discussed later) as done by Diebler et al. (2001). Volatile compounds released by artificial mouths are generally transferred to extraction and analytical instruments for analysis. However, it should be acknowledged that since the model systems represent uninterrupted exhaling, they do not represent the rhythm of breath cycles and therefore cannot be representative of breath-by-breath volatile release. It is perhaps due to the lack of a model mouth

inclusive of all the characteristics features of the human mouth and the eating process, less variation is exhibited by these models than by human subjects (Krause et al., 2011). Moreover, human subjective issues such as palatability, fatigue and safety are also eliminated.

1.4.1.4 Electronic Nose

Flavor analysis consists of not only identification and/or quantification of volatiles but it is also important to understand how the identified aroma contributes to the overall perception. Although various sensory methodologies exist for this purpose, it is both expensive and time consuming. The electronic nose has been developed as an alternative to analyze complex vapors in their native form in much the same way as the human olfactory system. The device consists of sensor arrays that represent sensors in the nose and can be used to monitor volatile compounds (Pearce et al., 1993). The sensors are based on conducting composites which respond to a variety of chemical classes and change resistance on exposure to a vapor. Response produced by such change in resistance is then converted to a signal using a computer software analogous to the way the brain converts sensory stimuli in the olfactory system into a response. Despite its lack of sensitivity and specificity in comparison to human nose, the electronic nose can be useful for quality control and flavor assessment functions such as assessing rancidity, authenticity, blending and so on (Hodgins, 1997). Bleubaum et al. (2002) compared sensory results with an electronic nose for apple juices and found high correlations suggesting that the electronic nose can be used for quality control and assurance. They

further pointed out the importance of identifying the factors determining the quality of a food product.

1.4.1.5 Identification of sensory relevant compounds by Gas Chromatography-Olfactometry (GC-O)

Continuous development in analysis techniques has resulted in identification of thousands of volatiles in foods. However this does not imply that all of these compounds are responsible for aroma and may not be sensorily relevant (Lin and Rouseff, 2001). In order to identify compounds that impact aroma, Fuller et al. (1964) developed the method of gas chromatography-olfactometry (GC-O) in which humans are interfaced with a GC system and a sample of vapor is split between an instrumental detector such as FID of a mass spectrometer and a human assessor. Since the human nose is very sensitive towards aroma with a theoretical odor detection limit of about 10^{-19} moles (Reineccius, 1994), GC-O is a suitable method to detect trace compounds or off-odors in foods. Using humans as the detector can however pose serious problems due to possible fatigue and sensory adaptation. GC-O studies have shown that on one hand, a big peak obtained in a GC method does not necessarily mean it contributes to aroma (Eyres et al., 2005); on the other hand, compounds present in too low concentration to be detected by instrument can be important contributors of aroma (Valim et al., 2003; Eyres et al., 2005). Several chemists have unsuccessfully attempted to use GC-O data to re-create synthetic models of natural aromas by blending different odorants (Flath et al., 1973; Durr and Schobinger, 1981). Such inconsistency in aroma quality between the original and the resulting aroma models has often been attributed to either lack of odorants that are only detectable by

GC-O and not by the GC detector or incorrect quantitative data. (Grosch, 2001). However the research group of Buttery et al. (1990, 1995) reported preparation of aroma models for fresh tomato and tomato paste based on results obtained by GC-O. Results from sensory panel showed that the aroma models were very similar to the original samples. This shows that it requires careful attention to perform analysis and evaluate data in order to obtain useful results from GC-O.

1.4.1.6 In-vivo measurement of aroma

Flavor perception is determined by aroma compounds released from the food matrix during consumption. When a food material is introduced in the mouth, it is modified by various conditions in the mouth such as mastication, saliva, temperature, semi-dynamic air flow and possible adsorption in the mouth mucosa (Tayor and Linforth, 1996; Buettner and Schieberle 2000; Piggott and Schaschke, 2001; Buettner et al., 2002). Such a series of events eventually affects volatile release (Overbosch et al. 1991; Harrison 1998). Therefore the techniques to measure aroma release during eating needs to consider the dynamic nature of the process.

1.4.1.6.1 Real-time analysis of aroma in expired breath: APCI-MS and PTR-MS

As already mentioned numerous times in this literature, flavor release and perception is a dynamic phenomenon that changes as a food is introduced in mouth. Among the many techniques devised for decoding flavor release during eating, methods developed by the groups of Taylor and of Hansel et al. have been used extensively for over two decades (Linforth and Taylor 1993; Hansel et al., 1995) and have been termed

as atmospheric pressure chemical ionization-mass spectrometry (APCI-MS) and Proton Transfer Reaction-Mass Spectrometry (PTR-MS) respectively. Both techniques have been developed and used quite parallel with each other but certainly not without limitations.

Such on-line monitoring of aroma with time or "time-release profile" is possible only if there is an instrument that is sensitive enough to detect trace amounts of aroma (parts per million to parts per trillion) present in human breath as well as high resolving power to characterize a large number of compounds present in a food aroma (Taylor et al., 2000). Since analysis is to be conducted on a breath-by-breath basis, the system should be capable of sampling at a very fast rate in order to collect enough data for each breath (Taylor et al., 2000). All these requirements were fulfilled by directly sampling volatiles from human breath to mass spectrometers by both the Taylor and Hansel groups. Another issue with direct analysis was that the presence of air and water in breath reduces the performance of certain mass spectrometric sources such as electron impact (EI) (Linforth and Taylor, 1993). Besides, EI techniques are known to result in fragmentation, making data interpretation difficult. Therefore, chemical ionization technique (CI) was used since it is a soft ionization technique giving rise to fewer fragments (Taylor and Linforth, 2003). Also, it is based on proton-transfer reactions in which a primary reactant or precursor ion, such as water (H_3O^+) transfers a proton H^+ to the volatiles in the sample mixture (Hansel et al, 1995). Such reaction has three-fold advantage: firstly, the proton affinity of water is higher than that of components of air but lower than those of most of the volatile aroma compounds, ensuring that protons are transferred only to the volatiles

of interest (Lindinger et al., 1998a). Secondly, the energy of the reagent ion is sufficient to ionize the analyte of interest but not to fragment it further, giving fairly simple spectra (Taylor et al., 2000). Thirdly, water is a necessary element for the analysis, not a hindrance as used to be the case earlier (Taylor et al., 2000).

While these are some common principles for APCI-MS and PTR-MS, the major difference lies in the way charge is transferred from the precursor ion to the analyte. In APCI-MS method, the entire process leading to the formation of analyte ion, i.e. formation of precursor ion, charge transfer and declustering all take place in the same region (Taylor and Linforth, 2003). Sunner et al. (1988) found that based on gas phase basicity, some volatile compounds are able to be ionized to a greater extent than others, leading to incorrect quantification. The inventors of PTR-MS methodology came up with a solution for this problem. Instead of carrying out all ionizations at one region, they separated the process in two different regions. They generated the precursor ion in one region and mixed the precursor ion with analyte ion in a specially designed drift tube set at precise temperate and pressure so as to control the rate of reaction and therefore ensure the same extent of ionization (Lindinger et al., 1998b). PTR-MS has also been shown to have higher sensitivity (parts per tillion) if the dwell time for analysis is set high, i.e. several seconds (Lindinger et al. 1998b). However, if the purpose of analysis is to monitor volatiles breath by breath, increasing dwell time reduces the rate of data collection especially with the presence of multiple ions to be monitored. APCI, on the other hand, is sensitive to parts per billion levels with a dwell time as low as 20 milli-seconds, making it a method of preference if faster data acquisition rate with higher

sensitivity is required (Taylor and Linforth, 2003). Additionally, the sensitivity of APCI can also be increased simply by increasing the sample flow rate whereas the flow rate for PTR is kept constant.

In short, both the methods have some advantages and limitations associated. Depending on the availability and the purpose of study, a large number of works have been done with both methods. These methods have been used to measure aroma release in vitro (Linforth et al., 1996; Deibler et al., 2001; Yeretzian et al. 2003) as well as in vivo (Linforth and Taylor, 1998; Mayr et al., 2002; Dunphhy et al., 2006). In addition, they have been useful to examine the effects of factors affecting release and perception (Weel et al., 2002; Saint-Eve et al. 2006), monitoring aroma changes during physiological and chemical processes (Yeretzian et al., 2000; Turner et al., 2002; Hodgson et al. 2003).

1.4.2 Taste

For a long time, taste research has been neglected in favor of aroma research. The reason is partly because of the general thought that aroma is the major, if not the sole, contributor to flavor. This view however has been repudiated by research showing that taste plays an import role for flavor perception along with aroma, and both of these inputs have been found to interact with each other. Therefore more researchers are shifting their focus in decoding the role of taste in flavor perception and ultimately understanding the mechanisms. A pioneer in this field of study is Dr. Thomas Hofmann from Technische Universitat Munchen, who according to the website of the university, has published over

a hundred research articles on taste studies (Scientific Publications, 2012). Selected publications of Dr. Hofmann are presented in Table 1-5. Another notable scientist who did extensive research on taste of wine is Dr. Ann C. Noble (Arnold et al., 1980; Fischer and Noble 1994b; Noble, 1995).

Although gas chromatography is a method suitable for samples that easily volatilize, it has also been used to analyze taste compounds. When this is the case, the taste analytes are derivatized so as to render them volatile. Analysis of sugars, polyols, acids and amino acids in aprictots was carried out using GC-MS after they underwent trimethylsilylation (Katona et al., 1999). Similarly, gas chromatographic determination of the sugar polyols: sorbitol, mannitol, and xylitol present in chewing gum and sorbitol mints were achieved by derivatization of the aqueous extract with pyridine-acetic anhydride to the corresponding peracetates (Daniels et al., 1982).

Due to the novelty of the area of taste research, it is not yet possible to sample and measure taste release continually in real time. There have been attempts at accessing changes in conductivity associated with salts and pH (Jack et al., 1995; Davidson et al. 1998). Analogous to electronic mouth for aroma analysis, taste compounds can also be identified by using electronic tongue based on an array of lipid/polymer membranes. When in contact with tastants, the membranes produce output by changing their electrical properties (Toko, 1998).

Another approach for in-mouth measurement of taste compounds during eating is sampling in saliva and analysis by liquid chromatography coupled with mass

spectrometry. This is perhaps one of the best methods available for the analysis of changes in concentrations of tastants like sugars, salts, phenolics and acids, however it is rather tedious to perform them. Davidson et al. (2000) developed two methods for in-vivo sampling of non-volatiles in saliva. In one method, as panelists masticated chewing gum, saliva was sampled from their mouths at discrete time points with cotton swabs. In the second method, saliva was sampled continuously with the help of a motor and barrel attachment to wind a 1.5mm wide ribbon through the mouth during eating. The length of the ribbon was then measured and weighed. After it was dried, it was divided into sections representing certain time period during eating so as to provide information about time-release. Saliva samples obtained from such methods are generally extracted with solvents and analyzed by using APCI-MS (Davidson et al., 2000).

The use of liquid chromatography-mass spectrometry (LCMS) seems to be the primary mode of analysis for taste compounds in food. Availability of a number of columns and various forms of mass spectrometric methods have made this technique versatile and capable of analyzing wide range of compounds available in food (Careri et al., 1998).

Table 1-5. Selected publications on taste research by Dr. Hofmann's research group.

Title	Author(s)	Date of Publication
Taste dilution and biomimetic *in vitro* assays-novel keys to unlock the secrets of food taste.	Hofmann et al	2003
Structural and sensory characterizationof compounds contributing to the bitter off-taste of carrots (Daucus carota L.) and carrot puree.	Czepa and Hofmann	2003
The taste activity concept: a powerful tool to trace the key tastants in foods.	Hofmann	2004
Identification of the astringent taste compounds in black tea infusions by combining instrumental analysis and human bioresponse.	Scharbert et al.	2004
Taste-active Maillard reaction products – The "tasty" world of nonvolatile Maillard reaction products.	Hofmann	2005
Molecular and gustatory characterization of the impact taste compounds in black tea infusions.	Hofmann et al.	2006
Identification of bitter off-taste compounds in the stored cold pressed linseed oil.	Bruhl et al.	2007
Molecular insights into the chemistry producing harsh bitter taste compounds of strongly roasted coffee.	Hofmann et al.	2008
Three TAS2R bitr taste receptors mediat the psychophysical responses to bitter compounds of hops (Humulus lupulus L.) and beer.	Intelmann et al.	2009
Quantitative studies on the influence of the bean roasting parameters and hot water percolation on the concentrations of bitter compounds in coffee brew.	Blumberg et al.	2010
Quantitative analysis of taste-active glutamyl peptides in foods using HPLC-Ms/MS.	Dunkel and Hofmann	2011
Structural and sensory characterization of key pungent and tingling compounds from black pepper (Piper nigrum L.)	Dawid et al.	2012

1.4.3 Flavoromics: a comprehensive approach to predict flavor

Despite the multimodal nature of flavor perception, flavor research has been primarily focused on volatiles. Taste research is slowly gaining interest, however most works are still focused on individual area, with little or no ability to correlate the different stimuli with sensory results. It has been recently been argued that by conducting non-targeted research in conjunction with chemometrics, flavor prediction can be done (Charve et al., 2011). Such approach of comprehensive and holistic study has been termed as flovoromics (Reineccius, 2008) or flavour metabolomics (de vos et al., 2008) and presented in a PhD dissertation to predict flavor of mandarin juice (Charve, 2011). The idea is to study ideally all low molecular weight compounds in foods (both volatile and non-volatile) as they can all act as stimuli for human perception. Conducting such research requires the availability and expertise of handling powerful instruments such as UHPLC-TOF-MS, GC-TOF-MS, and specialized data processing and statistical modules.

1.4.4 Molecular biology and biotechnology

Among numerous fields of application, molecular biology and biotechnology also are being used to understand flavor perception. As discussed in this section, although there are many techniques available for study of both aroma and taste, they all have their limitations and therefore cannot replace human nose or mouth. This has led to the introduction of flavor science to methods borrowed from new molecular biology and biotechnology.

Learning from molecular biology, culture of olfactory receptor neurons or cloning of receptor genes in the olfactory epithelium and tongue can provide insights into the complex process of perception by humans (Zotterman, 1967; Ramming et al., 1993; Vargas and Lucero, 1999). Molecular biology has been instrumental in understanding taste transduction by identification and cloning of a number of proteins involved in signal transduction (McLaughlin et al., 1992 a,b; Margolskee, 1993). This has led to discovery of taste specific proteins such as G protein as well as provided insights into mechanisms of taste (Heck et al. 1984; Avenet et al. 1988). Genes encoding the taste receptors have been identified (Max et al., 2001; Intelmann et al., 2009). This provides new avenues for discovery of taste compounds that activate or inhibit specific taste receptors.

Another alternative for detection of flavor in food that is gaining more exposure is the use of biosensors. They are small devices that utilize biochemical reactions to detect a chemical or biochemical species with the help of biological detection unit (Cock et al, 2009). Because they include biological elements such as antibody, enzyme, tissue, membrane or even whole cells, detection by biosensors is highly selective and therefore considered to be more reliable than any other sensors. Biosensors have been used to monitor desirable as well as undesirable flavor qualities in various foods as an indicator of quality. A biosensor based on electrochemical oxidation of NDAPH has been used to monitor concentrations of the undesired byproduct diacetly during fermentation of beer. Since NADPH plays an important role in reduction of diacetyl, the concentrations of the substrate diacetyl could be calculated by measuring the reduction in its oxidation potential (Vann and Shephard, 2005). Due to high sensitivity, selectivity, ease of

operation, automation and short test duration, biosensors are also applicable for real time analysis of flavor (Rasooli 2001). This combined with the availability of biosensors (Asav and Akyilmaz, 2010) that can detect the presence of sugars and polyols (Takamizawa et al. 2000) offers the potential for use of biosensor for measuring concentration of sweeteners released in vivo during consumption of confectionery such as chewing gum, which is otherwise a very difficult and time-consuming task. Biosensors are also being thought of as alternative to sensory panel studies which could be subjective even after the tedious process of panel training and standardizations. Such an attempt has been done by Pioggia et al. (2007) who used three different types of sensors to access the sensory quality of solutions containing different concentrations of stimuli for all the five basic tastes.

1.4.5 Sensory Measurement

Although there are many instrumental techniques available for analysis of different aspects of flavor, true assessment of sensory perception can only be done by sensory tests. Such tests give qualitative information as to any difference(s) in flavor sensation between products as well as quantitative information as to how big or small that difference is. In order to obtain meaningful and robust data, rigorous training and assessment of the panelists is very important. Also, a good knowledge of statistical analysis methods is as vital for understanding the results of sensory tests.

Sensory tests can be broadly divided into three types: discrimination, analytical intensity rating and consumer tests (Noble and Lesschaeve, 2006). Discrimination tests

give qualitative sensory information about two or more products. Samples are presented to the panelists who are asked to determine if one is perceptibly different from the other or not (Lawless and Heymann 1998). It is also a popular practice to determine thresholds of the stimuli to be measured. This is useful in studies where sensitivity of panelists is an important factor or when inter-individual differences are known to affect result. The intensity rating tests, on the other hand, can provide both qualitative and quantitative sensory information about food products. Descriptive sensory analysis is a sophisticated method that can provide a complete sensory description of products. Sensory attributes of complex samples such as wine, coffee, cheese and many others has been characterized and quantified by using descriptive sensory analysis (Piggott and Mowat 1991; Kotseridis et al, 2000; Alasalvar et al. 2003). It involves identification and naming of all possible sensory attributes of the product being tested by discussion among the panelists. The panelists are then trained to quantify the intensity of the selected stimuli by use of reference standard solutions. However, with the knowledge of dynamic nature of flavor release during consumption of food, time-intensity (TI) sensory analysis has become a method of choice in many studies. The most obvious and popular application of TI methodology is to monitor temporal aspect of flavor release during food consumption. Davidson et al., (2000) combined TI with simultaneous measurement of in-nose concentration of menthone by APCI-MS in mint flavored chewing gum.

1.5 The Work Presented

In the work presented, we have examined the influence of different properties of polyols on flavor delivery in sugar-free chewing gum. In all the studies, we have trained people to masticate the gum samples to monitor the release profiles of volatiles in their breath and the non-volatiles in the expectorated saliva. We tested the influence of polyols with different water solubility on flavor delivery and complemented the instrumental results with sensory. We also studied the effect of particles sizes of two polyols on flavor delivery and perception of sweetness. Lastly, to investigate mechanisms of flavor delivery, we performed mass balance analysis of the volatile and non-volatile flavor compounds released during a 12-min mastication period.

Chapter 2

Research Objectives and Hypotheses

Study 1 (Chapter 3): Investigation of the effect of polyols on flavor release in sugar-free chewing gum during mastication.

Objective 1: Monitor the real-time release of different volatile compounds in chewing gums made with different polyols in-vivo.

Null Hypothesis: There will be no difference in release of the different volatile compounds in chewing gums made with mannitol, sorbitol and xylitol during mastication by three trained panelists.

Objective 2: Monitor the real-time release of polyols and high intensity sweeteners in chewing gums made with different polyols in-vivo.

Null Hypothesis: There will be no difference in release of the polyols and high intensity sweeteners during mastication of chewing gums made with mannitol, sorbitol and xylitol.

Objective 3: Conduct sensory analysis to access the perception of overall aroma and sweetness in chewing gums made with different polyols.

Null hypothesis: There will be no difference in the perception of overall aroma and sweetness during mastication of chewing gum made with mannitol, sorbitol and xylitol.

Study 2 (Chapter 4): Effect of particle size of sorbitol and mannitol on flavor release in sugar-free chewing gum

Objective 1: Monitor the real-time release of different volatile compounds in chewing gums made with sorbitol and mannitol of different particle sizes.

Null Hypothesis: There will be no difference in the release of different volatile compounds during mastication of chewing gum made with different particle sizes of sorbitol (62 and 224 μm) and mannitol (57 and 184 μm).

Objective 2: Monitor the real-time release of high intensity sweeteners in chewing gums made with sorbitol and mannitol of different particle sizes.

Null Hypothesis: There will be no difference in the release of the two high intensity sweeteners acesulfame K and aspartame during mastication of chewing gum made with different particle sizes of sorbitol (62 and 224 μm) and mannitol (57 and 184 μm).

Objective 3: Conduct sensory analysis to evaluate the perception of sweetness in chewing gums made with sorbitol of different particle sizes.

Null Hypothesis: There will be no difference in the perception of sweetness during mastication of chewing gum made with different particle sizes of sorbitol (62 and 224 μm).

Study 3 (Chapter 5): Mass Balance of Taste and Aroma Components in Sugar-free Chewing Gum

Objective 1: Monitor the amounts of volatile compounds released in exhaled breath and saliva, and the amount remaining in the gum bolus during mastication of chewing gum made with sorbitol of different particle sizes.

Null Hypothesis: There will be no difference in the amount of volatile compounds released in the exhaled breath and saliva, and the amount remaining in the gum bolus during mastication of chewing gum made with different particle sizes of sorbitol (62 and 224 μm).

Objective 2: Monitor the release of the high intensity sweeteners in saliva and the amount retained in the gum bolus during mastication of chewing gum made with sorbitol of different particle sizes.

Null Hypothesis: There will be no difference in the release of the two high intensity sweeteners acesulfame K and aspartame in saliva and the amount remaining in the gum bolus during mastication of chewing gum made with different particle sizes of sorbitol (62 and 224 μm).

CHAPTER 3

Influence of polyol-type on flavor release during mastication of sugar-free chewing gum

This section is presented as an exploratory study to investigate how the use of different polyols may affect release of volatiles and non-volatiles during mastication of sugar-free chewing gum. Three chewing gum samples were formulated with three polyols (sorbitol, xylitol and mannitol) that differed in water solubility. Three trained panelists masticated the samples for 12 min period. APCI-MS analysis of the expired breath showed that chewing gum formulated with mannitol had a higher aroma release profile than when formulated with xylitol and sorbitol (the least release). LC/MS analysis of the expectorated saliva showed that polyol release depended on their corresponding solubility in water; concentration of the most water soluble polyol (sorbitol) was the highest in saliva and that of the least water soluble polyol (mannitol) was the least. Similarly, a significantly higher release profile for the HIS (aspartame and acesulfame K) for gums containing mannitol and xylitol were also reported. Instrumental results for both aroma and HIS release were supported by sensory time-intensity analysis of the samples. In summary, formulating gums with polyols differing in water solubility altered the release of taste and aroma components.

3.1 INTRODUCTION

Chewing gum is the fastest growing confectionery product in the US (Blischok, 2010). Qualities such as low caloric output and more specifically, non-cariogenic property have made the use of polyols in chewing gum manufacturing more popular than sucrose. Like sucrose, polyols also impart sweetness to products and are known to alter texture and flavor attributes. However, there is limited knowledge on the mechanism by which polyols alter flavor delivery in confectioneries such as chewing gum.

Chewing gum is primarily composed of a water insoluble gum base phase and a water soluble sugar/polyol phase, roughly in the ratio of 1:3. The volatile aroma compounds, which constitute about 0.5-1% of the total gum weight, are distributed between these two phases. Phase distribution of aroma compounds is related to compound hydrophobicity, i.e. compounds that are more hydrophobic have greater solubility in the gum base phase. Alternately, compounds that are less hydrophobic are more likely to be distributed in the water soluble sugar/polyol phase.

De Roos et al. (1994) developed physico-chemical models to predict flavor release from chewing gum based on the gum-water (saliva) and air-water (saliva) partition coefficients for a wide range of hydrophobic compounds. They described flavor release as a two-step process in which a strong relationship was found between the rate of flavor release and the gum base to water partition coefficient (log cP) during the first five min of mastication (as the water soluble components are dissolving in saliva). However, after 5 min as the water soluble components are depleted, the relationship between the gum base to water partition coefficient and the rate of flavor release was not

as strong. They suggested that release during this time occurs by extraction of the non-polar gum base and is diffusion controlled.

Similarly, Harrison (2000) developed a mathematical model that also relates flavor release with the gum to saliva partition coefficient. The model predicted a faster release for compounds with a low gum to saliva partition coefficient but a constant rate for those compounds with high gum to saliva partition coefficient. Sostmann, et al. (2003) calculated the affinity of four series of homologous flavor compounds with gum base ingredients. They found that with increasing hydrophobicity of the flavor compounds, a higher binding strength was found for each of the gum base ingredients. Fisker et. al. (2006) also showed there is more release of fruit flavors when using more polar gum base indicating lesser affinity of the aroma compounds to the polar gum base. All these studies focused on explaining the rate of flavor release as dictated by hydrophobicity of compounds and gum base.

As discussed in detail in Chapter 1, the perceived flavor intensity of chewing gum is influenced by sweetness. Davidson et al. (1999) investigated the effect of sucrose release on the perceived mint flavor intensity of chewing gum. They found that flavor perception by panelists followed the release of sucrose rather than that of menthone. In other words, the presence of sweetness enhanced the perception of menthone although the release of menthone was constant, suggesting interaction at the cognitive level. Potineni and Peterson (2008) also found that the release of cinnamaldehyde from chewing gum was correlated to sorbitol release rather than what would be predicted from the calculation of gum to water partition coefficient. They further showed that when sorbitol was not present, the release of cinnamaldehyde was much lower, as would be

predicted based on the log P value (2008). They proposed that cinnamaldehyde reacted with sorbitol in the gum and formed transient hemiacetals which were converted back to cinnamaldehyde during mastication, thereby enhancing the perceived flavor intensity. Similarly, Fisker et. al. (2006) compared the effect of polyols on flavor release for fruity chewing gums. They reported a higher release rate of fruit flavors from chewing gum containing mannitol and xylitol as bulk sweeteners in comparison to sorbitol gums. Haahr, et. al. (2006) also reported that the release for mint flavor from xylitol gums was higher than from sorbitol gums; mint flavor perception was also higher in xylitol gums. All these studies on effect of polyols on flavor release in chewing gums have used very simple systems with one or two aroma compounds. Since the aroma in a real chewing gum is composed of a number of compounds, it is important to investigate the effects of polyols on combinations of aroma compounds.

The objective of this study was, therefore, to investigate the effect of different polyols on the release of fruit flavors from chewing gum. For this, three commonly used polyols – sorbitol, mannitol and xylitol were used to make sugar-free chewing gum with fruit aroma blends. The release kinetics of aroma, polyols and high intensity sweeteners (HIS) were analytically measured and compared to perceived aroma and taste attributes by a trained sensory panel.

3.2 MATERIALS AND METHODS

Chewing gum. Three different chewing gum formulations were made in the form of sticks. The samples varied in polyols used (sorbitol, mannitol or xylitol). The chewing gum formulation consisted of gum base (27g/100g), powdered polyols (40g/100g),

maltitol-sorbitol syrup (31.745g/100g), high intensity sweetener (mixture of acesulfame K and aspartame 0.475g/100g), aroma blend (0.5g/100g) lecithin (0.13g/100g), and flavor solvent (0.15g/100g; MCT). The aroma blend included ethyl acetate (5.81%), ethyl butyrate (29.07%), cis-3-Hexenol (3.49%), benzaldehyde (29.07%), limonene (29.07%) and ethyl isovalerate (3.49%).

Chewing gum was made by softening the gum base pellets (Cafosa, Barcelona, Spain) in a forced air oven at approximately 75˚C. The maltitol-sorbitol syrup was added to the mixing bowl of a mixer containing two overlapping sigma blades (READCO mixer, Read Standard, York, PA, USA) heated to the temperature of approximately 60˚C via a circulating bath. The softened gum base was then added to the mixer along with about 1/3rd of powdered polyol and lecithin, and mixed well for 1 minute. Rest of the polyol was poured into the mixer, followed by high intensity sweeteners. The flavor blend was added carefully avoiding splashing and allowed to mix with the rest of the ingredients for another 5 minutes. The final chewing gum dough was rolled using a rolling pin to an average thickness of 0.16cm with the help of a thickness bar. The sheet was then cut into small size sticks, wrapped in plastic and aluminum foil. The samples were finally sealed in Mylar® bags and stored in freezer (-4˚C) until further use.

Measurement of Polyols and HIS Release from Chewing Gum during Mastication

The concentrations of non-volatiles, i.e. polyols (sorbitol, mannitol, xylitol) and HIS (aspartame, acesulfame K) were measured in expectorated saliva of three trained panelists during mastication of chewing gum samples for a period of 12 min. Saliva collection and analyses were done in triplicates. The panelists were asked to chew gums at their natural chewing rate and expectorate the saliva generated into sample cups with

lids at 0, 15, 30, 45, 60, 75, 90, 120, 180, 240, 360, 540 and 720 sec for each polyol treatment. Saliva samples (0.01g) were transferred to 2mL centrifuge tubes containing 1mL of 0.1% formic acid in acetonitrile:water (75:25 v/v) . The acidified samples were then centrifuged at 11,700 rcf for 15 min with a temperature maintained at 5°C. The supernatant (700µm) was transferred into 2mL amber vials containing 10µm of 5000ppm solution of butyl-4-hydroxybenzoate to be used as an internal standard. Samples were analyzed by using LC/MS. The concentration of polyols and HIS in gums were determined by using external standard curves at 50, 60, 250, 300, 400 µg/mL for sorbitol; 25, 100, 300, 400, 500, 600 µg/mL for xyliltol; 50, 150, 350, 550, 750 µg/mL for mannitol; 0.1, 1, 10, 50, 100 µg/mL for aspartame and acesulfame K, plotted versus peak area (r^2>0.99).

Liquid Chromatography Mass Spectrometry (LCMS). Analysis of sorbitol was conducted in triplicate using a Micromass Quattro micro mass spectrometer (Waters Corp., Milford, MA, USA) coupled to a Shimadzu HPLC system (Shimadzu Scientific Instruments, Columbia, MD, USA) consisting of two pumps (LC-10ATvp), degasser (DGU-14A), an autosampler (SIL-10Ai), an ion sabre control unit and a heater (Waters Corp., Milford, MA, USA). Analysis was carried out with a Restek Pinnacle II Amino column (Restek Corp., Bellefonte, PA, USA; 5µm, 150 x 2.1 mm i.d.) as isocratic runs with 0.5mL/min flow rate and 0.5µL injection volume. The mobile phase used was mixture of acetonitrile and water in the ratio of 75:25 v/v. The MS operation parameters were: APCI negative mode, capillary voltage was 3.7 kV, source temperature was 100°C and desolvation temperature was 400°C. Data was collected in multiple reaction monitoring (MRM) mode; dwell time was 0.6 sec; transition ion was m/z 89←181 for

sorbitol (collision 20V, cone 15V), 89← 151 for xylitol (collision 20V, cone 15V) and m/z 92← 193 was monitored for the internal standard butyl-4-hydroxybenzoate (collision 14V, cone 30V). Analysis of mannitol was done on a CARBOSep Coregel 87 C Fast column (Transgenomic Inc., Omaha, NE, USA; 20µm, 100 x 7.8 mm i.d.). The mobile phase was water with a flow rate of 0.32mL/min. Injection volume was 40µL and the column temperature was maintained at 85°C. Mannitol was detected in MRM mode by monitoring the transition 59←181 (collision 20, cone 15V, 0.6sec dwell time) in negative APCI mode.

Analysis of HIS was conducted on a UPLC/MS system (Waters Quattro Premier XE, Milford, MA, USA) equipped with a sample manager and a binary solvent manager by using an Acquity UPLC BEH C18 column (Waters Corp., Milford, MA, USA; 1.7µm, 50 x 2.1 mm i.d.) by injecting 1.5 µL sample at a flow rate of 0.23 mL/min. A binary solvent system with 0.1% formic acid (A) and methanol (B) was used as the mobile phase, with a linear gradient of B in A starting at 25% B in A (0-2 min), increasing to 95% B in A (2-3 min), and finally decreasing to 25% B in A (3-4 min). Acesulfame K and the internal standard butyl-4-hydroxybenzoate were detected in ESI negative mode whereas aspartame was detected in positive mode. Mass spectrometer parameters were as follows: 0.6 sec dwell time, inter-channel delay of 0.02 sec, inter-scan delay of 0.1 sec, capillary voltage was 3.8 kV, source temperature was 100°C and desolvation temperature was 350°C. Dwell time for all analytes was set to 0.6second. Data was collected in multiple reactions monitoring (MRM) mode. For acesulfame K, ion m/z 82←162 was monitored with collision energy of 14V and cone of 25 V. Similarly, m/z 120←295 was

monitored for aspartame (collision 22V, cone 20 V) and m/z 92←193 was monitored for the internal standard ((collision 25V, cone 35V).

Measurement of Volatile Release from Chewing Gum during Mastication

Aroma release profiles during mastication of chewing gum samples were monitored by breath-by-breath analysis with atmospheric pressure chemical ionization-mass spectrometry (APCI-MS) as previously described (Schober and Peterson, 2004). Panelists were asked to chew a piece of gum at their normal chewing frequency while breathing normally but keeping their mouths closed to prevent loss of volatiles from mouth. The exhaled breath from the nose was directly and continuously sampled via an interface of a deactivated capillary column (0.53mm i.d.) heated at 90°C to the mass spectrometer (ZMD 4000 Micromass, Waters, Milford, MA, USA) at intervals 0-4, 6-7, 9-10 and 12-13 min. The panelists were chewing throughout the 13 min period however were given time away from the instrument to prevent fatigue due to limited mobility while being interfaced with the APCI-MS. The APCI operating conditions were as follows: SIM mode in positive ionization; breath sampling flow rate was 190mL/min; block temperature was 100°C; corona discharge was 4 kV. Aroma compounds were monitored in their protonated forms, i.e. $[M+H]^+$ ions with the exception of menthol, which was measured in its dehydrated form, i.e. $[M-H_2O]^+$ ion. The ions monitored were ethyl acetate (m/z 89), ethyl butyrate (m/z 117), ethyl isovalerate (m/z 131), cis-3-hexenol (m/z 83), benzaldehyde (m/z 107), and limonene (m/z 81).

Quantification of the aroma compounds was done by standard calibration curves obtained by injecting increasing volumes of a standards mixture into an airtight water-jacketed 1.1L deactivated glass vessel. Temperature inside the vessel was maintained at

40°C and standards were held for 4 minutes with constant stirring (300 rpm) prior to interfacing to the APCI-MS using the same operating conditions as described above. The standards mixture was made of ethyl acetate (0.36 μL/mL pentane), ethyl butyrate (0.41 μL/mL pentane), limonene (0.22 μL/mL pentane), benzaldehyde (0.22 μL/mL pentane), ethyl isovalerate (0.34 μL/mL pentane), and cis-3-hexenol (0.10 μL/mL pentane). The volumes of standards mixture used for calibration were as follows: 1.1, 3.1, 10.5, 16.6, 30, 50, 100 μL. Calibration curves were generated by plotting the peak height (ion intensity) against ng/L air for each compound used ($r^2 > 0.99$).

Time-Intensity Sensory Analysis. Seven panelists (3 males and 4 females aged in the mid to late twenties) were trained for assessing sweetness and overall flavor intensity of chewing gum samples presented in a randomized way. Each panelist evaluated the samples by rating the intensity of sweetness and overall flavor on 20 point line scales, where a rating of 20 indicated maximum intensity. Intensity ratings of sweetness were made on the standard sucrose scale (Meilgaard et al., 1999). Studies (Taylor et. al., 2003; Murphy et.al. 1977) have shown that sweetness affects the overall sensory flavor perception and panelists tend to confuse sweetness with aroma when both of these modalities are introduced simultaneously. Therefore, two separate gum samples were used to access sweetness and overall flavor so that only one attribute could be rated at one time. In addition, the panelists blocked their nostrils with nose clippers to avoid sniffing of the aroma compounds during accessing sweetness.

For flavor intensity, various food products were used as references (Saltines premium unsalted cracker-2, Mott's unsweetened apple sauce-5, Minute maid orange juice-7, Welch's grape juice-10, Big red cinnamon gum-12). Before assessing the gums

for flavor intensity, panelists were also asked to smell the gums to get familiarized with the fruity note in the samples. Data were collected at 0, 15, 30, 45, 60, 75, 90 sec, 2, 3, 4, 6, 9 and 12 min in duplicates. Data from all panelists were averaged and plotted against time, with a 95% confidence interval.

Quantification of Aroma Compounds in Chewing Gum. For each sample, 0.5 ± 0.007 g chewing gum was soaked in 1mL of hexane and mixed using a Vortex-2- Genie (VWR Scientific, NY, USA) vortex shaker for five consecutive shakes; samples were prepared in triplicates. The mixture was then centrifuged at 11,700 rpm for 10 min (Brinkman Instruments Inc.) and 0.7 mL supernatant was added to 1mL methanol. The methanol-supernatant mixture was centrifuged at 11,700 rpm for 10 min and 1mL supernatant was collected. The methanol extract (300µL) containing butyl isovalerate (as internal standard; 2500mg/L) was analyzed by gas chromatography/mass spectrometry coupled with a flame ionization detector (GC-FID) and a mass selective detector (MSD).

Gas Chromatography/Mass Spectrometry (GC-MS). Aroma compounds were analyzed by using a Hewlett-Packard 6890 gas chromatograph equipped with a mass selective detector, a split/splitless injector, flame ionization detector (FID), autosampler, and a fused-silica capillary column (DB-wax, 60m, 0.25 mm i.d., 0.3µm film thickness, J&W Scientific, Agilent Technologies, Inc., CA, USA). The GC operating conditions were as follows : 1µL of sample was injected in split mode (1:20); inlet temperature was 200°C, oven program was 35°C for 2 min, then increased at 10°C/min to 180°C and then at 30°C/min to 230°C. Data was collected in selective ion monitoring mode (SIM) and ions monitored were m/z 61 for ethyl acetate, m/z 71 for ethyl butyrate, m/z 67 for cis-3-

hexenol, m/z 77 for benzaldehyde, m/z 68 for limonene, m/z 43 for ethyl isovalerate and m/z 85 for the internal standard butyl isovalerate.

Particle size of polyols. To characterize the polyols, their particle sizes prior to being incorporated into chewing gum were measured using a Beckmann Coulter – tornado dry powder system (Beckmann Coulter, Inc.). Samples in the form of dry powder were placed in the sample holder to fill about half of the holder. A high shear force generated by a change in direction of air was used to disperse the dry powder, and was then delivered to the particle analyzer via vacuum. Measurements were taken in triplicate for each polyol.

Statistical Analysis. The average of the triplicate analyses and 95% confidence intervals were calculated using Excel (Version 2010, Microsoft). Analysis of variance (ANOVA) was used to calculate statistical differences in release of aroma and taste compounds, and respective sensory ratings at different time points within same and between different gum samples ($\alpha \leq 0.05$). When a statistical significance was determined, analysis was followed by Fisher's Least Significant Difference (LSD) to determine differences between samples. Analyses were done using Statistix statistical software (V. 9.0, Analytical Software, Tallahassee, Fl).

3.3 RESULTS AND DISCUSSION

Before analytical and sensory analysis, the concentrations of the aroma compounds in the three gums formulated with different polyols were determined. No significant difference ($\alpha = 0.05$) in initial aroma concentrations were reported for each compound among these samples (Table 3-1).

To investigate the influence of polyol-type on flavor release in chewing gum, the release profiles for the aroma compounds, HIS and polyols were monitored. The release profiles for aroma compounds (monitored from the exhaled breath for 13 min) are shown in Figure 3-1 for one panelist; similar results were observed for the other two panelists (Appendix A). Since inter-individual differences in flavor release profiles are known to occur (Overbosch et. al., 1991), all data were interpreted as a separate block for each of the three panelists. There were similar trends in the results obtained from all three panelists. In general, for all aroma compounds with the exception of limonene, the release profiles can be divided into two phases: 0-4 min and 5-13 min. During the first phase (0-4min), a higher concentration of the aroma compounds were detected in exhaled breath for gums containing mannitol in comparison to xylitol and sorbitol, whereas after 4min, the influence of polyol-type was less obvious (Figure 3-1). As anticipated, the release profile of limonene did not change considerably throughout the entire mastication period because it is entrapped in the gum base more than in the polyol phase due to its high log P value. Release of compounds like limonene is controlled by log P and is slower but more prolonged (Harrison, 2000).

To further understand the release kinetics of aroma compounds from chewing gum, the average maximum aroma concentration measured in the exhaled breath from 0-4 min (max1) and 6-13 min (max2) are shown in Table 3-2. A ratio of these two parameters indicates the rate at which aroma delivery changes during mastication. A value greater than 1 indicates that the release is rapid initially and slows with time whereas a value lesser than 1 suggests that the compound releases slowly and has a stable release over a longer time period. For compounds with log $P < 2$, the max1/max2 values

were greater than 1 indicating rapid release initially similar to the release profile of polyols (Fig 3-2) suggesting influence of polyol on the release of these compounds. The max1/max2 ratio for benzaldehyde is more than twice that of all the other compounds suggesting a rapid initial release, esp. in the gums containing sorbitol. However for limonene (log P >2; see Table 3-3), the max1/max2 ratio was less than 1, i.e. it has a steady release throughout the entire mastication time period or were not influenced by polyol.

The HIS and polyol release profiles (monitored in expectorated saliva) for the three chewing gum types for one panelist are shown in Figure 3-2. Based on their water solubility, the polyols were released differently (Figure 3-2a). Similar to the profiles of aroma compounds in Figure 3-1, sorbitol and xylitol release can also be divided into two distinct parts: a rapid release during the first four min and decline thereafter. As a gum is masticated, saliva is generated as a response to the presence of stimuli (polyols) and the polyols dissolve in the saliva based on how soluble they are. Due to high water solubility of sorbitol and xylitol (Table 3-4), there is an initial spike in the concentration of these polyols in water, however after 4 min, most of these polyols are depleted thereby the release is in steady state. Mannitol, however did not depict such initial spike and decline in its saliva concentration but is uniformly delivered due to its low water solubility (18g/100g water). Consequently, the concentration of mannitol in saliva is the least among all the three polyols studied.

The HIS release profiles for the same panelist are shown in Figure 3-2b and 3-2c. In contrast to the release profiles of polyols in Figure 3-2a, the profiles for the release of HIS suggest a slower release in the gums containing sorbitol than the gums containing

xylitol or mannitol. Xylitol gums showed higher release of HIS until 2 min and the concentration dropped thereafter whereas the concentration in mannitol gums was the highest of all the three polyols until 4 min. From this observation, it can be hypothesized that delivery of HIS is affected by the water solubility of the bulk polyol used. The polyol whose water solubility is similar to that of the HIS will have a higher loading of the sweetener. Since the water solubility of mannitol, acesulfame K and aspartame (Table 3-4) are very similar, it is likely that there is more distribution of the two HIS over mannitol than over xylitol or sorbitol. HIS distributed at the surface of the polyol would be anticipated to improve the extractability during mastication versus being entangled in the gum base. Consequently, the concentration of HIS in saliva was found to be the highest in gums formulated with mannitol.

Although it was not the purpose of this study, particle sizes of the three polyols were measured to characterize the samples as shown in Table 3-5. The mean particle size of mannitol powder was found to be the smallest, followed by xylitol and sorbitol, which possibly suggests the role of surface area of polyol particle in addition to polyol-type, and will be subsequently investigated in Chapter 4.

To determine if changes in the delivery of HIS and aroma observed by instrumental analysis was significant enough to cause differentiation in flavor perception of the samples with three polyol treatments, time-intensity sensory analysis was conducted. Average sensory ratings from all the panelists are shown in Figure 3-3. Influence of polyol-type on overall flavor perception was observed throughout the 12 min mastication of the gums (Figure 3-3a). Mannitol gums were perceived to be the most flavorful gum, correlating to instrumental data for release of aroma compounds in

mannitol gums (Figure 3-1). Xylitol gums were also rated to be more flavorful than sorbitol gums initially whereas sorbitol gums were rated the lowest in terms of overall perceived flavor intensity. In case of sweetness perception (Figure 3-3b), xylitol gums were rated to be the sweetest gums until 45 sec of mastication. This correlates to both the relative sweetness of xylitol compared to other polyols (Table 3-4) as well as higher delivery of HIS in these gums (Figure 3-2b and 3-2c). Despite low sweetness of mannitol, the gum containing this polyol was comparable in sweetness to that of sorbitol gums suggesting the role of higher delivery of HIS on the perceived sweetness intensity.

Based on the findings in this study, it can be concluded that the influence of polyol-type on the release of both the volatiles and non-volatiles was observed; sensory perception was affected accordingly. Gums containing the least water soluble polyol (mannitol) resulted in higher release of aroma and HIS whereas using the most water soluble polyol (sorbitol) resulted in lower concentrations of both aroma and HIS in exhaled air and saliva respectively.

Table 3-1. Concentration of the aroma compounds (µg/g gum) in the original gum samples. Each data is the average of triplicates along with 95% confidence intervals.

Concentration (µg/g gum)*						
Gum	**Ethyl Acetate**	**Ethyl Butyrate**	**Cis-3-hexenol**	**Benzaldehyde**	**Limonene**	**Ethyl Isovalerate**
Sorbitol	190 ± 45	1281 ± 186	235 ± 74	2121 ± 432	2893 ± 571	211 ± 54
Mannitol	228 ± 56	1262 ± 205	201 ± 78	2188 ± 530	2765 ± 375	224 ± 43
Xylitol	212 ± 34	1308 ± 293	219 ± 56	2088 ± 444	2709 ± 491	195 ± 39

*No statistical significance for the concentrations of aroma compounds among the three gums.

Table 3- 2. Maximum Average Concentration of aroma compounds monitored from the nose at 0-4 and 6-13 min from chewing gum during mastication. Each data is the average of triplicates along with 95% confidence intervals.

In-nose compound concentration (ng/L air)						
	Ethyl Acetate			**Ethyl Butyrate**		
Sample	max 1 (0-4min)	max 2 (6-12min)	Ratio (max1/max2)	max 1 (0-4min)	max 2 (6-12min)	Ratio (max1/max2)
Sorbitol	685 ±95	453 ±262	1.51	754 ±176	692 ±233	1.09
Xylitol	1249 ±460	1150 ±298	1.09	1032 ±399	379 ±79	2.72
Mannitol	1425 ±548	811 ±325	1.76	915 ±566	561 ±279	1.63
	Cis-3-Hexenol			**Ethyl Isovalerate**		
	max 1 (0-4min)	max 2 (6-12min)	Ratio (max1/max2)	max 1 (0-4min)	max 2 (6-12min)	Ratio (max1/max2)
Sorbitol	123 ±45	98 ±13	1.26	154 ±38	181 ±68	0.85
Xylitol	82 ±32	63 ±18	1.30	170 ±77	184 ±59	0.93
Mannitol	168 ±86	51 ±31	3.26	276 ±87	215 ±131	1.28
	Benzaldehyde			**Limonene**		
	max 1 (0-4min)	max 2 (6-12min)	Ratio (max1/max2)	max 1 (0-4min)	max 2 (6-12min)	Ratio (max1/max2)
Sorbitol	272 ±59	71 ±19	3.82	604 ±146	681 ±365	0.89
Xylitol	160 ±74	74 ±29	2.17	656 ±219	698 ±147	0.94
Mannitol	295 ±170	123 ±37	2.40	932 ±484	907 ±479	1.03

Table 3-3. Predicted Log P values for aroma compounds, polyols and high intensity sweeteners

Predicted log P of aroma, polyols and HIS[a,b]					
Aroma	**log P**	**Polyol**	**log P**	**HIS**	**log P**
Ethyl Acetate	0.29	Sorbitol	-2.94	Acesulfame K	-0.31
Ethyl Butyrate	1.37	Mannitol	-2.94	Aspartame	-0.39
Cis-3-Hexenol	1.48	Xylitol	-2.4		
Ethyl Isovalerate	1.7				
Benzaldehyde	1.78				
Limonene	4.57				

[a]Predicted by ChemDraw Ultra software
[b]SRC PhysProp Database (http://www.syrres.com)

Table 3-4. Properties of polyols and HIS[a]

Taste Component	Water solubility at 20˚C (g/100g water)	Sweetness (Sucrose=1)
Sorbitol	235	0.6
Xylitol	62	1
Mannitol	18	0.5
Acesulfame K	27	160-200
Aspartame	Slightly soluble	200

[a]Wilson (2007)

Table 3-5: Particle size of polyols used in this study with standard deviations. Each data is the average of triplicates along with 95% confidence intervals.

Polyol	Mean Particle Size µm
Mannitol	52.5 ± 2.8
Xylitol	84.8 ± 4.4
Sorbitol	261.3 ± 6.4

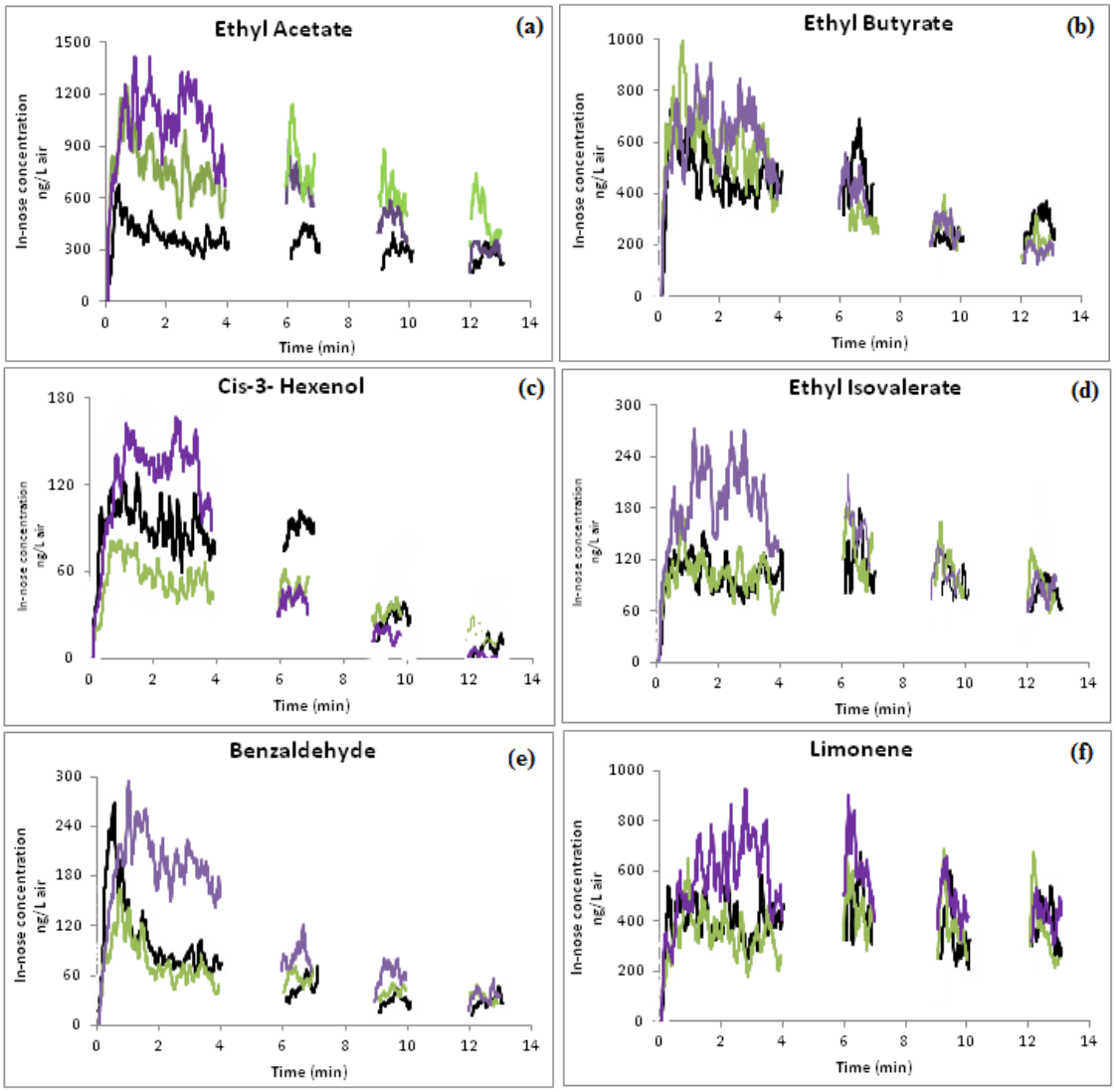


Figure 3-1. Breath release profiles of **(a)** ethyl acetate, **(b)** ethyl butyrate, **(c)** cis-3-hexenol, **(d)** ethyl isovalerate, **(e)** benzaldehyde, and **(f)** limonene for one panelist. Each curve has the release data of the aroma compound from the polyols: sorbitol (—), xylitol (—), and mannitol (—), and represents the mean of three replicates subsequently smoothed by a 5sec moving average trend line.

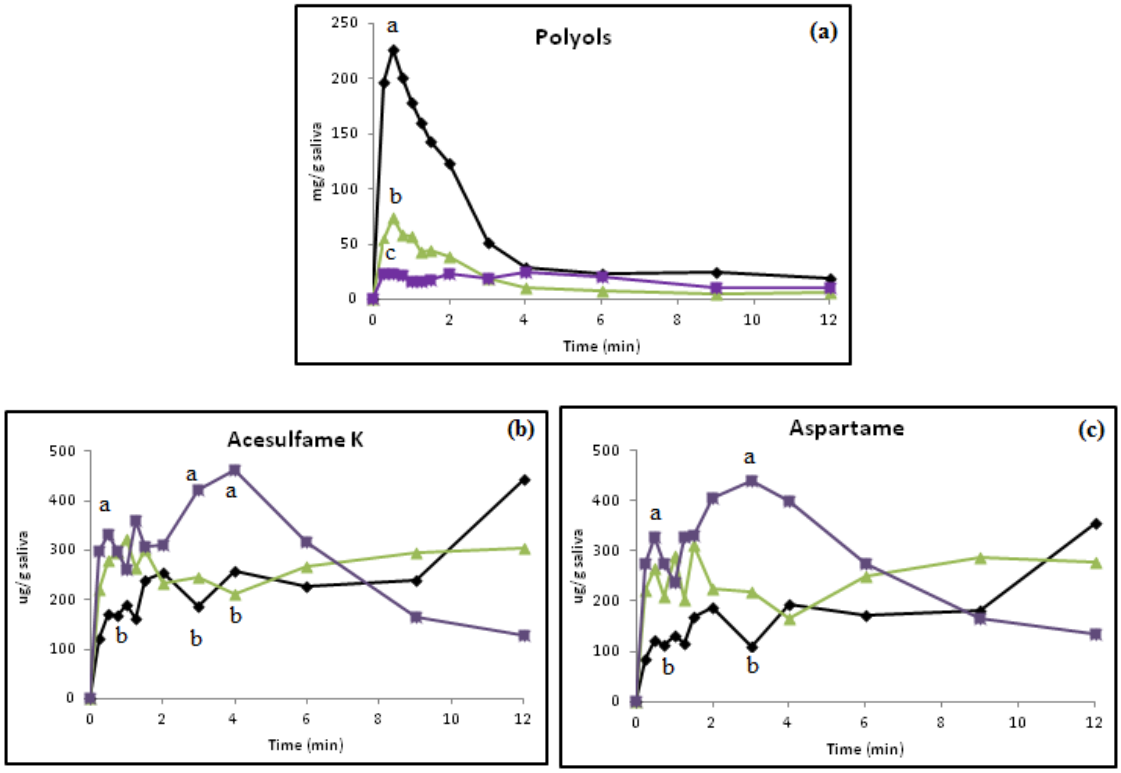


Figure 3-2. Release profiles of **(a)** polyol, **(b)** acesulfame K. and **(c)** aspartame for one panelist from chewing gum samples made with sorbitol (◆), xylitol (▲), and mannitol (■). Different letters indicate statistical significance (α=0.05).

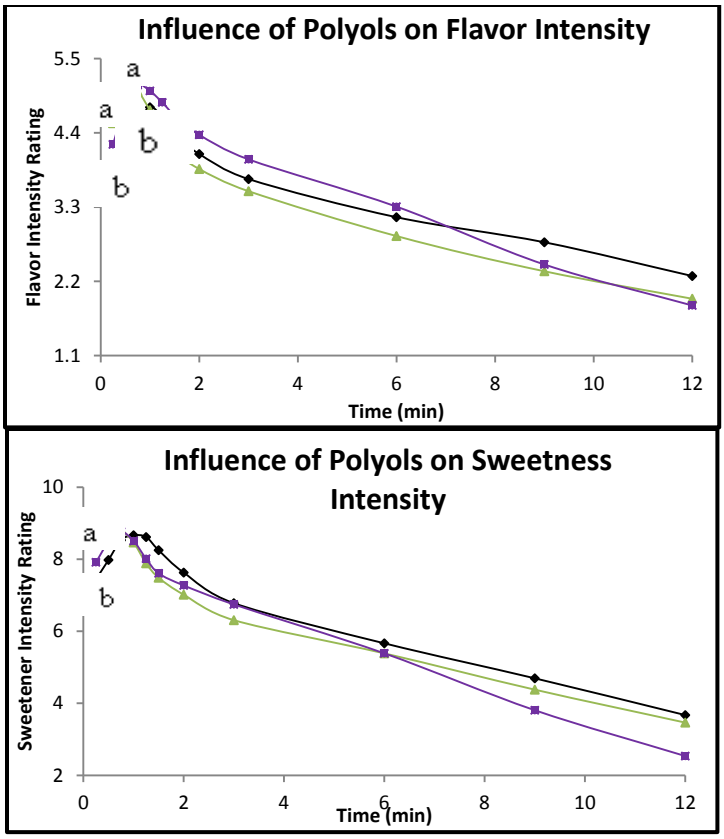


Figure 3-3. Time-intensity sensory perception profiles of overall flavor, and sweetness of gums made with sorbitol (◆), xylitol (▲), and mannitol (■). Each curve represents the average of ratings for triplicates of 7 trained panelists. Different letters indicate statistical significance (α=0.05).

CHAPTER 4

Effect of particle size of sorbitol and mannitol on flavor release in sugar-free chewing gum

The influence of polyol-type and particle size on the flavor release profile in sugar-free chewing gum was investigated *in vivo*. Four chewing gum samples with an average particle size of 62 or 246μm (sorbitol) and 57 or 184μm (mannitol) were analyzed. Two trained panelists masticated the samples at a controlled chewing rate for 12 min period. APCI-MS analysis of the expired breath reported chewing gum formulated with mannitol, in general, had a higher aroma release profile than when formulated with sorbitol. LC/MS analysis of the expectorated saliva reported the HIS (aspartame and acesulfame K) had a significantly higher release profile for the smaller polyol particle size samples for polyol formulated gums; the release rate of polyol was not significantly changed by the particle size. Sensory time-intensity analysis of the sorbitol samples was also in agreement with the HIS delivery, the smaller particle size sorbitol gum was significantly higher in perceived sweetness intensity (compared the larger particle sized sample). In summary, unique polyol-flavor interactions were reported to alter flavor delivery in chewing gum; the aroma compounds were mainly influenced by the polyol-type whereas the HIS by particle size (surface area).

4.1 INTRODUCTION

Maintaining and improving the quality of foodstuff is a constant demand of the food industry. Defining parameters that help define or predict food properties is consequently an important area of food research. One parameter that has been utilized for monitoring food quality is the particle size of ingredients. This physical attribute can influence many food/ingredient properties such as flowability of a bulk powder, the rate of dissolution, mouthfeel and even product stability (Bancarz et.al., 2008). Numerous studies (Servais et.al., 2002; Afoakwa et.al., 2008) and patents (Ribadeau et.al., 1996; Kaiser and Purwo, 2000) have indicated that maintaining the correct particle size plays an important role in the quality of many food products; however the application is still very much empirically designed.

Chocolate is an example of a food where the effect of particle size on product quality has been extensively studied. In general, in order to maintain and enhance the sensory attributes associated with chocolates the particle size of cocoa, sugar and milk solids should not exceed 30 μm but cannot be finer than 7 μm (Bancarz et.al., 2008). Otherwise, chocolate will be perceived as either too gritty or too sticky, both of which are undesirable attributes associated with larger and finer particle sizes respectively. Do et. al. (2007) showed that optimizing the particle size distribution can help improve rheological properties, melting behavior, and hardness of chocolates with low fat content.

Others have reported that blending particles of different sizes can be a simple strategy for improved flavor. One such study concluded that when at least one high quality coffee ground to a fine particle size was mixed with low quality coffee ground to a coarse particle size (difference in sizes by approximately 200-300 μm), the final

product had an improved and balanced flavor character (Clifton and Uadlowsky, 1973). The authors suggested that reduction in particle size of coffee improved the extraction efficiency of coffee during brewing, which resulted in an enhanced flavor quality. Another study showed that when making chewing gum, sorbitol can be used as a blend of two powders of different particle sizes (75 and 200 μm) rather than of uniform size (Chapdelaine et.al., 1989). They found that it improved the processing as well as the texture of the final product.

Several patents have suggested that modulating the particle size of the water soluble phase of the chewing gum can improve taste and aroma properties. Patel (1992) claimed that when more than 60% of the particles of sucrose used to make sugar gums are less than 45 μm in diameter, the sweet sensation in the gum is increased. A similar claim was reported by Cherukuri and Raman (1990) for the use of fructose with particle size of less than 212 μm. Similarly, a study conducted for maltitol concluded that when 50% of the particles were smaller than 90 μm, the resultant gum resulted in improved taste and aroma in terms of impact and duration (Kaiser and Purwo, 2000). All these examples imply that particle size of the water-soluble sugar/polyol phase in the chewing gum can be modified in ways that are beneficial for sensory as well as physical properties of the finished product. These authors suggested the reduction of the polyol particle size provides an easy and cost efficient way to improve flavor as compared to other methods such as flavor encapsulation, modification of gum base composition, and increasing the flavor load.

Although particle size has been reported to influence the flavor performance of foodstuff, little information is known about the mechanisms related and how this attribute

influences the release kinetics of the flavor stimuli. The objective of this study was to investigate the influence of particle size of two polyol formulated chewing gum samples (sorbitol and mannitol) on the delivery of both taste and aroma compounds in correlation to the sensory perception. It was hypothesized that as the particle size was decreased, highly polar flavor compounds, such as the high intensity sweeteners (HIS), would release more rapidly during mastication. As the particle size is decreased the surface area would increase and it was predicted the amount of HIS distributed on the particle surface would increase and therefore less HIS would be entrapped by the gum base during manufacture. The release of polyol, such as sorbitol, occurs relatively faster than flavor (i.e. aroma) from the gum base so a higher loading of HIS on the polyol surface would be predicted to increase delivery during mastication. Sorbitol and mannitol were selected because they range approximately 1 order of magnitude in water solubility as well as both are common polyols utilized in chewing gum and confections.

4.2 MATERIALS AND METHODS

Chewing gum. Four different chewing gum samples were formulated with either sorbitol or mannitol (Cargill, Inc., MN, USA) with different average particle size, sorbitol (62 or 246 μm) or mannitol (57 or184 μm). The different particle sizes were obtained from the same sample but separated by mechanical sieving. The formulation consisted of gum base (Cafosa, Barcelona, Spain) (25.9%), powdered polyols (34.74%), sorbitol syrup (35.73%), high intensity sweetener (mixture of acesulfame K and aspartame, 1.22%), and aroma blend (2.42%). The model aroma blend consisted of ethyl butyrate (10%), benzaldehyde (10%), limonene (1.06%), menthone (23.37%), menthol (51.36%) and 2-isopropyl-N,2,3-trimethylbutyramide (4.24%). The compounds used for the model

chewing gum aroma mixture samples were selected to provide a range in physical properties (see Table 1).

Chewing gum samples were made by general procedures described in Chapter 3. In brevity, gum base pellets were softened in a forced air oven at approximately 75˚C. Sorbitol syrup was added to the mixing bowl of a mixer containing two overlapping sigma blades (READCO mixer, Read Standard, York, PA, USA) heated to the temperature of approximately 60˚C via a circulating bath. The softened gum base was then added to the mixer along with about 1/3rd of powdered polyol and mixed well for 1 minute. Rest of the sugar alcohol was poured into the mixer, followed by HIS. The flavor blend was added carefully avoiding splashing and allowed to mix with the rest of the ingredients for another 5 minutes. The final chewing gum dough was rolled using a rolling pin to an average thickness of 0.16cm with the help of a thickness bar. The sheet was then cut into small size sticks, wrapped in plastic and aluminum foil. The samples were finally sealed in Mylar bags and stored in freezer until further use.

Laser Diffraction Analysis. Particle size distribution of the powdered polyols (treatments) was determined by laser diffraction technique by using a Malvern Mastersizer 2000 (Malvern Instruments Ltd., UK). Polyol samples were dispersed in silicone oil and stirred at 3000 rpm for 2 min before taking measurements.

Environmental Scanning Electron Microscopy (ESEM). ESEM was conducted to measure the polyol particle size (sorbitol and mannitol) in the four gum samples. In order to avoid movement interference from the volatiles during imaging, samples were dried in a vacuum oven at ambient temperatures for 3 days. The dried samples were sputter-coated with gold-palladium and observed in a Hitachi S3500N variable pressure scanning

electron microscope (Hitachi High-Technology Corp., Tokyo, Japan) at an accelerating voltage of 5kV.

Quantification of Aroma Compounds in Chewing Gum Samples. Three gum pieces were randomly selected for each chewing gum treatment and 0.5 ± 0.007 g of each piece was dissolved in 1mL of hexane using a vortex shaker (Vortex-2- Genie, VWR Scientific, NY, USA) for five consecutive shakes. The hexane mixture was then centrifuged at 11,700 rcf for 10 min (Brinkman Instruments Inc.) and 0.7 mL supernatant was added to 1mL methanol. The methanol-supernatant mixture was centrifuged at 11,700 rcf for 10 min to facilitate the precipitation of the gum base polymers, and 1mL supernatant was collected. The hexane-methanol supernatant (300µL) containing methyl hexanoate (as internal standard; 2500mg/L) was analyzed by gas chromatography/mass spectrometry coupled with a flame ionization detector (GC-FID) and a mass selective detector (MSD). Data was collected in selective ion monitoring (SIM) mode. Ions monitored were m/z 88 for ethyl butyrate, m/z 106 for benzaldehyde, m/z 68 for limonene, m/z 112 for menthone, m/z 95 for menthol and m/z 74 for the internal standard methyl hexanoate.

Measurement of Polyol and HIS Release from Chewing Gum during Mastication. The concentrations of the non-volatiles: sorbitol, mannitol, acesulfame K and aspartame were measured in expectorated saliva of two panelists during mastication of each chewing gum treatment for a period of 12 min. The panelists were trained to chew and expectorate saliva in a specific manner: chewing frequency of 60/min (regulated with a metronome) and expectorate saliva into 20mL cups with lids at 0, 15, 30, 60, 120, 180, 360 and 720 secs. The first three samplings of saliva were done at short intervals since

the polyols are known to release quickly during this period. However, due to such frequent samplings of saliva, it may make the mouth environment dry. So, two different pieces of gums were masticated for each replicate of a treatment: the first gum was masticated for the entire 12 minutes and saliva expectorated for all the time points excluding the 15sec; the second piece of gum was used to collect expectorated saliva at 0 and 15sec only. Saliva samples (0.01g) were immediately transferred into 2mL centrifuge tubes containing 1mL of 0.1% formic acid in acetonitrile: water (80:20 v/v) along with butyl-4-hydroxybenzoate (70µg/mL) and sucrose (100µg/mL) to be used as internal standards for HIS and polyol, respectively. The samples were then centrifuged at 11,700 rcf for 5 minutes. The supernatant was transferred into 2mL amber vials and frozen prior to analysis. The polyol concentrations were determined using external standard curve at 1, 10, 50, 100, 300, 500 µg/mL for sorbitol; 1, 10, 25, 100, 300 µg/mL for mannitol; and 0.1, 1, 10, 30, 50, 100 µg/mL for aspartame or acesulfame K water plotted versus peak area (r^2>0.99).

LCMS. Analysis of sorbitol was conducted in triplicate using a UPLC/MS system (Waters Quattro Premier XE, Milford, MA, USA) equipped with Waters Acquity sample manager and binary solvent manager. Analysis was carried out with a UPLC BEH Amide column (1.7µm, 100 x 2.1 mm i.d, maintained at 35°C) as isocratic runs with 0.12mL/min flow rate and 0.3µL injection volume. The mobile phase used was mixture of acetone and water with 0.05% ammonium hydroxide (A: 80/20 acetone/water; B: 30/70 acetone/water) as 94% mobile phase A and 6% mobile phase B. The MS operation parameters were: ESI negative mode, capillary voltage was 2.8 kV, source temperature was 120°C and desolvation temperature was 350°C. Data was collected in multiple

reaction monitoring (MRM) mode; dwell time was 0.15 sec; transition ion was m/z 89←181 for sorbitol (collision 14V, cone 30V) and m/z 179← 341 was monitored for the internal standard sucrose (collision 14V, cone 30V).

Analysis of mannitol was done in a Micromass Quattro spectrometer coupled to a Shimadzu HPLC system consisting of two pumps (LC-10ATvp), degasser (DGU-14A), an autosampler (SIL-10Ai), an ion sabre control unit and Waters Millipore heater (CTO-10ACvp). Separations were done on a CARBOSep Coregel 87 C Fast column (Transgenomic, 20um, 100 x 7.8 mm i.d.). The mobile phase was water (100%) with a flow rate of 0.32mL/min. Injection volume was 40µL and the column temperature was maintained at 85°C. Mannitol was monitored as the ion m/z 59 with collision 15V, cone 20V and 0.6 second dwell time in negative APCI mode.

Analysis of the HIS was also conducted on the UPLC/MS with an XTerra C18 column (3.5µm, 100 x 2.1 mm i.d.) with the column temperature maintained at 50°C. A binary solvent system with 0.1% formic acid (A) and methanol (B) was used as the mobile phase, with a linear gradient of B in A starting at 10% B in A (0-2.3 min), increasing to 95% B in A (2.3-4.1 min), and finally decreasing to 10% B in A (4.1-5.7 min). Acesulfame K and the internal standard butyl-4-hydroxybenzoate were detected in ESI negative mode whereas Aspartame was detected in positive mode. Mass spectrometer parameters were as follows: inter-channel delay of 0.02 second, inter-scan delay of 0.1 second, capillary voltage was 3.8 kV, source temperature was 100°C and desolvation temperature was 350°C. Dwell time for all analytes was set to 0.1second. Data was collected in multiple reactions monitoring (MRM) mode. For acesulfame K, ion m/z 82←162 was monitored with collision energy of 14V and cone of 25 V. Similarly,

m/z 120←295 was monitored for aspartame (collision 22V, cone 20 V) and m/z 92←193 was monitored for the internal standard (collision 25V, cone 35V).

Measurement of Volatile Flavor Release from Chewing Gum during Mastication. Aroma release profiles during mastication of chewing gum samples was monitored by performing breath-by-breath analysis by atmospheric pressure chemical ionization-mass spectrometry (APCI-MS) as previously described (Schober and Peterson 2004). Panelists were asked to masticate the samples as described in the 'Measurement of Polyol and HIS Release from Chewing Gum during Mastication' section. The exhaled breath from the nose was directly and continuously sampled via an interface set at 90°C to the mass spectrometer (ZMD 4000 Micromass, Waters, Milford MA) at intervals 0-4, 6-8, and 10-12 minutes. The panelists were chewing throughout the 12 min period however were given time away from the instrument to prevent physical strain – panelist had limited mobility while interfaced with the APCI-MS. The APCI operating conditions were as follows: SIM mode in positive ionization; breath sampling rate was 190mL/min; block temperature was 100°C; corona discharge was 4 kV. Aroma compounds were monitored in their protonated forms $[M+H]^+$ except for menthol for which the dehydrated $[M-H_2O]^+$ions were used instead. The ions monitored were limonene (m/z 81), ethyl butyrate (m/z 117), benzaldehyde (m/z 107), menthol (m/z 139), menthone (m/z 155) and 2-isopropyl-N,2,3-trimethylbutyramide (m/z 172). Quantification of the aroma compounds were done by standard calibration curves obtained by injecting increasing volumes of standards into an airtight water-jacketed 1.1L deactivated glass vessel. Temperature inside the vessel was maintained at 40°C and standards were held for 4 minutes with constant stirring (300 rpm) prior to interfacing to the APCI-MS using the same operating

conditions as described above. The calibrant was prepared in pentane as a mixture of limonene (0.07μL/mL pentane), ethyl butyrate (0.48μL/mL), benzaldehyde (0.18μL/mL), menthol (1.1μL/mL), menthone (0.26μL/mL) and 2-isopropyl-N,2,3-trimethylbutyramide (0.27μL/mL). Calibration curves were generated by plotting the peak height (ion intensity) for each set of starndard injections against ng/L air for each compound (all compounds reported $r^2 > 0.99$).

Sensory Time-Intensity Sweetness Analysis. Analysis of sweetness perception during mastication was conducted for the two sorbitol chewing gum samples of different particle size. Analysis was restricted to sorbitol gums since mannitol gums were found to have excessive trigeminal cooling which made it difficult to rate sweetness. Nine panelists (4 males and 5 females) were recruited and trained to rate sweetness intensity based on a sucrose scale described elsewhere (Meilgaard et.l., 1999). Sucrose solutions of 2, 5, 7, 10 and 16% were prepared in bottled water and were used as references. The panelists were also categorized based on their sensitivity towards high intensity sweeteners. The panelists were presented with two solutions, each containing the same concentration of sorbitol (50mg/mL) with two levels of aspartame and acesulfame K: 500 μg/mL and 1000 μg/mL prepared in bottled water. Based on the intensity ratings, panelists were divided into two groups: those who were able to significantly rate the solution with 1000 μg/mL at a higher intensity and those who were unable to do so.

Panelists were trained to chew at a frequency of one chew per second and regulated by a metronome. They were also instructed not to swallow saliva 10 seconds prior to rating sweetness intensity to avoid variation in concentration of the sweeteners in mouth due to different volume of saliva generated over time. Digital timers were

provided to every individual to ensure ratings were done at these times: 0.5, 1, 2, 3, 6, 9 and 12 min. Evaluations were conducted with nose-clips. Tests were done in duplicates over two sessions on the same day. During each session, panelists evaluated two samples in random order with at least 15 min interval in order to recover from fatigue due to the first analysis. Crackers and water were provided to cleanse the palate.

Statistical Analysis. The average of the triplicate analyses and 95% confidence intervals were calculated using Excel (Version 2010, Microsoft). Analysis of variance (ANOVA) was used to calculate statistical differences in concentrations of aroma in nose and taste compounds in saliva at different time points within same and between different gum samples ($\alpha \leq 0.05$). When a statistical significance was determined, analysis was followed by Fisher's Least Significant Difference (LSD) to determine differences between samples. Analyses were done using Statistix statistical software (V. 9.0, Analytical Software, Tallahassee, Fl).

4.3 RESULTS AND DISCUSSION

Chewing gum was formulated with polyol ingredients differing in average particle size to investigate the role of this physical attribute on flavor delivery. The average particle size and distribution for each polyol ingredient is shown in Table 4-1. In general the 'large' polyol samples were approximately 3.5x larger in diameter in comparison to the 'small' polyol samples. It was anticipated that the relative difference in particle size would be maintained in the chewing gum after manufacture or the mechanical (mixing) and thermal processing (60^{o}C) steps. To support this idea, environmental scanning electron microscopy (ESEM) was used to visualize the size distribution of the polyols in the chewing gum samples. ESEM permits the observation of samples in their native state

without requiring any coatings of conductors or insulators (Donald, 1998). Images of the four samples are shown in Fig 4-1 with the same magnification (100x) for direct comparison. Close examination of the sorbitol images shows a needle-like particle structure that is absent in the mannitol samples. This physical characteristic of sorbitol particles further supports the structures measured in the ESEM images was indeed polyol particles (sorbitol or mannitol). In general larger particles were observed in the chewing gum samples formulated with the larger particles of both mannitol (Fig. 4-1a) and sorbitol (Fig 4-1c), as compared to the smaller particles sizes (Fig. 4-1b and 4-1d). This implied that the initial polyol particle size was transferred to the final chewing gum products.

Prior to analytical and sensory analysis, the concentrations of the aroma compounds in the four chewing gum samples (different particle sizes of sorbitol and mannitol) were determined. No significant differences ($\alpha = 0.05$, Table 4-3) were reported for each compound between these samples.

To investigate the influence of polyol-size and -type on flavor release in chewing gum, the release profiles for the aroma compounds, HIS and polyol were monitored over a 12-min mastication period. For the aroma compounds, the release profiles (monitored directly from the exhaled breath) for the large and small size mannitol or sorbitol formulated chewing gums are shown in Fig 4-2 and 4-3, respectively for one panelist; similar trends were found for the second panelist (data not shown). In general, a higher concentration of the aroma compounds were detected in exhaled breath for gums made with mannitol in comparison to sorbitol, regardless of the particle size (Fig 4-2, 4-3). Changing the particle size of the polyol did not show any definitive trends on aroma

release; however select compounds did appear to have a higher flavor release when formulated with a larger particle size polyol, specifically limonene and 2-isopropyl-N,2,3-trimethylbutyramide.

The HIS and polyol release profiles (monitored from the expectorated saliva) for the large and small size mannitol or sorbitol formulated chewing gums are shown in Figures 4-4 and 4-5, respectively for one panelist (data for panelist 2 shown in Appendix B). In contrast to the release of the aroma compounds, chewing gums made with small particle size polyol were found to have higher concentration of the two high intensity sweeteners in saliva. Because the particle sizes of mannitol and sorbitol were not identical in the current study, a direct comparison between the two polyol-types was limited by this confounding variable. For the aroma delivery analysis, because particle size did not appear to influence the aroma release, comparison of the polyol-type was considered possible. The release of the polyol into the saliva is shown in Figure 4-4 and 4-5. As predicted, the concentration of mannitol in saliva was less than that of sorbitol in gums based on the solubility of each polyol in water. No distinct differences in polyol release were reported for chewing gum formulated with different particle sizes.

These findings indicated unique polyol-flavor interactions influenced the delivery of the aroma compounds in comparison to the HIS in chewing gum. A possible explanation for these two apparently different mechanisms of flavor delivery in chewing gum can be related to the compound solubility. The aroma compounds were more hydrophobic in comparison to the HIS (see Table 4-2) and thus within chewing gum the aroma component would have a greater distribution in the gum base than with the polyol material. It was assumed that aroma release of chewing gum during mastication was

mainly by water extraction from the exposed surface of the gum base, not by diffusion through the gum base. A polyol material that enhanced the generation of new gum base (surface) in the oral cavity during mastication likewise would be expected to have a higher rate of aroma delivery. Mannitol is less water-soluble than sorbitol, and consequently the release of mannitol is also slower during mastication (Figure 4-4 and 4-5). A chewing gum with solid polyol particles would be anticipated to provide mechanical stress on the gum base during chewing, and facilitate the exposure of new gum base surface. Because mannitol does not dissolve as fast in the mouth during mastication, it was suggested to provide more mechanical stress on the gum base during chewing (versus sorbitol), therefore improving the aroma extraction efficiency and causing a higher concentration of aroma in the exhaled breath.

For the release of HIS in chewing gum, a different mechanism of delivery was suggested. Because HIS are hydrophilic, increasing the surface area of the polyol phase (by decreasing the particle size) favored a higher distribution of HIS loading at the polyol surface. HIS distributed at the surface of the polyol would be anticipated to improve the extractability during chewing versus being entangled in the gum base and consequently resulting in a higher concentration in the saliva.

Sensory time-intensity evaluation of the chewing gum formulated with large and small sorbitol particles was further conducted to define if the noted changes in HIS delivery (Fig 4-5b and 4-5c) influenced sweetness perception. Because differences in the sensitivity of high intensity sweeteners among the population are known (Zhao and Tepper, 2007) the panelists were further screened for their ability to detect different concentrations of aspartame and acesulfame K released during mastication of gums. Two

solutions containing 500μg/mL and 1000μg/mL of aspartame and acesulfame K were chosen to mimic the range of changes in release of each sweetener from the gums. Six out of the nine panelists could detect significant differences in the sweetness of the two sweetener solutions with different concentrations. The panelists that were sensitive to HIS were selected to conduct further time-intensity sensory analysis of the sweetness intensity for both the large and small sorbitol chewing gum samples (see Fig 4-6). Gums formulated with smaller particle of sorbitol were perceived to be significantly ($\alpha = 0.05$) sweeter than those made with the larger particle size at time 0, 1 and 2 minutes. This finding correlated with instrumental data for release of HIS in saliva shown in Fig 4-5. However, after 2 minute, the panelists could not differentiate the sweetness in the two gums despite varying release shown by instrumental data in Fig 4-5. At this point, due to the continuous exposure of HIS in the mouth because of gum chewing, sensory adaptation might have occurred which diminished the ability of the panelists to differentiate between different stimuli.

In summary, unique polyol-flavor interactions were found to alter flavor delivery in chewing gums. The HIS delivery was reported to be influenced by the particle size of polyols (surface area); the smaller particle size gums showing higher HIS delivery compared to the large particle size gums, which was translated into higher sweetness perception. Aroma delivery on the other hand, was found to be mainly influenced by the polyol-type but not the particle size.

Table 4-1. Particle size distribution and average diameter of polyol particles, as measured by laser diffraction.

Polyol	d(0.1) µm	d(0.5) µm	d(0.9) µm	Volume weighed mean D[4,3] (µm)
Sorbitol Large	106	224	426	246
Sorbitol Small	19	47	121	62
Mannitol Large	41	136	389	184
Mannitol Small	18	48	110	57

Table 4-2. Log P and water solubility of flavor components in chewing gum.

Compound	logP[a,b]	Water Solubility[b,c] (mg/L at 25°C)
Ethyl Butyrate	1.37	4900 @20°C
Benzaldehyde	1.78	6950
2-isopropyl-N,2,3-trimethylbutyramide	2.48	460
Menthone	2.87	688
Menthol	3.40	456
Limonene	4.57	7.57
Sorbitol	-2.94	235@20°C (g/100mL)
Mannitol	-2.94	18@20°C (g/100mL)
Acesulfame K	-0.31	27@20°C (g/100mL)
Aspartame	-0.39	Slightly soluble

[a]Predicted by ChemDraw Ultra Software
[b]SRC PhysProp Database (http://www.syrres.com)
[c]Wilson, 2007

Table 4-3. Concentrations of aroma compounds (μg/g gum) in the unmasticated gum samples. Each data is the average of triplicates along with 95% confidence intervals.

Concentration (μg/g gum)*					
Gum	**Limonene**	**Benzaldehyde**	**Ethyl Butyrate**	**Menthone**	**Menthol**
Sorbitol Large	259 ± 15	1559 ± 106	1096 ± 94	3745 ± 321	10718 ± 1090
Sorbitol Small	264 ± 6	1562 ± 104	1096 ± 58	3629 ± 560	10646 ± 1337
Mannitol Large	261 ± 6	1508 ± 183	1102 ± 135	3613 ± 243	10688 ± 846
Mannitol Small	263 ± 16	1518 ± 72	1072 ±90	3575 ± 222	10678 ± 288

*No statistical significance for the concentrations of aroma compounds between the four gums.

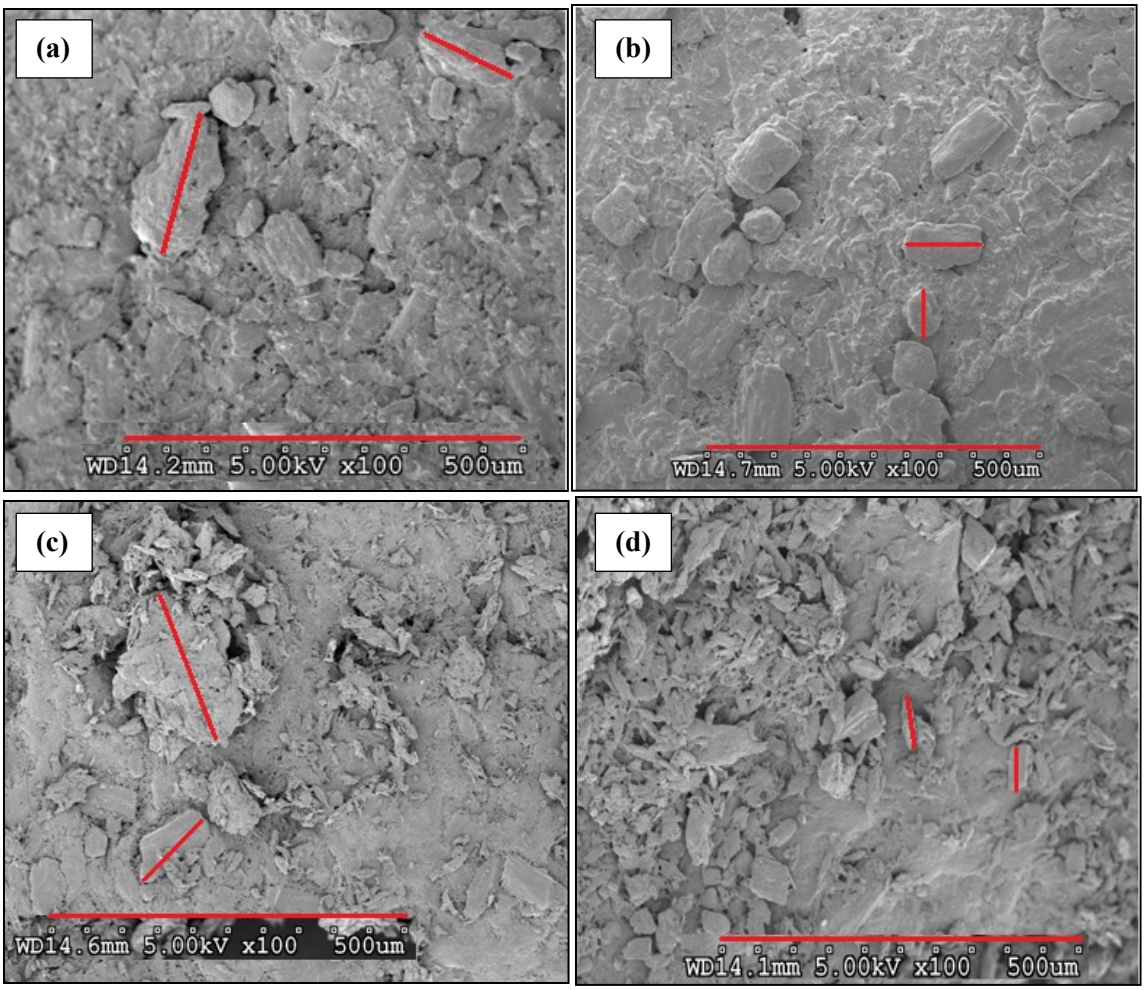
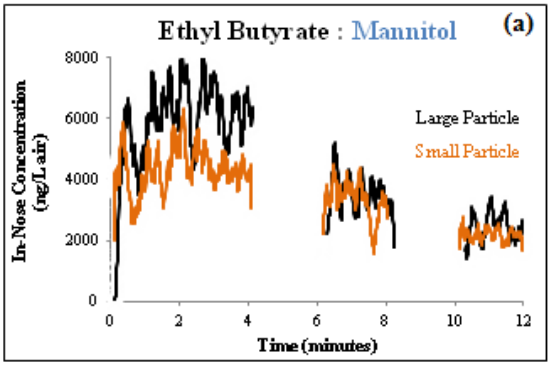
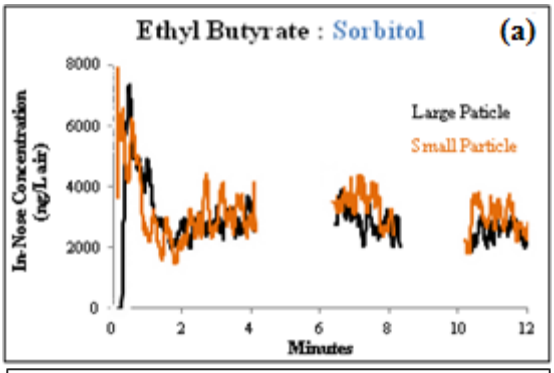
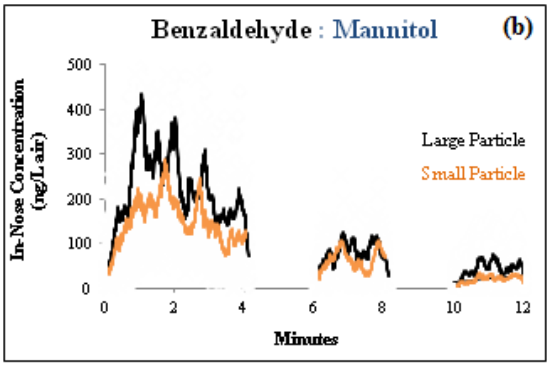
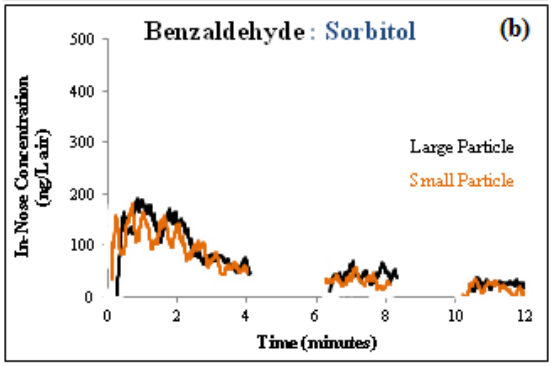


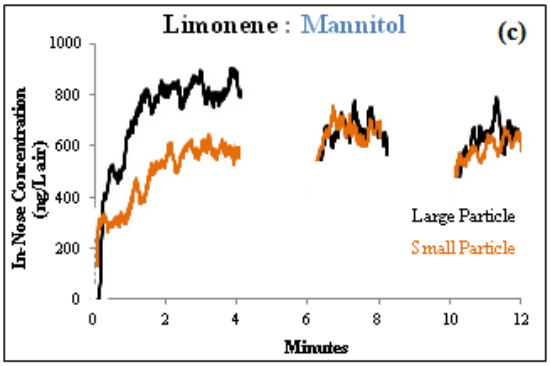
Figure 4-1. ESEM microscopy images of polyol particles in chewing gum: **(a)** mannitol large, **(b)** mannitol small, **(c)** sorbitol large, **(d)** sorbitol small at 100x magnification. The scale shows a length of 500 µm.

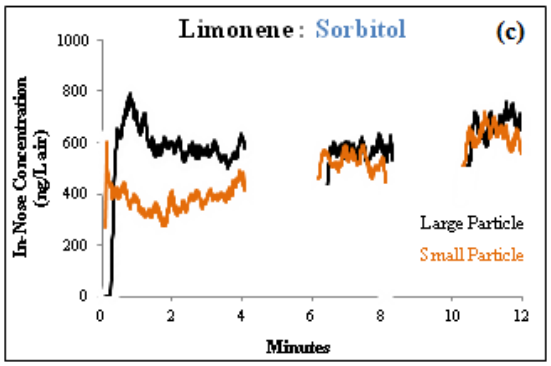

Ethyl Butyrate : Mannitol
(a)
In-Nose Concentration (ng/Lair)
Large Particle
Small Particle
Time (minutes)

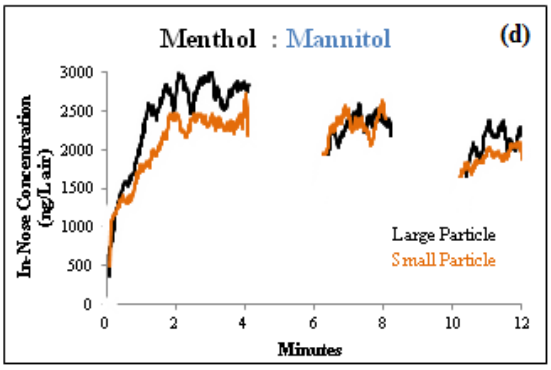

Ethyl Butyrate : Sorbitol
(a)
In-Nose Concentration (ng/Lair)
Large Paticle
Small Particle
Minutes

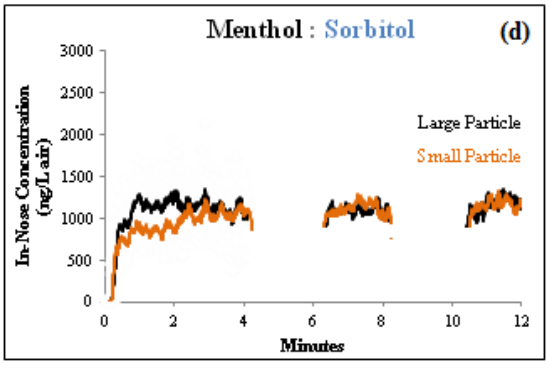

Benzaldehyde : Mannitol
(b)
In-Nose Concentration (ng/Lair)
Large Particle
Small Particle
Minutes


Benzaldehyde : Sorbitol
(b)
In-Nose Concentration (ng/Lair)
Large Particle
Small Particle
Time (minutes)


Limonene : Mannitol
(c)
In-Nose Concentration (ng/Lair)
Large Particle
Small Particle
Minutes


Limonene : Sorbitol
(c)
In-Nose Concentration (ng/Lair)
Large Particle
Small Particle
Minutes


Menthol : Mannitol
(d)
In-Nose Concentration (ng/Lair)
Large Particle
Small Particle
Minutes


Menthol : Sorbitol
(d)
In-Nose Concentration (ng/Lair)
Large Particle
Small Particle
Minutes

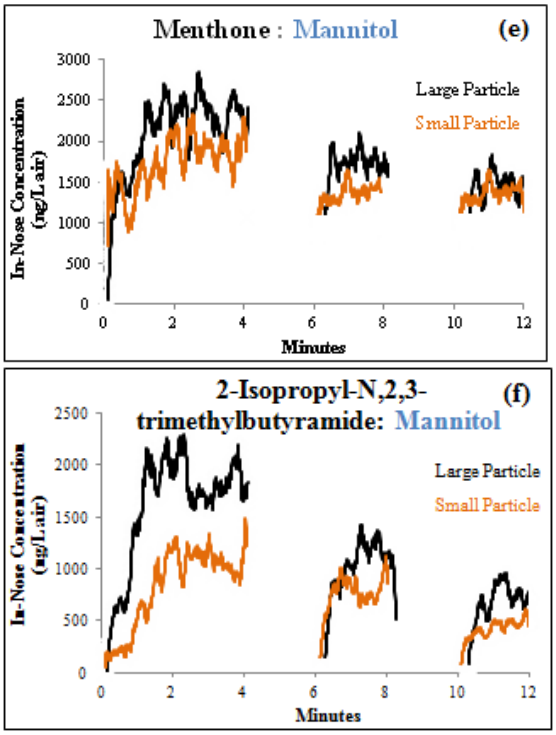


Figure 4-2. Breath release profiles of **(a)** ethyl butyrate, **(b)** benzaldehyde, **(c)** limonene, **(d)** menthol, **(e)** menthone and **(f)** 2-isopropyl-N,2,3-trimethylbutyramide for one panelist for gums made with mannitol, large particle size (—) and small particle size (—). Each plot is the average of triplicates.

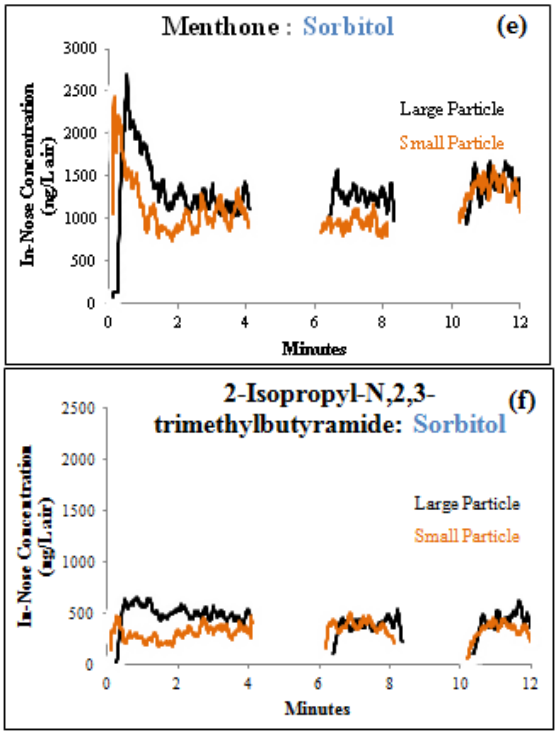


Figure 4-3. Breath release profiles of **(a)** ethyl butyrate, **(b)** benzaldehyde, **(c)** limonene **(d)** menthol, **(e)** menthone, and **(f)** 2- isopropyl-N,2,3-trimethylbutyramide for one panelist for gums made with sorbitol, large particle size (—) and small particle size (—). Each plot is the average of triplicates.

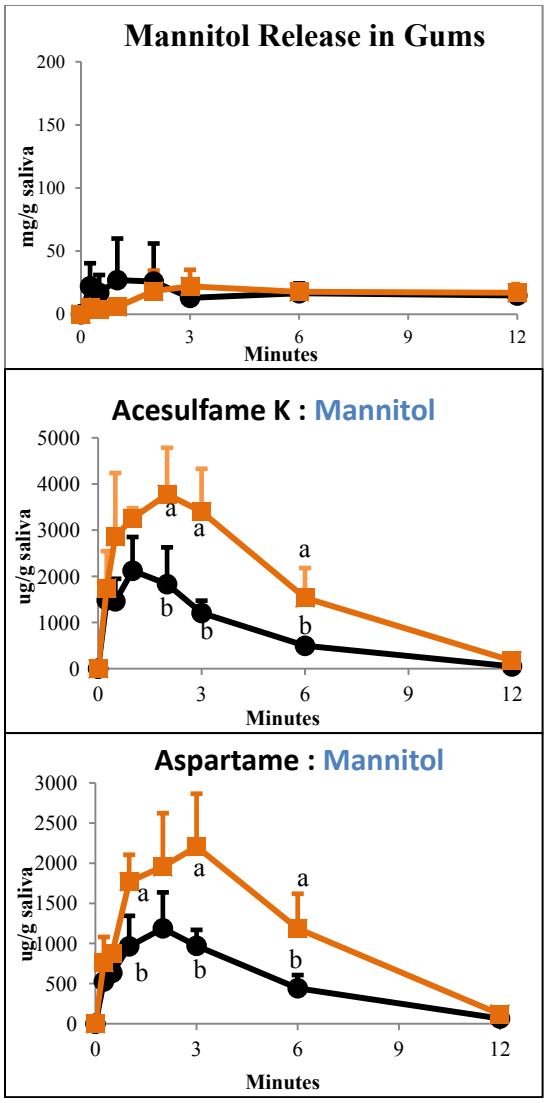


Figure 4-4. Release profiles of mannitol, acesulfame K, and aspartame in saliva for for one panelist for gums made with large (●) and small (■) particle size. Each data point is an average of three replicates with ± 95% confidence interval. Different indicate statistical significance.

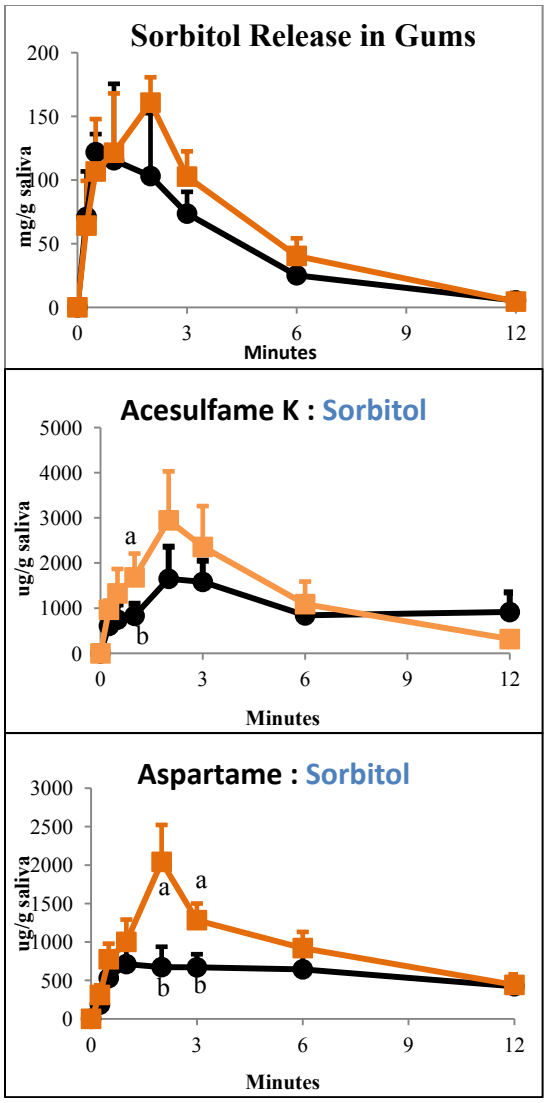


Figure 4-5. Release profiles of sorbitol, acesulfame K, and aspartame in saliva for one panelist for gums made with large (●) small particle size (■). Each data point is an average of three replicates with ± 95% confidence interval. Different letters letters indicate statistical significance.

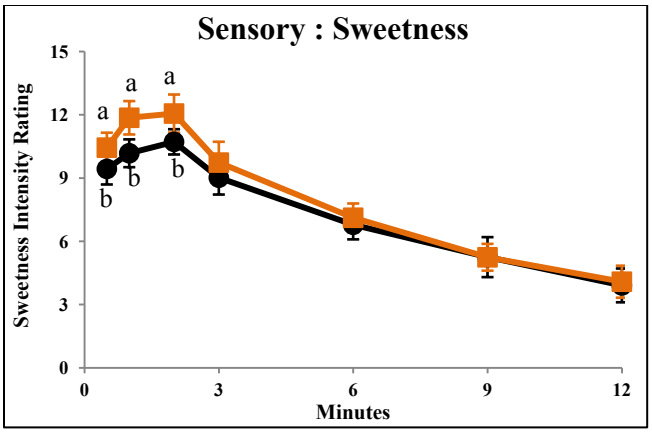


Figure 4-6. Sweetness perceived by six panelists while masticating gums made with sorbitol large (●) and sorbitol small (■). Each data point is an average of duplicate intensity ratings of six panelists with 95% confidence interval. Different letters indicate statistical significance.

CHAPTER 5

Delivery of Taste and Aroma Components in Sugar-free Chewing Gum: Mass Balance Analysis

To investigate mechanisms of flavor delivery in sugar-free chewing gum a mass balance analysis of the volatile and non-volatile flavor compounds released during a 12-min mastication time period was conducted. The mass and release rate of the volatile compounds (ethyl butyrate, benzaldehyde, menthol, menthone and limonene) were monitored in the exhaled breath, in the saliva and the gum bolus; whereas the non-volatiles compounds (sorbitol or mannitol, aspartame and acesulfame K) were monitored in the expectorated saliva and the gum bolus. In general, the percent recovery of the volatile compounds released during mastication was lower or was less quantifiable in comparison to the more polar non-volatile compounds. For the volatile compounds analyzed, the medium hydrophobicity region of compounds showed the lowest % recovery (< 10%) and suggested biological absorption was the main quantitative route of delivery in the oral cavity. The aroma release profile in the exhaled breath was reported not to be influenced by the compound concentration in the saliva or gum bolus during mastication but was suggested to be mainly controlled by residual levels of these compounds in the oral cavity or the lungs. Conversely, the percent recovery of the non-volatile compounds released from the chewing gum during mastication was relatively high (> 90%). The release profile of both the polyol (sorbitol) and HIS compounds were reported not to be concentration dependent during the first 4 minutes of mastication. This suggested the polyol and HIS were physically entrapped in the gum base and released by becoming exposed to the oral cavity during chewing (mechanical stress) for extraction during mastication.

5.1 INTRODUCTION

Flavor is an important characteristic that dictates the quality and acceptance of a food product. In order for flavor to be perceived, the components responsible for the desired sensations need be released from food matrix to either saliva (taste) or headspace in the oral cavity (aroma) and transported to the appropriate receptors (Taylor, 2002). Therefore it is not simply the amount of flavorings present in the food product that is important for flavor perception but the proportion of the initial concentration that is released. Factors influencing flavor release have been extensively studied using various food products including chewing gum as models (Kendal, 1974; Overjo et.al., 2004; Potineni and Peterson, 2008).

Chewing gum can be viewed as a two-phase system primarily composed of a water insoluble gum base and a water soluble sugar/sugar alcohol phase approximately in the ratio of 1:3, with aroma compounds (0.5-1%) distributed between each phase based on the compound affinity. In general, the aroma compounds have been mainly associated with the gum base, based on hydrophobic interactions (De Roos et.al. 1994; Harrison, 2000; Sostmann et.al., 2003). Compounds with a higher affinity to the gum base have a lower release rate, and vice versa. Others have related aroma release to the polyol phase. Potineni and Peterson (2008) reported that cinnamaldehyde release in chewing gum during mastication was correlated to the release of the sugar alcohol phase as well as from the gum base. They concluded that cinnamaldehyde reacted with sorbitol in the chewing gum to generate more polar hemiacetal reaction products that were convert back to free cinnamaldehyde and sugar alcohol in the alkaline conditions of the oral cavity, specifically the hard palate. Consequently these more polar transient

hemi-acetal compounds resulted in a more rapid release rate of cinnamaldehyde than predicted based on the affinity of cinnamaldehyde for the gum base.

Oral processing conditions have also been related to the flavor delivery of chewing gum. Haar et al. (2004) investigated the release of peppermint flavor compounds as a result of effect of oral functions such as chewing frequency (CF), masseter muscle activity (MMA), chewing force (CFO) and saliva flow rate (SFR). They found that the concentration of aroma compounds in the expired air was directly related to the MMA and CF but inversely related to the saliva flow rate (more dilute). Guinard et.al. (1997) investigated the role of saliva flow rate on aroma release and perception from cherry-flavored chewing gum. They reported a significant positive correlation between saliva flow and time to reach maximum intensity of sweetness and cherry flavor (T_{max}) but did not observe significant effect on the maximum perceived intensity (I_{max}) or the duration ($T_{duration}$).

The absorption of aroma compounds in the oral cavity has also been reported. Hussein et al. (1983) analyzed aqueous mouth rinses of solutions containing various aroma compounds to determine the extent of absorption in the mouth. They found compound retention in the mouth ranged among the compounds analyzed and that some compounds were also degraded by hydrolysis. Similarly, Buettner et al. (2000) found higher recoveries of aldehydes and esters in spit-off samples of orange juice and model solutions with increasing polarity suggesting lower adsorption in the mouth, hence minimizing losses during mastication. They also found that lowering the concentration of a compound as well as increasing mastication time from 1 to 5sec significantly increased the percent recovery.

Further insight into mechanisms of flavor delivery of chewing gum has also been investigated by quantifying the release of the flavor stimuli during mastication. While such approach provides information about flavor released from food, it does not take into account what portion of flavor is still present in the food and hence not released. By conducting mass balance on flavor release during mastication, one can get further insights into factors governing delivery. However, for most food products, performing a mass balance of flavor delivery is challenged since foods are swallowed following mastication. Chewing gum is an exception where the bolus can be recovered and analyzed. Because of this benefit chewing gum has been used to investigate quantitative aspects of flavor delivery in the oral cavity during mastication.

Haahr et al. (2004) conducted mass balance on the aroma release of peppermint-flavored chewing gum. Out of the original 20 mg flavor load for menthol and menthone, they were able to trace 1.1mg of each compound in saliva and expired air after 14 min mastication. They also reported that at least 18 mg of these compounds were still retained in the chewing gum bolus after 4 min of chewing but the authors did not mention the remaining mass after the end of the 14 min chewing time period. Similarly, Kendall (1974) also found that about 60% of methyl salicylate was remaining in the gum cud after 30 minutes of mastication or only about 40% of the flavor is released during this time regardless of the concentration of flavoring added to the gum. While a few studies have provided some insight into the release of aroma compounds, little information is available on release of taste compounds (sweeteners) in chewing gum. Understanding the release of taste compounds is necessary to provide a more comprehensive view of flavor perception since it has been shown that the release of water-soluble taste compounds does

influence, not only the taste attributes, but also the perceived flavor intensity (Davidson et.al., 1999; Cook et.al., 2004).

The main objective of this study was to quantitatively monitor the release of the volatile and non-volatile flavor compounds in chewing gum (mass balance analysis) to provide further insight into mechanisms of flavor delivery.

5.2 MATERIALS AND METHODS

Chewing Gum Samples. Two different types of chewing gum samples (described in Chapter 4), with an average mass of 2.83 ± 0.02 g were made with sorbitol of different particle sizes: small (62μm) and large (224μm) The chewing gum formulation consisted of gum base (Cafosa, Spain) (25.9g/100g), powdered sorbitol (34.74g/100g), sorbitol syrup (35.73g/100g), high intensity sweetener (HIS, mixture of acesulfame K and aspartame 1.22g/100g), and aroma (2.42g/100g). The model aroma blend consists of ethyl butyrate (10%), benzaldehyde (10%), limonene (1.06%), menthone (23.37%), and menthol (51.36%). No significant differences were reported in the concentration of the aroma or HIS compounds between these samples.

Chewing gum samples were made by general procedures described in Chapter 3. In brevity, gum base pellets were softened in a forced air oven at approximately 75˚C. Sorbitol syrup was added to the mixing bowl of a mixer containing two overlapping sigma blades (READCO mixer, Read Standard, York, PA) heated to the temperature of approximately 60˚C via a circulating bath. The softened gum base was then added to the mixer along with about 1/3rd of powdered polyol and mixed well for 1 minute. Rest of the sugar alcohol was poured into the mixer, followed by HIS. The flavor blend was added carefully avoiding splashing and allowed to mix with the rest of the ingredients for

another 5 minutes. The final chewing gum dough was rolled using a rolling pin to an average thickness of 0.16cm with the help of a thickness bar. The sheet was then cut into small size sticks, wrapped in plastic and aluminum foil. The samples were finally sealed in Mylar bags and stored in freezer until further use.

Sample collection and processing. To conduct mass balance analysis, concentrations of volatiles and non-volatiles were determined in expectorated saliva, gum bolus and exhaled breath (only for volatiles). Each chewing gum sample was masticated by two panelists in triplicates for 12 min period. For the analysis of the chewing gum bolus and the expectorated saliva, sampling was done every min during the first four min (0-1, 1-2, 2-3, 3-4min) of mastication since studies have shown a rapid release of polyols and some hydrophilic aroma compounds during this initial period of gum mastication (Davidson et al., 1999; Cook et al., 2004; Potineni and Peterson, 2008). For the next 8 min of mastication, sampling was done over two periods, between 4-7 min and 7-12 min respectively. Consequently, six different gum pieces were chewed to obtain data for one sample.

For each time interval, chewing gum samples were masticated at the rate of 1 chew per second (regulated by metronome) without swallowing any saliva. The entire saliva was then expectorated in a 50mL plastic centrifuge tube containing 30mL deionized acidified (0.1% formic acid) water spiked with sucrose (200µg/mL) and sodium saccharin (200µg/mL) as internal standards for sorbitol and HIS, and 6mL chloroform spiked with methyl hexanoate (250µg/mL) as internal standards for aroma compounds. The resulting gum bolus was also collected in another centrifuge tube containing the same solvent system. Mass of both saliva and gum bolus were recorded.

The centrifuge tubes were then shaken in a lab-line orbit shaker (Lab-line Instruments, Inc., Melrose Park, Il) at 150 rpm for 4 hours at ambient temperature. This step helped in solubilization of gum base polymers for a complete extraction of the flavor components incorporated in this phase. To aid uniform exposure of the gum components with extraction solvent, the tubes were tilted to an angle of about 30°. After 4 hours, two distinct layers were visible, the upper aqueous layer containing the taste compounds (polyol and HIS), and the lower organic layer containing the aroma compounds. Aliquots from each layer (2mL) were filtered using 0.45µm pore size nylon filter for aqueous and Teflon filter for organic layers, and were then frozen until analysis. The organic layers were analyzed in neat condition whereas the aqueous layers were diluted with deionized water (1:10). The same procedure as above was repeated for all the other time points for all the samples. It was also ensured that there was at least half an hour gap between any two mastication periods of each panelist to minimize carry-over effects.

Preparation of calibration standards. All calibration standards were prepared and processed analogous to the chewing gum samples, in triplicates. Centrifuge tubes (50mL) were prepared in advance by adding 30mL deionized and acidified water and 6mL chloroform along with the same internal standards as used for the chewing gum samples described in 'sample collection and processing' section. A standard flavor mixture containing the five aroma compounds: limonene (4000µg/mL), ethyl butyrate (8000µg/mL), benzaldehyde (8000µg/mL), menthone (20,000µg/mL) and menthol (40,000µg/mL) was prepared in chloroform. A 300µL aliquot was added to the chloroform layer in the centrifuge tubes such that the final concentrations were 200, 400, 1000 and 2000 µg/mL for limonene, ethyl butyrate, benzaldehyde, menthone and

menthol respectively. Sorbitol (2.03g), acesulfame K (0.015g) and aspartame (0.0203g) were added to the aqueous layer). Gum base (0.75g) was added to simulate the chewing gum extraction conditions. The centrifuge tubes were then shaken using the lab-line orbit shaker (Lab-line Instruments, Inc., Melrose Park, Il, USA) for 4 hours at 150 rpm. Both the organic and aqueous layers (diluted 1:10) were filtered and frozen until further use. Just before analysis, appropriate aliquots of each layers were transferred to 2mL amber vials containing either water or chloroform as diluents, such that standards with these concentrations were formed: sorbitol (6.8, 67.7, 677, 3385, 6770 μg/mL), aspartame (3.66, 36.6, 366, 1830, 3660 μg/mL), limonene (0.2, 2, 20,100, 200 μg/mL), ethyl butyrate and benzaldehyde (0.4, 4, 40, 200, 400 μg/mL), menthone (1, 10, 100, 500, 1000 μg/mL) and menthol (2, 20, 200, 1000, 2000 μg/mL). Two stock solutions for internal standards were prepared: methyl hexanoate (2500 μg/mL chloroform), and sucrose and sodium saccharin (each containing 2000 μg/mL water). An aliquot of 10 μg/mL of each stock solution was added to all standards before analysis.

Analysis of Sorbitol and HIS in Residual Chewing Gum and Expectorated Saliva by Liquid Chromatography/Mass Spectrometry. The non-volatile (sorbitol and HIS) analysis was done in the original chewing gum, residual gum bolus after each sampling period and expectorated saliva in triplicates. Analysis was carried out on a UPLC/MS system (Waters Quattro Premier XE, Milford, MA, USA) equipped with Waters Acquity sample manager and binary solvent manager. For sorbitol analysis, a volume of 0.3μL sample was injected in a Acquity UPLC BEH Amide column (Waters Corp., Milfor, MA, USA; 1.7μm, 100 x 2.1 mm i.d, maintained at 35°C) to run isocratically with flow rate of 0.12mL/min. The mobile phase used was mixture of acetone and water with 0.05%

ammonium hydroxide (A: 80/20 acetone/water; B: 30/70 acetone/water) as 94% mobile phase A and 6% mobile phase B. The MS operation parameters were as follows: ESI negative mode, capillary voltage was 2.8kV, source temperature was 120°C and desolvation temperature was 350°C inter-channel delay of 0.02 second, inter-scan delay of 0.1 second,. Data was collected in multiple reaction monitoring (MRM) mode; dwell time was 0.15sec; transition ion was m/z 89←181 (collision 20, cone 15V) for sorbitol, and m/z 179← X341was monitored for the internal standard, sucrose (collision 25V, cone 35V).

The high intensity sweetener compounds were analyzed from the same extracts used for sorbitol analysis described above. Prior to analysis, samples were frozen in order to prevent degradation of aspartame over time. Separations were done with an XTerra C18 column (Waters Corp., Milford, MA, USA; 3.5µm, 100 x 2.1 mm i.d.) with the column temperature maintained at 50°C. Mobile phase was water containing 0.1% formic acid (A) and methanol (B) with a linear gradient of B in A starting at 10% B in A (0-2.3 min), increasing to 95% B in A (2.3-4.1 min), and finally decreasing to 10% B in A (4.1-5.7 min). Acesulfame K and the internal standard sodium saccharin were detected in ESI negative mode whereas Aspartame was detected in positive mode. Mass spectrometer parameters were as follows: inter-channel delay of 0.02 second, inter-scan delay of 0.1 second, capillary was set to 3.8kV, source temperature was 100°C and desolvation temperature was 250°C. Dwell time for all analytes was set to 0.4second. Data was collected in MRM mode. For acesulfame K, transition m/z 82←X162was monitored with collision energy of 14V and cone of 25 V. Similarly, m/z 120←X295was

monitored for aspartame (collision 22V, cone 20 V) and m/z 106←204 was monitored for the internal standard sodium saccharin (collision 35V, cone 16V).

Analysis of Aroma Compounds in Saliva and Chewing Gum Bolus during Mastication. Aroma compounds extracts were quantified by using a Hewlett-Packard 6890 gas chromatograph equipped with a mass selective detector, a split/splitless injector, flame ionization detector (FID), autosampler, and a fused-silica capillary column (DB-wax, 60m, 0.25 mm i.d., 0.3μm film thickness, Agilent Technologies, CA). The GC operating conditions were as follows : 1μL of sample was injected in splitless mode; inlet temperature was 200°C, oven program was 35°C for 2 min, then increased at 10°C/min to 180°C and then at 30°C/min to 230°C. Data were collected in selected ion monitoring (SIM) mode for these ions: m/z 88 for ethyl butyrate, m/z 106 for benzaldehyde, m/z 68 for limonene, m/z 112 for menthone, m/z 95 for menthol and m/z 74 for the internal standard methyl hexanoate.

Analysis of Aroma Compounds Released in Nose

The breath analysis data collected for the two sorbitol gums in Chapter 4 was used for the current study to measure the total mass of each aroma compound released over the 12 min mastication time period. Mass of aroma exhaled during a period can be calculated if area under the curve and the total volume of air exhaled are known (Eq 5-2). For each compound, areas under curves of the breath-by-breath release profiles were subsequently calculated by following the trapezoidal rule in equation 5-1 (Gearhart et.al., 1974). The average volume displacement of each breath (determined by water displacement method) and the number of breaths (counting acetone peaks in the breath analysis data) were calculated to determine the total volume of air displaced.

$$\text{Area under curve} = \sum \text{conc2} + \text{conc1})/2 * (\text{time2} - \text{time1})$$

Equation 5-1

Mass of aroma exhaled = (Area under curve) * (total volume of air exhaled)

Equation 5-2

Calculation of gum-aroma affinity (log cP). Log cP values are better representations of gum-aroma affinity than log P values since log cP values are calculated based on concentrations of aroma compounds in gum base and water as opposed to octanol and water. So, to determine the gum-aroma affinity, log cP values were calculated for each aroma compound with eq 5-3. Gum base (Cafosa) was ground into fine powder in a mortar and pestle using liquid nitrogen such that the resultant powder could be passed through #40 sieve (35 mesh, 0.0165 inches) for a uniform powder. The ground gum base (0.1g) was placed in a 20mL SPME headspace vial and 1mL deionized water was added. Fifty microliters of a 40mg/L stock solution of the aroma mixture (8mg/mL each for ethyl butyrate, benzaldehyde, menthol, menthone and limonene) was also added to the vial so that the resulting concentration of the aroma mixture was 2mg/L. Another vial was prepared in the same way without added gum base to use as the reference. Triplicate samples were prepared and incubated in a shaking incubator maintained at 37°C with 100 rpm for 4 days to ensure equilibrium.

$$\log cP = \log \frac{\text{concentration in gum base}}{\text{concentration in water}}$$

Equation 5-3

The equilibrated samples were analyzed by headspace SPME/GC method using an SPME fiber containing polydimethylsiloxane (PDMS) phase. Analysis was performed on a Hewlett-Packard 6890 GC equipped with a HP 6890 Mass Selective Detector (MSD), autosampler and a fused-silica capillary column (DB-wax, 30m, 0.25 mm i.d., 0.25 μm film thickness, Agilent Technologies, CA). Inlet temperature for the GC was 200°C and was used in splitless mode, oven program was 38°C for 2 minutes, then increased at 14.5°C/min to 230°C and held for 4 minutes; constant pressure of 13.72 psi (He). Samples were incubated at 37°C, extracted for 10 seconds and desorbed for 7 minutes. Detector was maintained at 250°C. Ions were monitored in the selective ion monitoring (SIM) mode. Ions monitored were m/z 88 for ethyl butyrate, m/z 106 for benzaldehyde, m/z 68 for limonene, m/z 112 for menthone, and m/z 95 for menthol. Quantification was done by external calibration curves obtained by adding 3, 10, 25 and 50 μL of the 40mg/L stock into 1mL deionized water, and plotting against peak areas (linear regression $r^2 > 0.98$).

Statistical Analysis. The average of the triplicate analyses and 95% confidence intervals were calculated using Excel (Ver 2010, Microsoft). Analysis of variance (ANOVA) was used to calculate statistical differences in concentrations of aroma in nose and taste compounds in saliva at different time points within same and between different gum samples ($\alpha \leq 0.05$). When a statistical significance was determined, analysis was followed by Fisher's Least Significant Difference (LSD) to determine differences between samples. Analyses were done using Statistix statistical software (V. 9.0, Analytical Software, Tallahassee, Fl).

5.3 RESULTS AND DISCUSSION

The saliva flow rates of the two panelists during 12 min mastication period were measured (Fig 5-1). While particle size of sorbitol was not found to influence saliva production, there were differences noted between the two panelists. Panelist 2 had significantly higher saliva flow rate than panelist 1 at all time intervals. The rates of saliva flow followed the release profile of sorbitol (Chapter 4); flow rates of both panelists significantly (α=0.05) peaked during the first 2 min of mastication and declined thereafter.

To investigate mechanisms of flavor delivery during mastication of chewing gum, mass balance analysis was conducted on the non-volatiles (sorbitol, HIS) and volatiles (aroma compounds) at continuous time intervals throughout the 12 min mastication period. Experiments were designed to facilitate sampling of the flavor components at the sites they were released as well as in the resultant gum bolus. As such the measurements of the non-volatiles were from the expectorated saliva and gum bolus whereas for the volatile flavor compounds were analyzed from the expectorated saliva, the exhaled breath and the gum bolus.

Analysis of sorbitol and HIS in the saliva and gum bolus during mastication is shown in Figure 5-2 and 5-3, respectively for panelist 1. Panelist 2 reported similar trends and absolute amounts of these taste compounds in the saliva and gum bolus during the 12min mastication time period (Appendix C) however because they had higher saliva flow rate, a lower saliva concentration was observed (data not shown). Sorbitol and HIS after 12 min were almost quantitatively released (80-93%) during the course (Table 5-1). Data for the intervals 4-7 and 7-12 min were pooled over 3 and 5 min respectively.

Overall, more than 86-97% of the sorbitol and HIS compounds were recovered from addition of the mass determined in saliva and gum bolus during mastication (Table 5-1).

Since saliva flow rate was found to be variable during the course of gum mastication (Fig 5-1), the absolute amounts of HIS released during the 12 min mastication were normalized for saliva and subsequently release rates were calculated (Fig 5-4). Overall, gums containing sorbitol small particle sizes had higher release rates for HIS compared to larger particle size gums, thus re-validating our findings in Chapter 4. Further examination of the HIS concentration profile in the saliva over 12 min suggested two phases: 0-4min and then 5-12min. Release rates during these two phases were significantly different from each other however were constant for the given phase suggesting HIS delivery was not concentration dependent but HIS were entrapped in the gum base and released by becoming exposed to the oral cavity during mastication. A faster rate of HIS delivery during the first phase (0-4min) of mastication caused a loss of 40-60% HIS from the chewing gum and the profile resembles the rapid release of sorbitol during the first 4 min (seen in Chapter 3&4) suggesting an interaction between HIS and sorbitol was responsible for the release kinetics observed. After 4 min, following the loss of sorbitol, rates of HIS release decreased significantly compared to the first 4 min (Fig 5-4).

The percent recovery for the five aroma compounds after the 12 min mastication time period is shown in Fig. 5-5. Based on equation 5-4, the overall recovery appears to be high with four of five compounds reporting a recovery greater than 80%. However, the proportion of each aroma compounds released during chewing was relatively low when compared to the polar sorbitol and HIS compounds (Table 5-1); the average %

aroma released for the large and small sorbitol chewing gums were 42, 16, 4, 6 and 16 for ethyl butyrate, benzaldehyde, menthol, menthone, limonene, respectively (% released was calculated by subtracting the mass of compound in gum bolus after 12 min of mastication from the initial mass of compound in chewing gum). Review of eq. 5-4 indicates if the aroma compound had a low % release (high concentration in the bolus) this would result in a high % recovery. To more quantitatively investigate the pathways of the aroma compounds during mastication, an adjusted % recovery was calculated as shown in Eq. 5-5. This later equation calculates the percent recovery based on the amount released (as measured by the loss from the gum bolus) and more appropriately illustrates major loss of each aroma compounds is occurring in the oral cavity, presumably due to absorption through the mucus lining in the oral cavity or lungs (Hussein et al., 1983, Buettner and Schieberle, 2000, Buettner et.al., 2002). For example, only approximately 4-6% of the menthol or menthone released during mastication was quantified in the exhaled breath and the saliva, indicating 94-96% was absorbed in the oral cavity or lungs. The amount absorbed in the oral cavity/lungs was also influenced by the 'solubility' or log cP with the highest absorption occurring at a log cP around 2 and showing lower absorption as this parameter increased and decrease; u-shaped response.

$$\%\, Recovery = \frac{\text{saliva} + \text{nosespace} + \text{gum bolus}}{\text{gum before mastication}}$$

Equation 5-4

$$\%\, Recovery\ (adjusted) = \frac{\text{saliva} + \text{nosespace}}{\text{gum before mastication} - \text{gum bolus}}$$

Equation 5-5

The quantities of each aroma compound in the gum bolus as well as the calculated rate of release into the saliva and nose during mastication are shown in Fig. 5-6 for panelist 1; similar data was observed for panelist 2 (data not shown). In general, although different compounds reported different release dynamics in the mouth there were also common trends. For all five compounds, the concentration in the gum bolus did not predict the concentration in the saliva over the 12min time period. For example the concentration of ethyl butyrate in the gum bolus did not decrease by any appreciable amount from 0-4min however the concentration in the saliva decreased by approximately 5-fold. Further review of the aroma concentration profile in the saliva over 12 min suggested the aroma delivery was occurring at a faster rate during the phase between 0-4min than from 5-12min; or had two different phases similar to HIS release profiles (Fig 5-4). For the first phase, the sorbitol particles were suggested to facilitate aroma delivery by applying mechanical stress on the gum base during mastication as previously suggested in Chapter 4. After 4 minutes, the loss of sorbitol (see Fig. 5.2) diminished this factor on mechanisms of aroma release.

The transport of aroma from food to the gaseous phase is commonly considered as a three phase process: from food to saliva to air, saliva concentration of aroma essentially acts as a reservoir for delivery into the air phase which is ultimately carried to the receptors in the olfactory epithelium. However the concentration of the aroma compounds in the exhaled breath from the nose was generally also not related to the concentration in the saliva, except for benzaldehyde. This can be likely explained by absorption of these compounds in the oral cavity or the lungs and suggested biological

absorption might be a major factor controlling aroma delivery as monitored from the exhaled air.

In summary, mass balance analysis of the volatiles and non-volatiles was useful in elucidating *in-vivo* flavor delivery mechanisms in sugar-free chewing gum. The HIS compounds were suggested to be 'entrapped' within the gum base and were released when exposed to the oral cavity during mastication. The delivery of aroma compounds were found to be influenced by the presence of the polyol particles as a result of more mechanical stress on the gum base to expose more surface area for release. Additionally, the absorption of the aroma compounds in the oral cavity or lungs was found to complicate aroma release in chewing gum.

Table 5-1: Percent recovery of sorbitol, aspartame and acesulfame K released from the initial chewing gum as monitored in the pooled saliva and the resultant bolus over a 12 min mastication time period.

Sorbitol Gum (Particle)	Compound	% Released[a]	% Recovery[b]
Large	Sorbitol	82	86
Small	Sorbitol	93	95
Large	Aspartame	67	92
Small	Aspartame	84	97
Large	Acesulfame K	87	94
Small	Acesulfame K	88	97

a = mass released/(mass in chewing gum – mass in gum bolus after 12 min)

b = (mass released in 12min+ mass in gum bolus after 12min)/mass in chewing gum

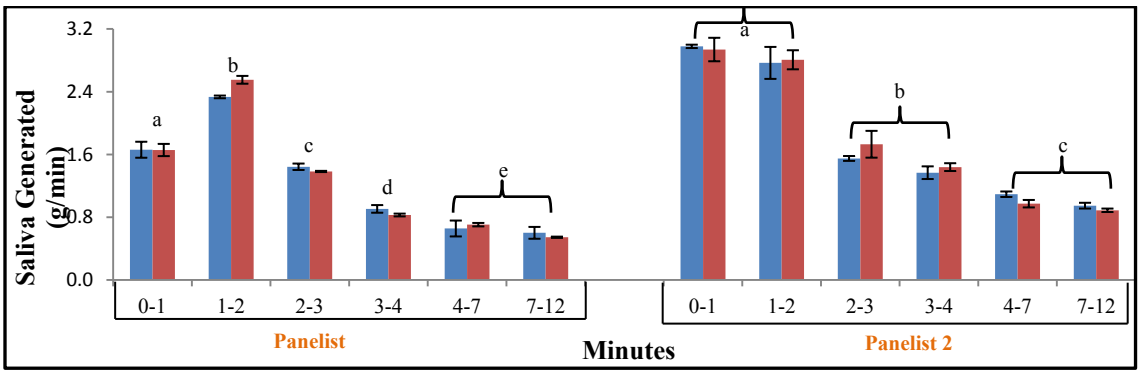


Figure 5-1: Saliva flow rates (g saliva/min) of two panelists during mastication of gums for 12 min. Each data is the average of three replicates and the error bar shows 95% confidence interval. The two gums are: sorbitol large particle size, (■) and sorbitol small particle size (■).Different letters indicate statistical significance (α=0.05)

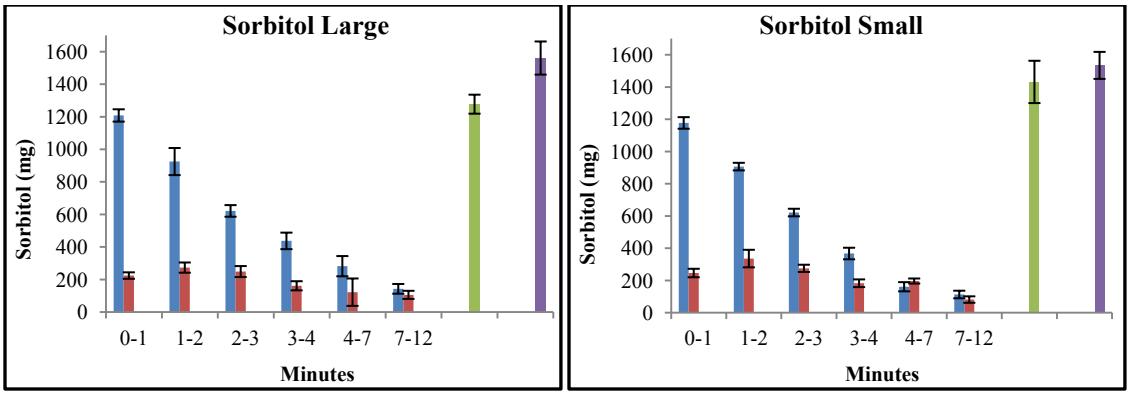


Figure 5-2: Mass balance of sorbitol in large and small particle size chewing gum. Masses in gum bolus ( ), saliva ( ), recovered ( ) and the gum ( ) samples over a12min mastication time period are presented. Average of three replicates and the error bar shows 95% confidence interval.

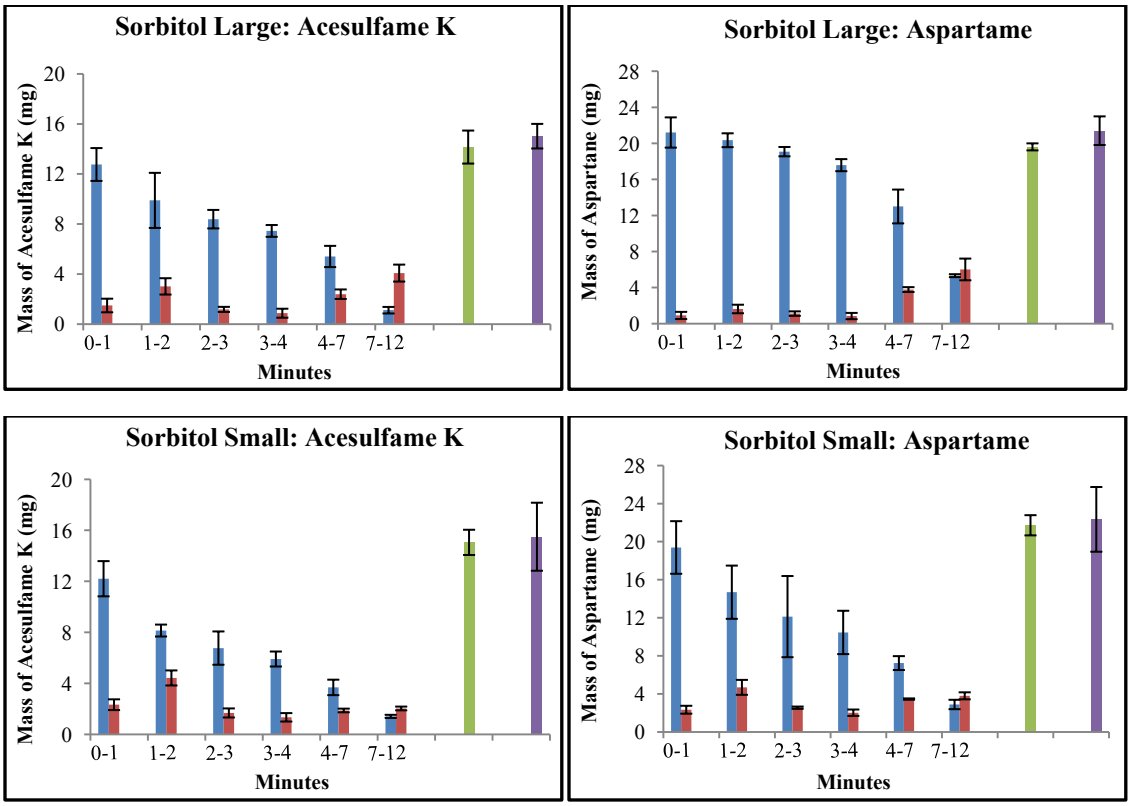


Figure 5-3: Mass balance of acesulfame K and aspartame in chewing gums formulated with large and small particles of sorbitol. Masses in gum bolus ( ), saliva ( ), recovered ( ) and the gum ( ) samples over a12 min mastication time period are presented. Average of three replicates and the error bar shows 95% confidence

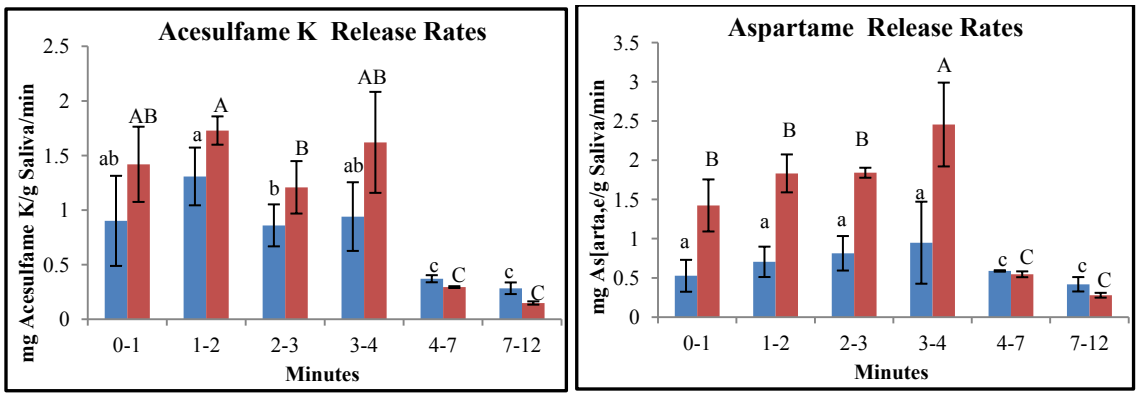


Figure 5-4. Release rates for acesulfame K and aspartame for one panelist over a 12min time period during mastication of sorbitol large (■), and sorbitol small (■),chewing gums. Each data is an average of triplicates with ±95% confidence interval; different letters in lower case indicate statistical significance for sorbitol large gums and in upper case indicate statistical significance for sorbitol small gums (α=0.05).

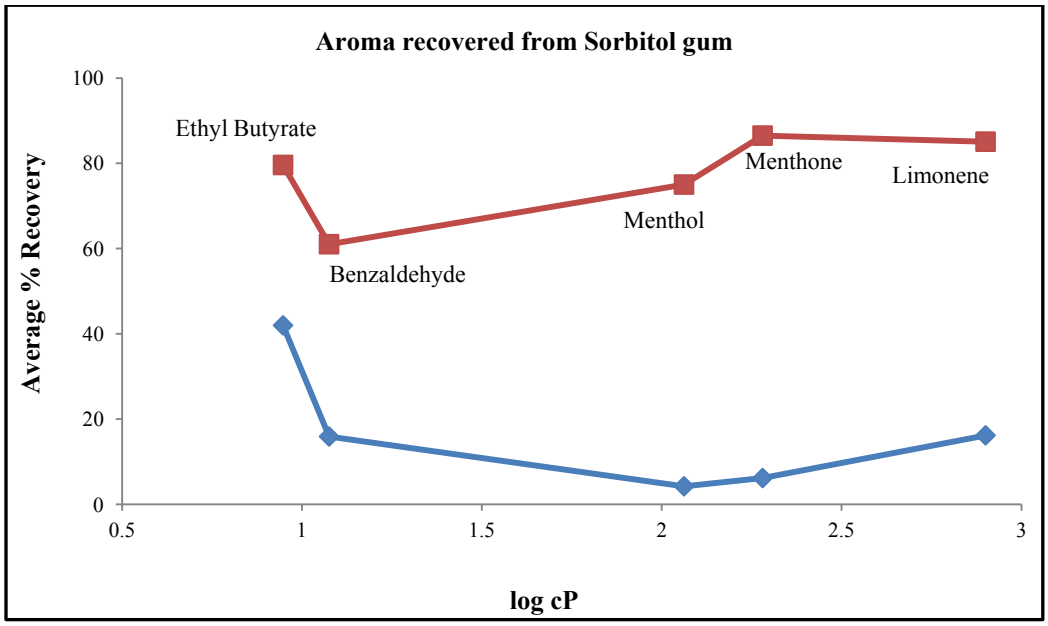
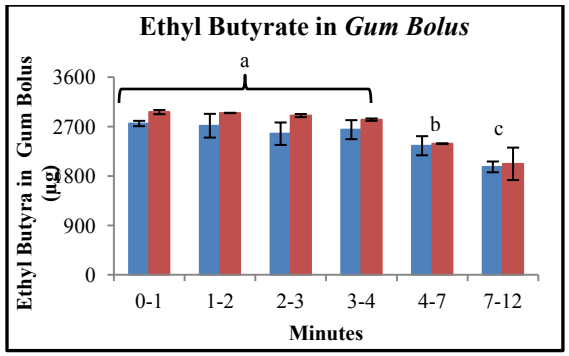
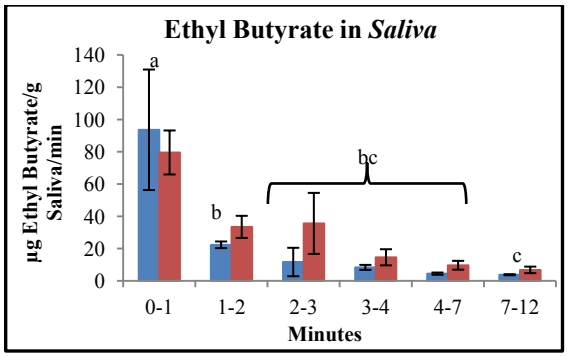
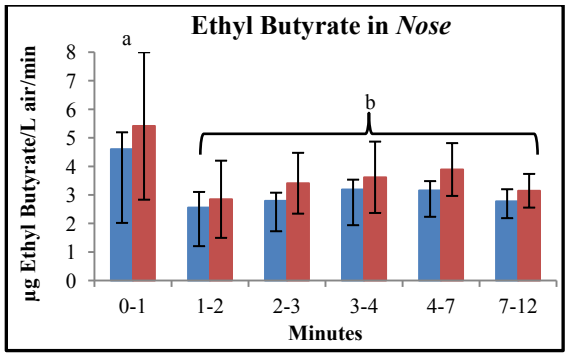
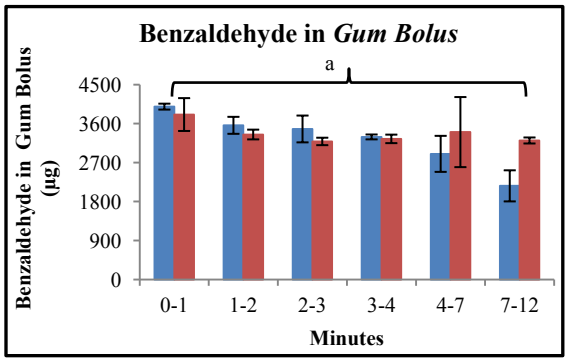


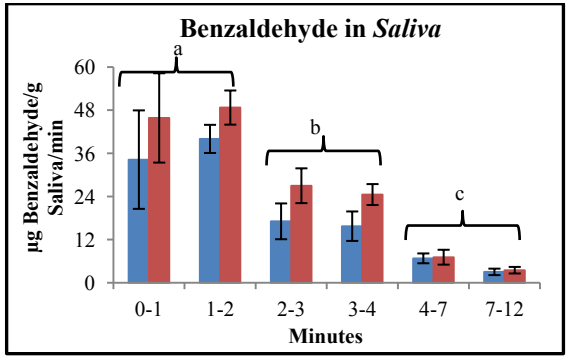
Figure 5-5. Average percent recovery of aroma compounds in the small and large particle sorbitol based on initial mass of aroma in gum before mastication (■), and based on the mass of aroma released from the gum (●), over 12 min of mastication by one panelist. Data shown are average of three observations for one panelist.

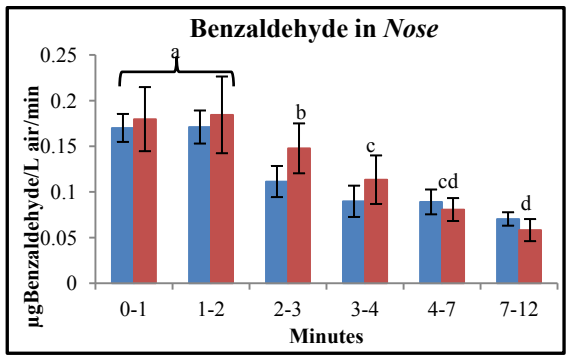

Ethyl Butyrate in *Gum Bolus*
Ethyl Butyra in Gum Bolus (µg)
3600
2700
1800
900
0
a
b
c
0-1
1-2
2-3
3-4
4-7
7-12
Minutes

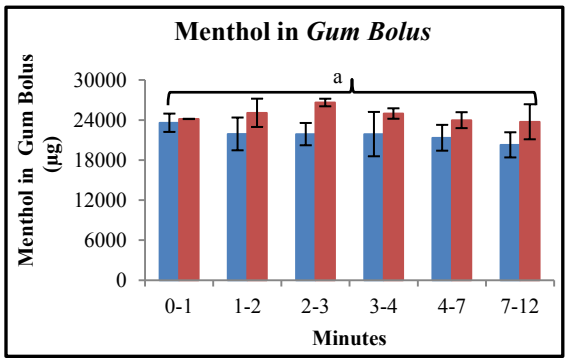

Ethyl Butyrate in *Saliva*
µg Ethyl Butyrate/g Saliva/min
140
120
100
80
60
40
20
0
a
b
bc
c
0-1
1-2
2-3
3-4
4-7
7-12
Minutes

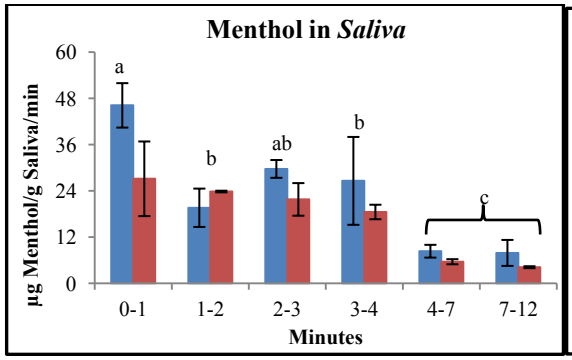

Ethyl Butyrate in *Nose*
µg Ethyl Butyrate/L air/min
8
7
6
5
4
3
2
1
0
a
b
0-1
1-2
2-3
3-4
4-7
7-12
Minutes

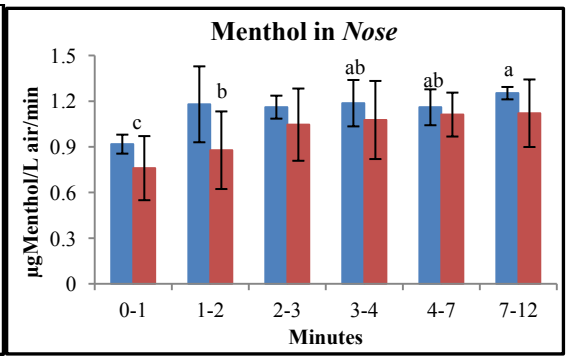

Benzaldehyde in *Gum Bolus*
Benzaldehyde in Gum Bolus (µg)
4500
3600
2700
1800
900
0
a
0-1
1-2
2-3
3-4
4-7
7-12
Minutes


Benzaldehyde in *Saliva*
µg Benzaldehyde/g Saliva/min
60
48
36
24
12
0
a
b
c
0-1
1-2
2-3
3-4
4-7
7-12
Minutes


Benzaldehyde in *Nose*
µgBenzaldehyde/L air/min
0.25
0.2
0.15
0.1
0.05
0
a
b
c
cd
d
0-1
1-2
2-3
3-4
4-7
7-12
Minutes


Menthol in *Gum Bolus*
Menthol in Gum Bolus (µg)
30000
24000
18000
12000
6000
0
a
0-1
1-2
2-3
3-4
4-7
7-12
Minutes


Menthol in *Saliva*
µg Menthol/g Saliva/min
60
48
36
24
12
0
a
b
ab
b
c
0-1
1-2
2-3
3-4
4-7
7-12
Minutes


Menthol in *Nose*
µgMenthol/L air/min
1.5
1.2
0.9
0.6
0.3
0
c
b
ab
ab
a
0-1
1-2
2-3
3-4
4-7
7-12
Minutes

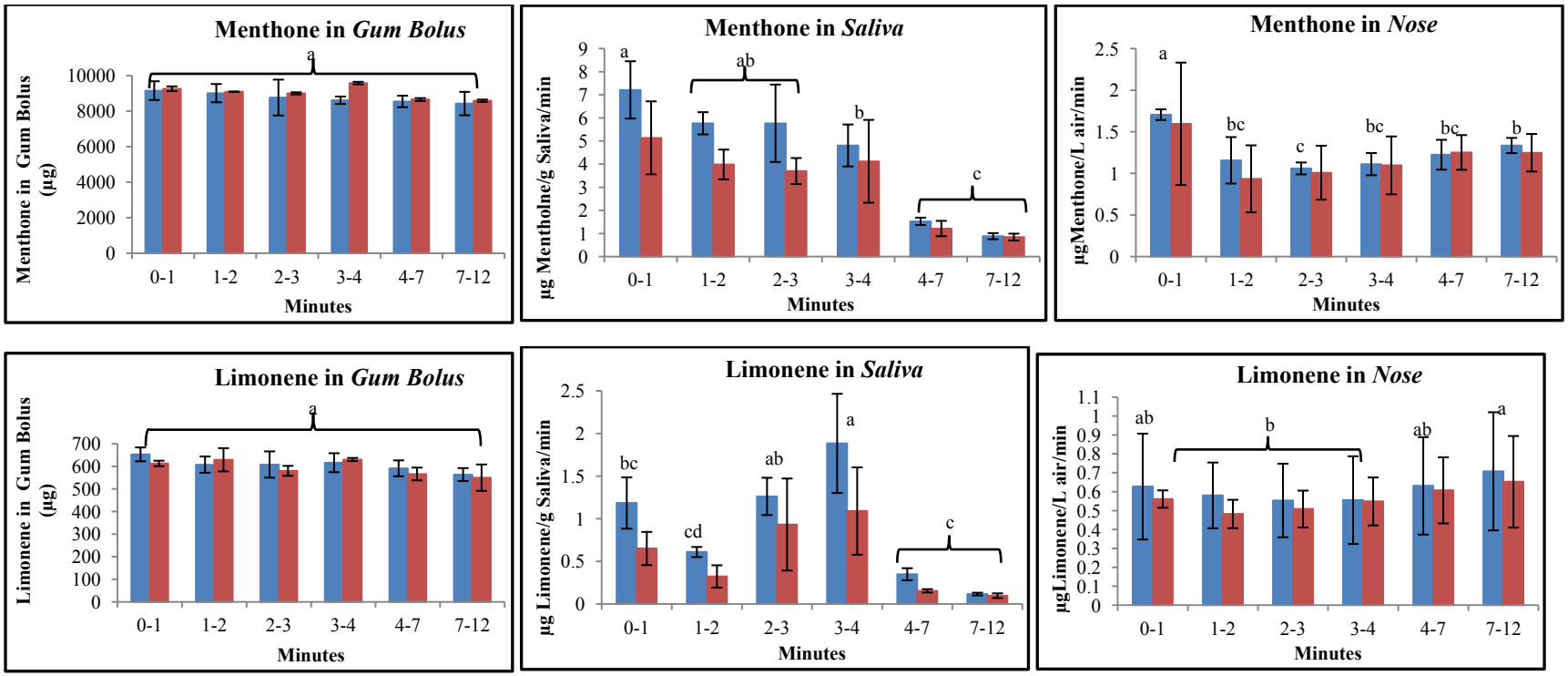


Figure 5-6. Mass balance of ethyl butyrate, benzyaldehyde, menthol, menthone and limonone in gum bolus, saliva and nose during mastication of chewing gum made with sorbitol large (■) and sorbitol small (■) particle sizes. Absolute mass in cud (µg) and rates of release in saliva (µg/g saliva/min) and nose (µg/L air/min) are presented. Each data is average of triplicates with ±95% confidence interval; statistical significance if different letters.

CHAPTER 6

Conclusions and Future Research

In the work presented, we have investigated the effect of polyol-type and their physical/chemical properties on delivery of volatile and non-volatile flavor components from chewing gum during mastication. We have shown that by choosing an appropriate polyol with certain water solubility and/or particle size, flavor release profiles can be controlled to cause significant impact on sensory perception. This is a relatively translatable technology for the food industry to improve flavor performance of chewing gum.

This work reported polyol material with a lower water-solubility (i.e. mannitol) resulted in increased delivery of HIS and aroma compounds. Such polyol was released more slowly from the chewing gum during mastication and thereby disrupted the gum base structure. This could enhance the exposure of gum base surface and facilitate flavor release. It was also supported that increasing the surface area of the polyol material increased the delivery of the polar HIS compounds indicating these materials were bound to the surface of the polyol and provided a new mechanisms of HIS delivery in related products. Finally, the observation that aroma delivery to the 'nose space' was largely controlled by absorption in the oral cavity and/or lungs further indicated the complexity of defining flavor release from foodstuffs.

Based on the observations for the current work two main future research topics are suggested. The first area would be to investigate more specifically the type of polyol-HIS interactions. This information could potentially be used to provide further strategies to designs polyol materials for flavor delivery. The second area would be to further define the mechanisms of aroma binding in the oral cavity. Considering absorption was

found to be a major ‘loss’ in terms of ‘flavor’ potential, defining technology to limit absorption would be a logical strategy to more effectively utilize the flavoring materials.

CHAPTER 7

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APPENDICES

Appendix A: Additional figures for Chaper 3

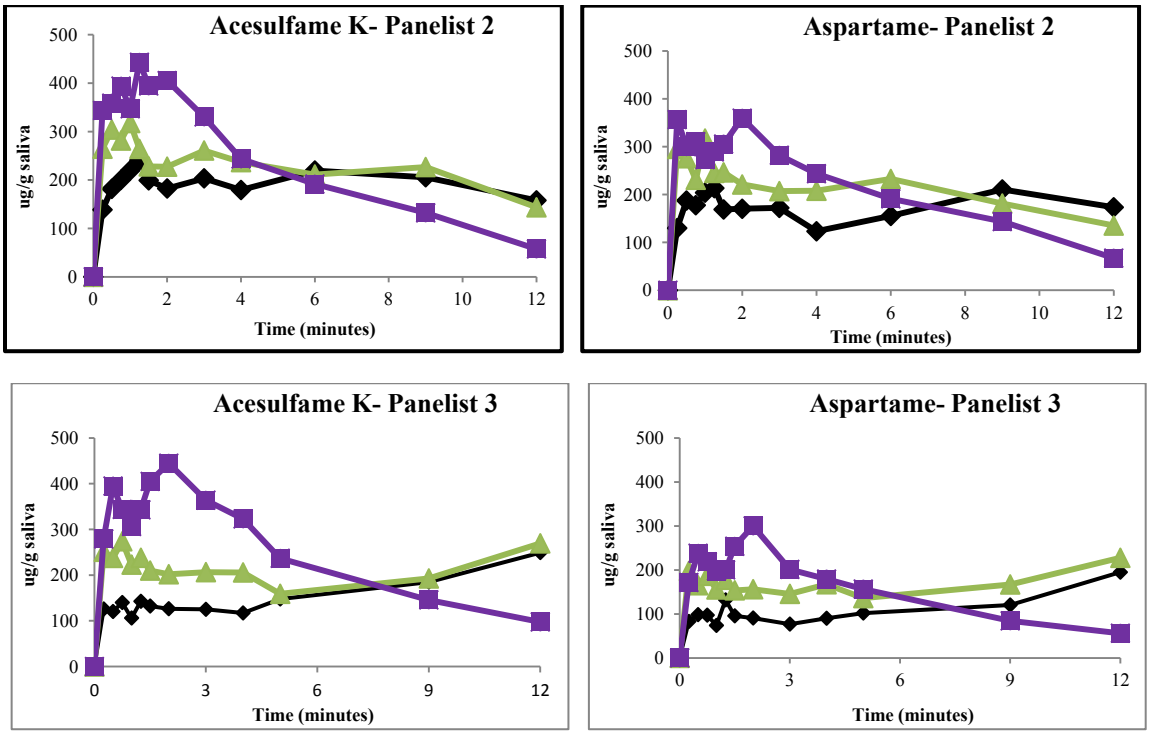


Figure 3-4. Release profiles of acesulfame K. and aspartame for panelist 2 and 3 from chewing gum samples made with sorbitol (◆), xylitol (▲), and mannitol (■). Each data point is an average of triplicate readings.

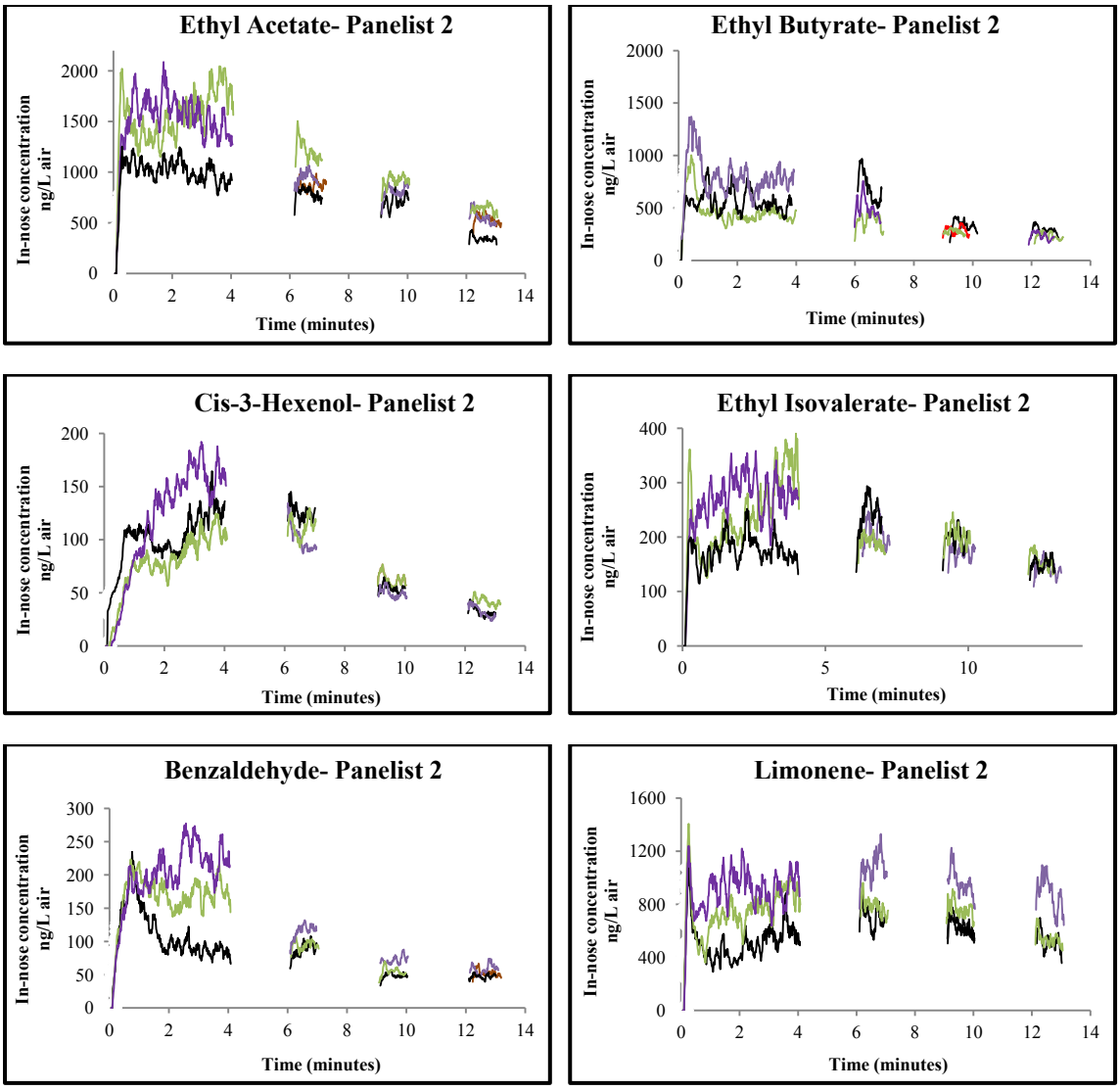


Figure 3-5. Breath release profiles of ethyl acetate, ethyl butyrate, cis-3-hexenol, ethyl isovalerate, benzaldehyde, and limonene for panelist 2. Each curve has the release data of the aroma compound from the polyols, sorbitol (—), xylitol (—), and mannitol (—), and represents the mean of three replicates subsequently smoothed by a 5s moving average trend line.

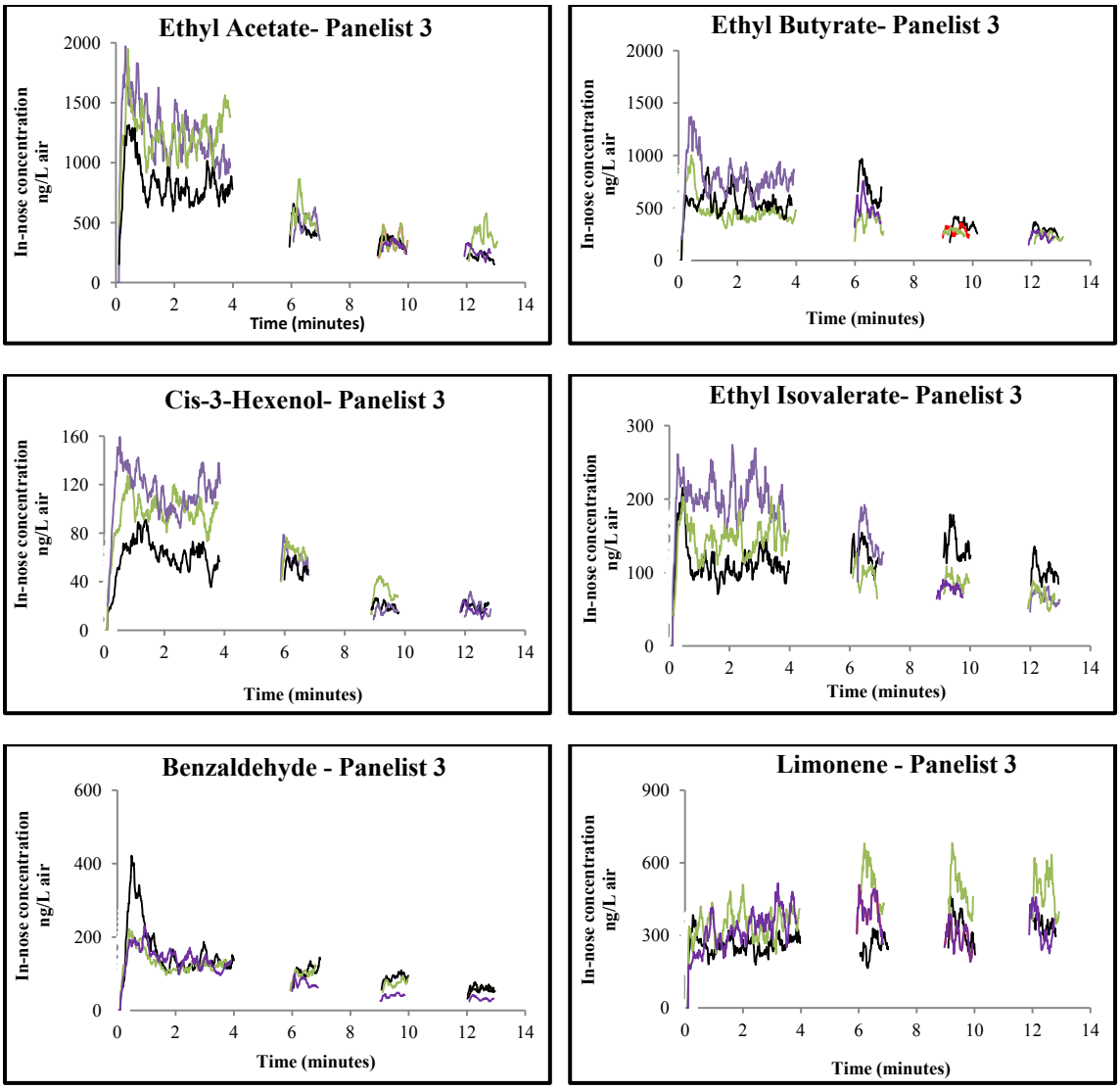
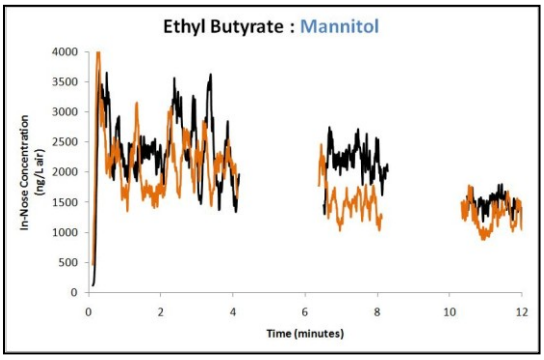
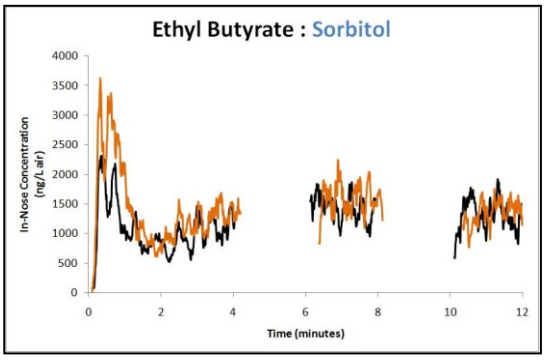
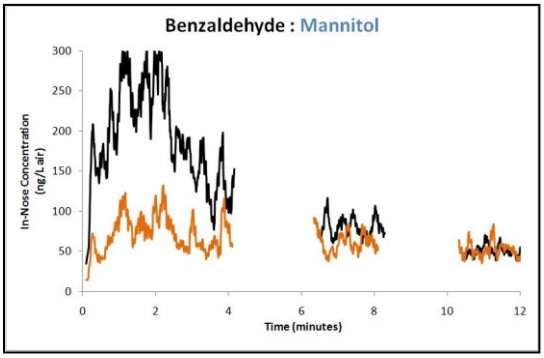


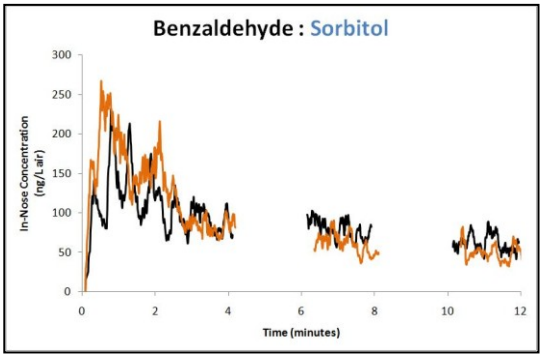
Figure 3-6. Breath release profiles of ethyl acetate, ethyl butyrate, cis-3-hexenol, ethyl isovalerate, benzaldehyde, and limonene for panelist 3. Each curve has the release data of the aroma compound from the polyols, sorbitol (—), xylitol (—), and mannitol (—), and represents the mean of three replicates subsequently smoothed by a 5s moving average trend line.

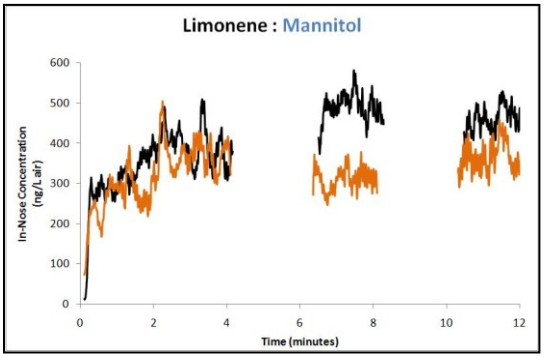
Appendix B: Additional Figures for Chapter 4











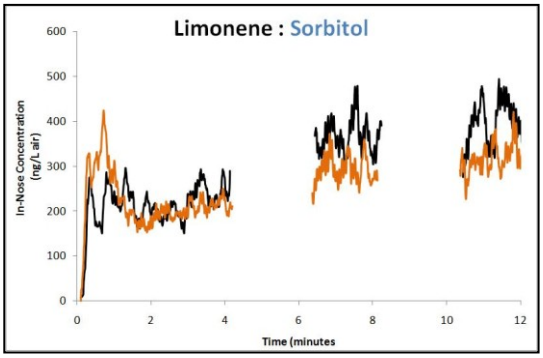


Figure 4-7a. Breath release profiles of ethyl butyrate, benzaldehyde, and limonene for panelist 2 for gums made with mannitol, large particle (—) and small particle (—); each curve is an average of triplicates.
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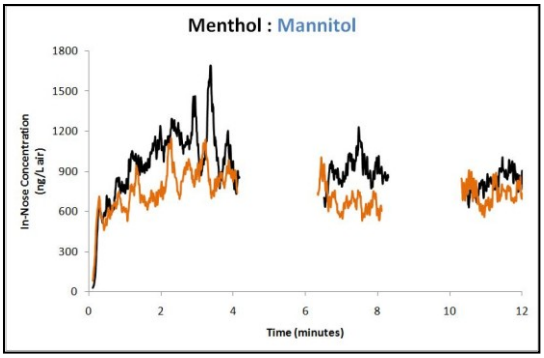
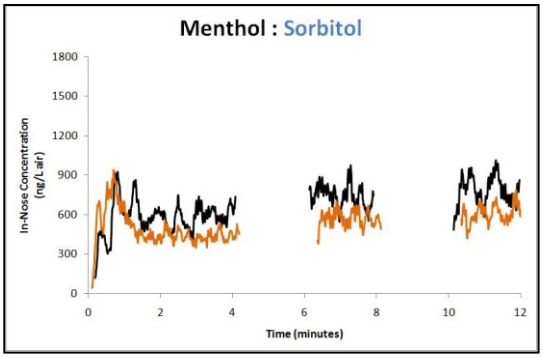
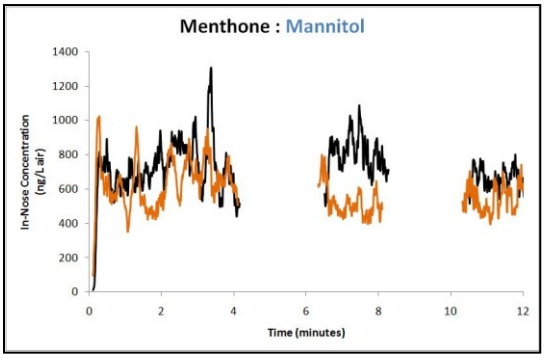
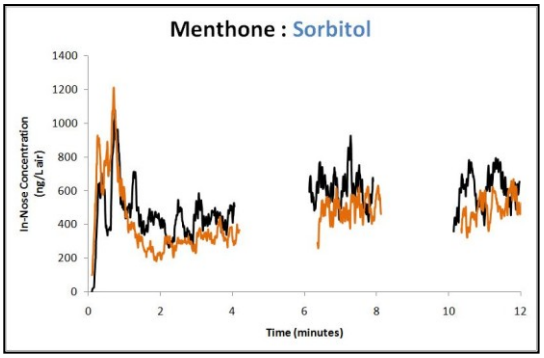
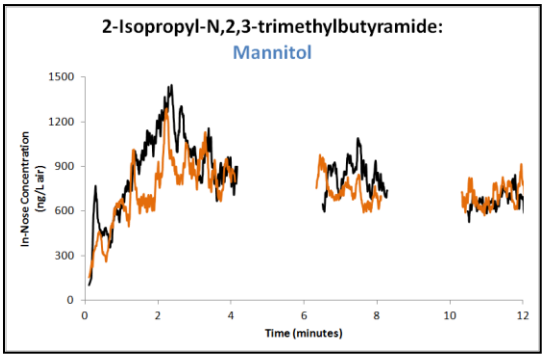
Figure 4-8a. Breath release profiles of ethyl butyrate, benzaldehyde, and limonene, for panelist 2 for gums made with sorbitol, large particle (—) and small particle (—); each curve is an average of triplicates.











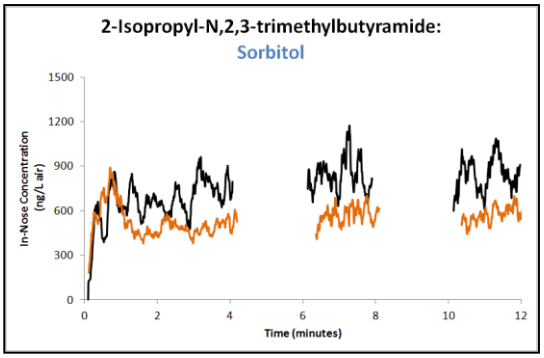
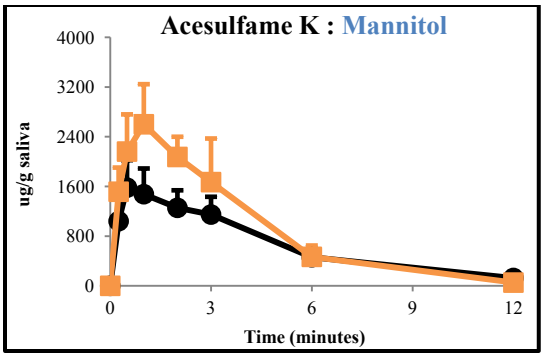
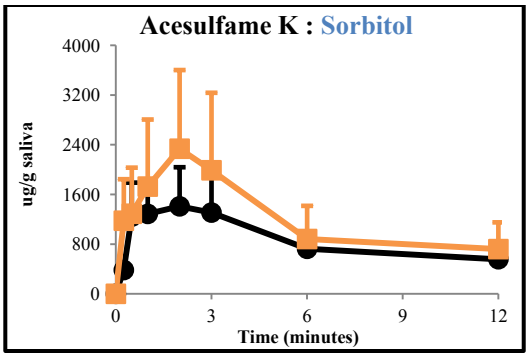


Figure 4-7b. Breath release profiles of menthol, menthone and 2-isopropyl-N,2,3-trimethylbutyramide for one panelist for gums made with mannitol, large particle size (—) and small particle size (—). Each plot is the average of triplicates.

Figure 4-8b. Breath release profiles of menthol, menthone, and 2-isopropyl-trimethylbutyramide for one panelist for gums made with sorbitol, large particle size (—) and small particle size (—). Each plot is the average of triplicates.





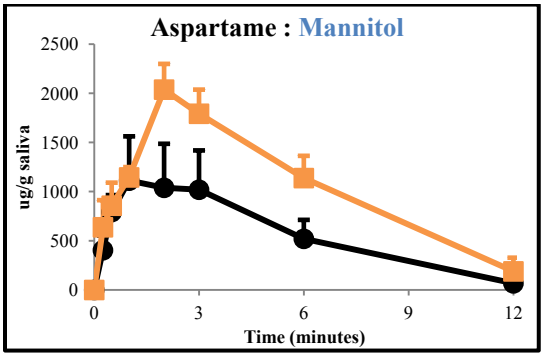


Figure 4-9a. Release profiles of acesulfame K, and aspartame in saliva for panelist 2 for gums made with mannitol large (●)and small (■)particle size. Each data point is an average of three replicates ± 95% confidence interval.

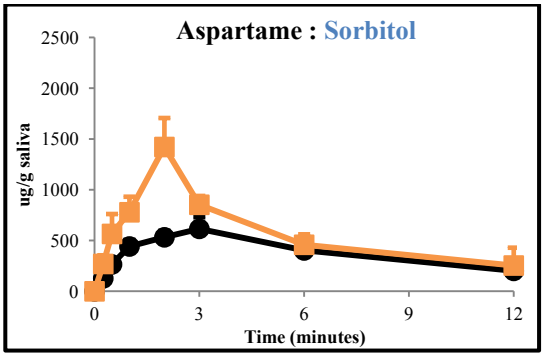


Figure 4-9b. Release profiles of acesulfame K, and aspartame in saliva for panelist 2 for gums made with sorbitol large (●) and small (■) particle size. Each data point is an average of three replicates ± 95% confidence interval.

Appendix C: Additional Figures for Chapter 5

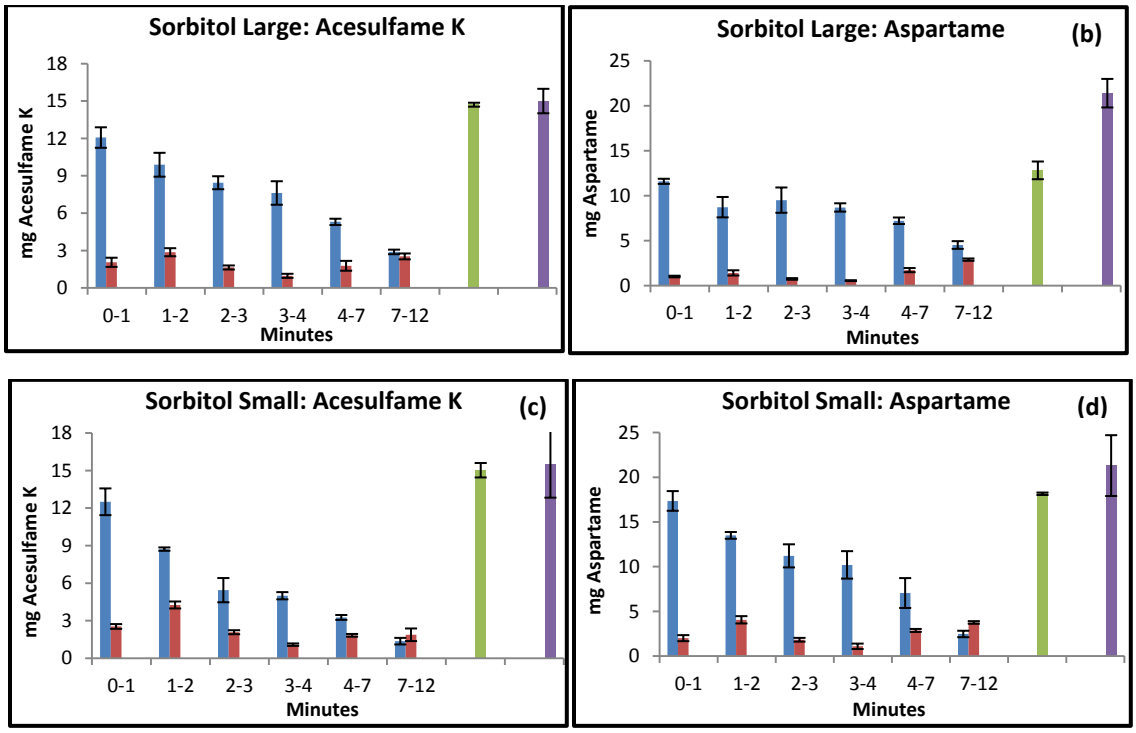


Figure 5-7: Mass balance of acesulfame K and aspartame in chewing gums formulated with large and small particles of sorbitol. Masses in gum bolus (■), saliva (■), recovered (■) and the gum (■) samples over a12 min mastication time period for panelist 2 are presented. Average of three replicates and the error bar shows 95% confidence

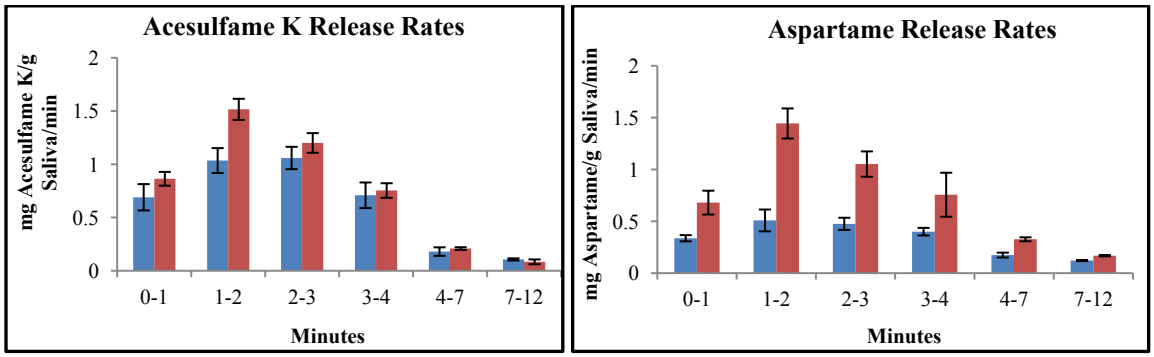


Figure 5-8. Release rates for acesulfame K and aspartame for panelist 2 over a 12min time period during mastication of sorbitol large (■), and sorbitol small (■),chewing gums. Each data is an average of triplicates with ±95% confidence interval.

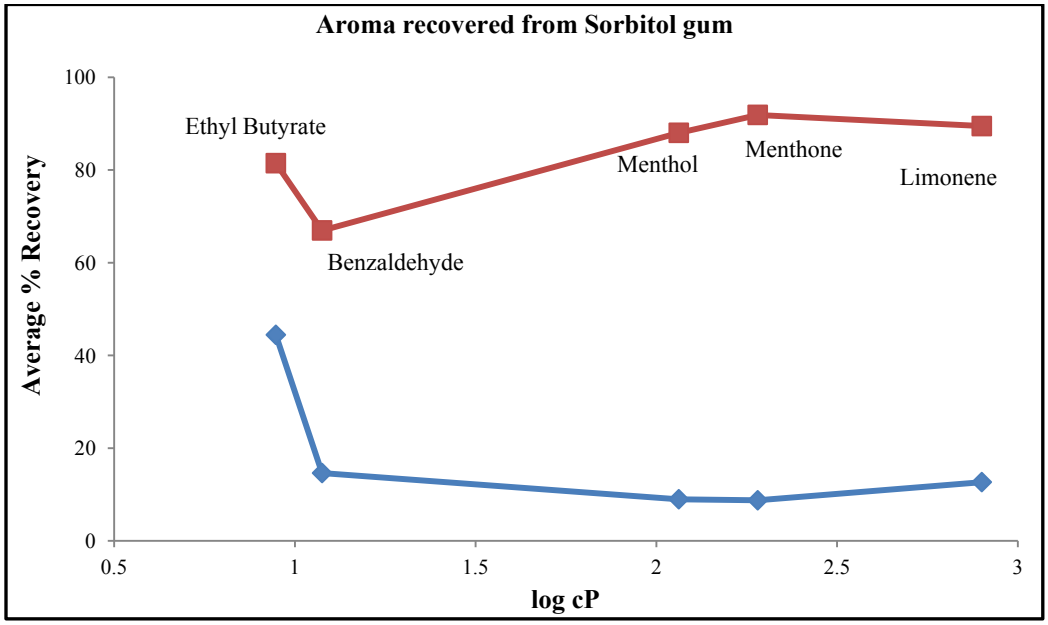
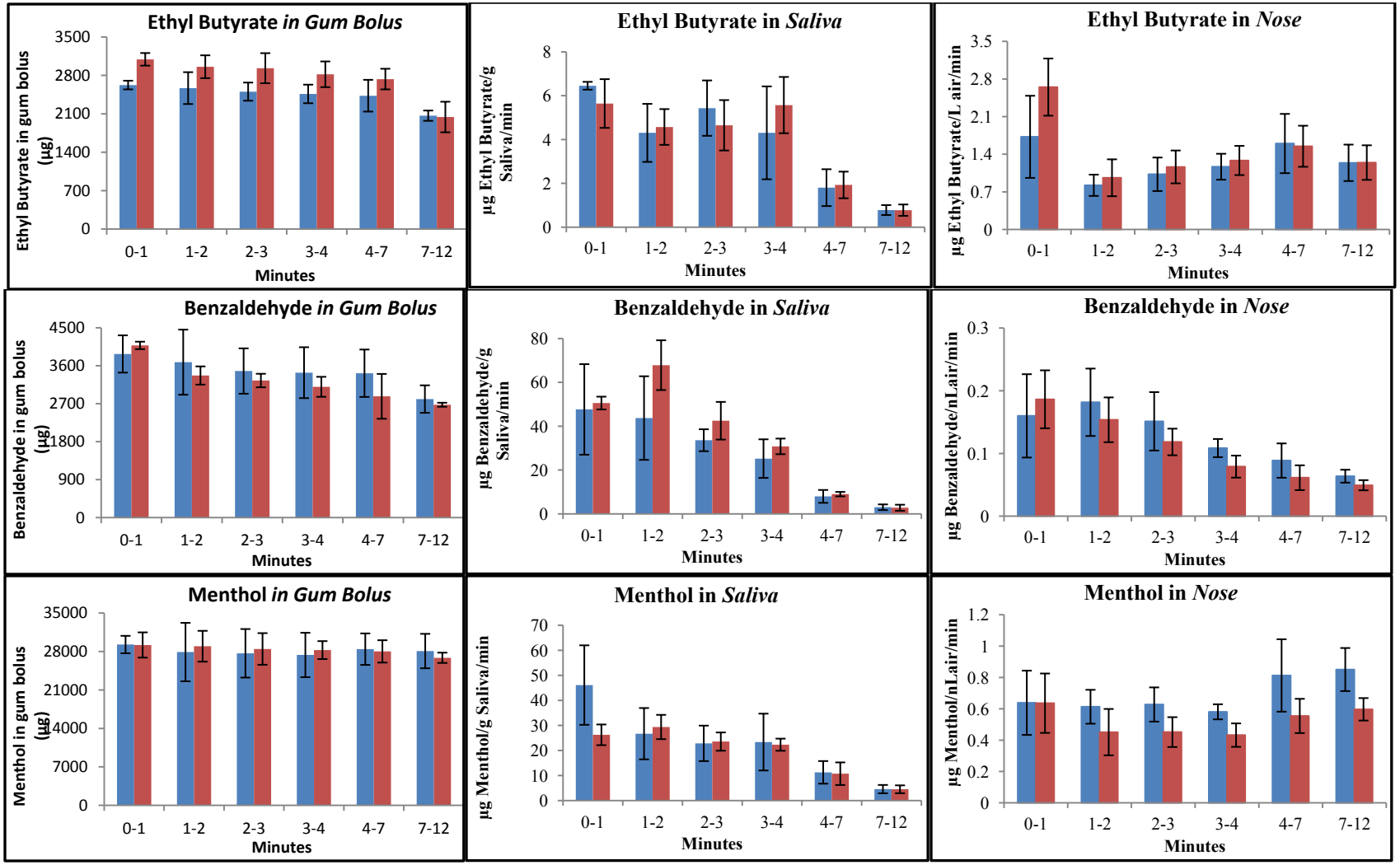


Figure 5-9. Average percent recovery of aroma compounds in the small and large particle sorbitol based on initial mass of aroma in gum before mastication (■), and based on the mass of aroma released from the gum (●), over 12 min of mastication by one panelist. Data shown are average of three observations for panelist 2.


Ethyl Butyrate *in Gum Bolus*
Ethyl Butyrate in gum bolus (µg)
3500
2800
2100
1400
700
0
0-1
1-2
2-3
3-4
4-7
7-12
Minutes
Ethyl Butyrate in *Saliva*
µg Ethyl Butyrate/g Saliva/min
8
6
4
2
0
0-1
1-2
2-3
3-4
4-7
7-12
Minutes
Ethyl Butyrate in *Nose*
µg Ethyl Butyrate/L air/min
3.5
2.8
2.1
1.4
0.7
0
0-1
1-2
2-3
3-4
4-7
7-12
Minutes
Benzaldehyde *in Gum Bolus*
Benzaldehyde in gum bolus (µg)
4500
3600
2700
1800
900
0
0-1
1-2
2-3
3-4
4-7
7-12
Minutes
Benzaldehyde in *Saliva*
µg Benzaldehyde/g Saliva/min
80
60
40
20
0
0-1
1-2
2-3
3-4
4-7
7-12
Minutes
Benzaldehyde in *Nose*
µg Benzaldehyde/nLair/min
0.3
0.2
0.1
0
0-1
1-2
2-3
3-4
4-7
7-12
Minutes
Menthol *in Gum Bolus*
Menthol in gum bolus (µg)
35000
28000
21000
14000
7000
0
0-1
1-2
2-3
3-4
4-7
7-12
Minutes
Menthol in *Saliva*
µg Menthol/g Saliva/min
70
60
50
40
30
20
10
0
0-1
1-2
2-3
3-4
4-7
7-12
Minutes
Menthol in *Nose*
µg Menthol/nLair/min
1.2
1
0.8
0.6
0.4
0.2
0
0-1
1-2
2-3
3-4
4-7
7-12
Minutes

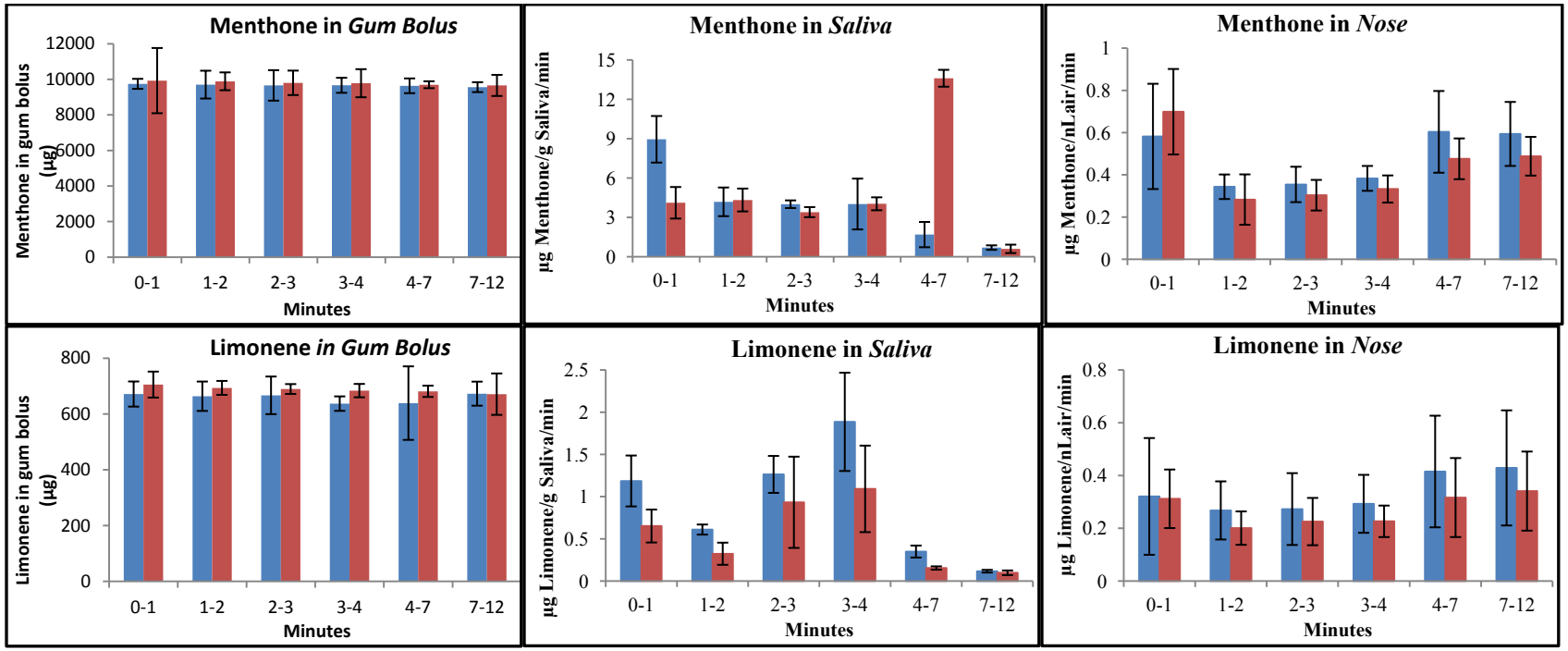


Figure 5-10. Mass balance of ethyl butyrate, benzyaldehyde, menthol, menthone and limonone in gum bolus, saliva and nose during mastication of chewing gum made with sorbitol large (■) and sorbitol small (■) particle sizes. Absolute mass in cud (µg) and rates of release in saliva (µg/g saliva/min) and nose (µg/L air/min) are presented. Each data is average of triplicates with ±95% confidence interval for panelist 2.